Heat-Induced Gelation of Faba Bean and Rice Protein Isolates with Egg White Powder and Hydrocolloids: Toward Hybrid Vegetarian Sausages

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Abstract

This thesis investigated the heat-induced gelation behaviour of two plant-based proteins, faba bean protein isolate (FBPI) and rice protein isolate (RPI), and their functional enhancement through the addition of egg white powder (EWP), modified waxy maize starch (MS), modified potato starch (PS), and methylcellulose (Me). The overarching goal was to develop gel matrices and sausage analogues with improved textural, rheological, and structural properties suitable for meat free alternatives. The experimental work was conducted in three stages. First, the minimum gelation concentrations (MGC) of FBPI and RPI were determined. Results showed that FBPI was able to form stable gels at 12% (w/w), whereas RPI required concentrations exceeding 15% (w/w) and exhibited limited self-gelling capacity. Subsequent, FBPI (12% (w/w)) and RPI (15% (w/w)) dispersions were blended with EWP across a range of ratios at pH 7 to investigate the synergistic effects between plant and animal proteins. Rheological analysis demonstrated that increasing EWP content significantly improved the storage modulus (G′) and reduced the loss factor (tan δ), indicating stronger and more elastic gel networks. Water-holding capacity (WHC), texture profile analysis (TPA), and SEM imaging confirmed that EWP incorporation led to firmer, more cohesive gels with finer, more homogenous microstructures. The second stage aimed to examine the integration of hydrocolloids (MS, PS, Me) into FBPI at 12% (w/w) and RPI at 15% (w/w) matrices to develop heat-induced composite gels. In FBPI systems, Me improved viscoelasticity, while starches contributed to firmness and WHC, with optimal results observed at 5% MS and 10% PS. RPI, which lacked intrinsic gelling capacity, responded more strongly to starch addition, particularly MS, which improved gel structure and stability even at lower concentrations. In the third stage, sausage formulations were developed with high (Me 1.6%, MS 5%, PS 10% (w/w)) and reduced hydrocolloid levels (Me 0.8%, MS 2%, PS 5% (w/w)). Sausages prepared with reduced hydrocolloid levels and supplemented with 8.8% (w/w) EWP (approximately 40% of total protein content) showed a significant increase in protein content and hardness Notably, improvements in chewiness was observed only in the FBPI-H-E sample. This research provides valuable insights into the development of meat free sausages with desirable textural properties, focusing on ingredient formulations and their effects on texture using FBPI and RPI. As sensory evaluation and flavour development were not within the scope of this study, further research is needed to address these aspects by incorporating flavourings to improve overall consumer acceptability.

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aw	Water activity
DSC	Differential scanning calorimetry
EAI	Emulsifying activity index
ESI	Emulsifying stability index
EW	Egg white
EWP	Egg white powder
FBPI	Faba bean protein isolate
FBPI-H	FBPI sausage prepared with 10% (w/w) PS, 5% (w/w) MS, and 1.6% (w/w) Me
FBPI-H-E	FBPI sausage prepared with 5% (w/w) PS, 2% (w/w) MS, 0.8% (w/w) Me, and 8.8% (w/w) EWP
G′	Storage modulus
G″	Loss modulus
HCl	Hydrochloric acid
IEF	Isoelectric focusing
LVR	Linear viscoelastic region
Me	Commercial methylcellulose (Methocel™ A4C)
MCG	Minimum gelation concentration
MS	Modified waxy maize starch (FIRM-TEX®)
NaCl	Sodium chloride
NaN_3	Sodium azide
OHC	Oil-holding capacity
pI	Isoelectric point
PS	Modified potato starch (PenBind® 850)
RPI	Rice protein isolate
RPI-H	RPI sausage prepared with 10% (w/w) PS, 5% (w/w) MS, and 1.6% (w/w) Me
RPI-H-E	RPI sausage prepared with 5% (w/w) PS, 2% (w/w) MS, 0.8% (w/w) Me, and 8.8% (w/w) EWP
SEM	Scanning electron microscopy
TDF	Total dietary fibre
TPA	Texture profile analysis
L*, a*, b*	Colour parameters: Lightness (L*), Redness (a*), Yellowness (b*)
WHC	Water-holding capacity

Chapter 1. Introduction

The increasing global demand for sustainable, ethical, and health-conscious food choices has intensified interest in plant-based protein alternatives. As consumers become more aware of the environmental impact and health implications associated with animal-derived foods, the food industry has responded by developing novel formulations that replicate the nutritional and functional characteristics of traditional meat products (Kyriakopoulou et al., 2021; Ishaq et al., 2022). Within this context, legume and cereal proteins, such as faba bean protein isolate (FBPI) and rice protein isolate (RPI), have emerged as promising plant-based ingredients due to their high protein content, functional versatility, and relatively neutral flavour profiles (Badjona et al., 2025; Agboola et al., 2005).

Despite these advantages, plant proteins often exhibit weaker gelling properties and lower water-holding capacities compared to their animal-based counterparts, which can compromise the texture and structural integrity of plant-based foods. To address these limitations, researchers have explored strategies involving the use of egg white powder, starches, and methylcellulose to enhance the gelation behaviour and mechanical strength of plant protein matrices (Zhao et al., 2024; Hamed et al., 2024). Egg white, in particular, is known for its strong gelling ability due to the presence of ovalbumin and ovotransferrin, which form cohesive protein networks under heat (Mine, 1995).

Previous studies have shown that composite systems combining plant and animal proteins can achieve synergistic effects, resulting in improved viscoelastic properties, water-holding capacity (WHC), and textural performance. Similarly, hydrocolloids and starches contribute to gel strength, stability, and moisture retention, playing critical roles in the formulation of plant-based meat analogues (Singh et al., 2024; Gong et al., 2024). However, a significant challenge in additive utilization is the inconsistency in functional performance, as variations between suppliers and production batches can lead to inconsistent performance and complicate product standardization. This is particularly relevant in clean-label and commercial plant-based applications, where minimal processing and reproducible quality are essential.

The overall aim of this thesis was to investigate the heat-induced gelation behaviour of FBPI and RPI and improve their functional performance through the incorporation of egg white, modified starches, and methylcellulose. These findings were used to develop plant-based and vegetarian sausage formulations with desirable texture and structural characteristics.

To achieve this aim, the specific objectives of the study were:

1. To investigate the gelation behaviour of FBPI and RPI at native pH from 5 to 15% (w/w) concentration
2. To investigate the effect of egg white powder addition on the gelation properties, viscoelastic behaviour, and structure of FBPI and RPI at pH 7.
3. To investigate the impact of incorporating methylcellulose, modified waxy maize starch, and modified potato starch on the rheological and structural properties of FBPI and RPI composite gels at pH 7.
4. To develop plant-based and vegetarian sausage formulations using a combination of plant proteins and hydrocolloids at both high and low concentrations, with egg white incorporated in the low-hydrocolloids formulations, and to evaluate their physicochemical, nutritional, and textural characteristics.

Chapter 2. Literature Review

This literature review provides an overview of the fundamental properties of faba bean protein isolate (FBPI) and rice protein isolate (RPI), with a focus on their functional characteristics relevant to heat-induced gelation. It also examines the role of functional additives, including egg white, methylcellulose, and modified starches, in improving gel formation and texture in plant-based systems. Relevant studies on the formulation of either gelation and plant-based meat analogues are summarized in Table 2.1 to contextualize the present research.

2.1 Rice protein isolate (RPI)

Rice is one of the most important food crops globally. It is grown in over 100 countries across all continents (Amagliani et al., 2017). Among major cereals such as wheat, maize, and barley, rice is distinguished by the high digestibility and biological value of its proteins. In fact, rice yields a higher proportion of usable protein than wheat, largely due to the quality of its protein composition (Eggum, 1979).

Proteins in rice play a vital role not only in its nutritional profile but also in determining its structural and functional properties. They are generally regarded as hypoallergenic and have been associated with beneficial health effects, including antioxidant, antihypertensive, anticancer, and anti-obesity activities. These characteristics make rice proteins an attractive ingredient for the development of food products with enhanced nutritional value (Amagliani et al., 2017).

Rice protein isolate (RPI) is commonly derived as a secondary product from the industrial processing of rice into starch syrups, lactic acid, monosodium glutamate, and alcohol. Despite its nutritional benefits, RPI has seen limited application in the food and pharmaceutical industries, which has hindered its broader commercial availability (Wang et al., 2016). Recent research efforts have focused on improving the solubility of RPI through enzymatic and chemical modifications. While these methods can enhance its functionality, they may also alter the protein's native structure, potentially affecting consumer perception and acceptance. Expanding the solubility and functionality of RPI is considered key to unlocking its full potential in food applications (Wang et al., 2016).

Building on the functional characteristics outlined above, Table 2.1 provides an overview of relevant studies investigating the gelation behaviour of RPI, FBPI, and plant-based protein systems formulated with functional additives.

Table 2.1 Relevant studies on gelation and plant-based meat analogues with rice protein isolate (RPI), faba bean protein isolate (FBPI), methylcellulose (Me), and modified starches (potato starch, PS; waxy maize starch, MS)

Reference	Protein/Starch System	Gelation Enhancer	Main Findings	Experimental Parameters
(Ji et al., 2024)	4% ultrasound-treated rice protein + 6% rice starch	Heat treatment; ultrasound pre-treatment of protein	The Ultrasound-treated rice protein–rice starch gels retained high water-holding capacity while reducing hardness by 62–78.3%. Viscosity and viscoelastic moduli (G′, G″) were lowered, and starch retrogradation was partially inhibited.	Prepared by alkaline extraction (pH 12), acid precipitation (pH 4.5), ultrasound treatment, and pH adjustment to 7. Heated at 65 °C for 20 min, then ramped to 95 °C at 1 °C/min.
(Nilsson et al., 2023)	FBPI + faba bean starch (varied ratios)	Thermal gelation (no external additives)	Higher starch content improved water binding, viscosity, and gel strength (hardness, fracture stress, modulus), forming tighter, more elastic networks. Protein-rich gels were softer with lower viscosity. Starch acted as the continuous phase in high-starch systems.	Formulations at 12% total solids (w/w), varying starch: protein ratios (e.g., S60P40 = 7.2% starch, 4.8% protein). Heated from 65 °C to 95 °C at 1.5 °C/min, held 30 min at 95 °C, cooled to 25 °C, and stored at 4 °C overnight.
(Liu et al., 2008)	Emulsion sausage using enzyme-modified potato starch as fat replacer	Heat-stable amylase modified potato starch	Adding 2–4% modified potato starch reduced fat content by up to 49% and maintained texture in reduced-fat sausages. At 2%, it improved tenderness and lightness, with a slight decrease in redness. Sensory results confirmed quality was retained.	Fat levels tested at 5%, 15%, and 30%; modified potato starch added at 2% and 4% (w/w).
(Chen et al., 2020)	8% native potato starch + whey protein isolate (WPI) fibrils	WPI fibrils prepared at pH 2 and incorporated at pH 3.5 or 6.8	Whey protein fibrils increased starch gel stiffness and G′ at pH 3.5 via electrostatic interactions but reduced compatibility and viscosity at pH 6.8. Fibrils raised gelatinization temperature, lowered enthalpy, increased brittleness, and caused sedimentation at high levels.	Starch: fibril ratios of 7:1, 6:2, and 4:4 (w/w). WPI fibrils prepared at 2% protein, pH 2, heated at 80 °C for 12 h, then adjusted to pH 3.5 or 6.8. Gels heated at 90 °C for 20 min with shaking.

(Saldanha do Carmo et al., 2021)	Faba bean protein concentrate	- -	High-moisture extrusion of faba bean protein produced fibrous, meat-like structures. Moisture content was the key factor affecting texture. Optimal firmness and elasticity were achieved at 130–140 °C with a water-to-feed ratio of 4 and feed rate of 11 rpm.	Extrusion at 130–140 °C, water-to-feed ratio of 4, and feed rate of 11 rpm (1.10 kg/h).
(Barbut, 2018)	Meat batters (25% fat (from beef + canola oil) with native and modified potato and corn starches (9 types)	Varied starch types	Five of six modified starches reduced cooking loss. Native corn starch showed incomplete gelatinization. One modified starch (MC-1) was incompatible, lowering texture and yield. Starches reduced fat loss; colour effects were minimal. Functionality varied by type and formulation.	Samples cooked from 25 °C to 70 °C.
(Aktaş & Gençcelep, 2006)	Sausage batters with sheep tail fat + 3% corn or potato starch	Gelatinized, solubilized/dispersed, and retrograded starches	Starch (3%) with phosphates and curing agents supported cohesive matrix formation. Modified starches improved emulsion stability and viscosity. Gelatinized starch performed best, while potato starch reduced jelly and fat separation more effectively than corn starch. No significant impact on pH or water-holding capacity.	Cooked in a 90 °C water bath until reaching an internal temperature of 72 °C.
Cavestany et al. (1994) (Carballo et al., 1995)	Bologna sausage (pork, fat trim, water, starch, and egg white)	Modified waxy corn starch (Clearam CH 20) and atomized-dried egg white	Starch enhanced binding, reduced cooking and purge loss, and increased hardness, chewiness, and penetration force. Egg white further improved texture but did not significantly affect binding.	Protein standardized to 12.5%. Modified waxy corn starch added at 0–10% (w/w); egg white added at 0–3% (w/w).

(Bakhsh et al., 2021)	Texturized soy isolate protein + varying methylcellulose (1.5-4%)	Methylcellulose levels: 1.5, 3 and 4% as heat-gelling binder	Increasing methylcellulose modified mechanical strength and water-holding capacity. At 3%, it provided the best texture balance. Higher levels (4%) reduced acceptability, likely due to over-gelling and phase separation. Beef control remained firmer overall.	Texturized soy protein paste heated at 120 °C for 2 hours before patty formation.
(Baig et al., 2024)	Mung bean protein + pea protein isolate + potato starch + methylcellulose	Heat-induced gelation (100 °C, 14 min); methylcellulose, gum arabic	Composite formulations showed heat-induced gelation. Hardness, springiness, and chewiness were influenced by protein and starch ratios (20–40%). Methylcellulose enhanced structure. Starch–protein synergy improved rheological and textural properties.	Steamed at 100 °C for 14 minutes.
(Lee, Oh, et al., 2022)	Rice protein isolate + soy protein isolate (25:75, to 100:0)+ 29% corn starch + 13% wheat gluten	Low-moisture extrusion; gluten for elasticity	Rice protein-rich formulations (75–100%) formed dense, compact extrudates with low porosity, expansion, and water absorption. Poor water affinity and protein aggregation (especially glutelin) reduced deformability and indicated higher elasticity and rigidity compared to soy-based samples.	Low-moisture extrusion (twin-screw)
(Kim & Chin, 2024)	Pork myofibrillar protein gel + reduced-fat + reduced-salt sausages +4% added faba bean protein isolate	Faba bean protein isolate (4% protein) alone or combined with microbial transglutaminase	Faba bean protein isolate increased shear stress, gel strength, and cooking yield in pork protein gels, indicating stronger matrix formation. It promoted protein aggregation and crosslinking. Combined with microbial transglutaminase, it reduced surface hydrophobicity and further enhanced gel strength through synergistic crosslinking.	Gels heated from 20 °C to 80 °C..

2.1.1 Chemical composition

RPI, commonly derived mainly from brown or whole grain rice, contains approximately 80–90% protein on a dry basis, depending on the extraction process and rice variety(Agboola et al., 2005; Amagliani et al., 2017). Cereal and legume proteins are typically classified into four types (albumin, globulin, prolamin, and glutelin) based on their solubility in different solutions, as described below. The major protein fractions of RPI include glutelins (80%), followed by smaller proportions of albumins, globulins, and prolamins, which are spatially distributed across the rice endosperm and bran layers (Bechtel & Juliano, 1980; Ogawa et al., 1987).

Rice proteins are primarily stored within the endosperm in the form of protein bodies: PB-I and PB-II. PB-I has a spherical, lamellar structure rich in prolamin, while PB-II exhibits a crystalline, irregular shape and is mainly composed of glutelin (Bechtel & Juliano, 1980). PB-II accounts for 60–65% of total endosperm protein, PB-I contributes 20–25%, and the remaining 10–15% consists of albumin and globulin dispersed throughout the cytoplasm (Amagliani et al., 2017; Ogawa et al., 1987).

Solubility fractions vary depending on rice type and extraction method. According to Adebiyi et al. (2009), brown rice contains approximately 5–10% albumin, 7–17% globulin, 75–81% glutelin, and 3–6% prolamin. In milled rice, these fractions shift to 4–6%, 6–13%, 79–83%, and 2–7%, respectively. Rice bran contains higher proportions of albumin and globulin, 24–43% and 13–36%, respectively, while glutelin remains the dominant fraction across all forms. Prolamin is consistently the minor fraction.

Among the rice protein fractions, albumin stands out for its high content of histidine and threonine, while prolamin is particularly rich in isoleucine, leucine, and phenylalanine. Globulin contributes the highest levels of sulphur-containing amino acids, such as methionine and cysteine, whereas prolamin has the lowest. Based on amino acid composition, albumin is generally regarded as having the highest biological value, while prolamin is considered the least nutritionally valuable (Amagliani et al., 2017).

Albumin is a water-soluble protein characterized by high solubility due to its net charge and the absence of extensive disulphide crosslinking. However, when extracted with water, it is often co-extracted with globulin. This is attributed to the solubilization of mineral ions that enhance globulin solubility. Isoelectric focusing (IEF) analysis of albumin reveals protein bands primarily within the pI range of 6.0 to 7.5 (Amagliani et al., 2017).

Globulin is soluble in salt solutions and has diverse pI profiles, with reported bands between pH 5.9 and 7.3 or at pH 4.3 and 7.9 depending on the method. Glutelin, the predominant protein in rice, is insoluble in water under neutral conditions but becomes soluble under acidic (pH < 3) or alkaline (pH > 10) environments. Structurally, glutelin comprises two polypeptide subunits: an acidic α-subunit (30–40 kDa) and a basic β-subunit (19–23 kDa), with respective pI (isoelectric point) ranges of 5.7–6.9 and 8.0–8.7 (Amagliani et al., 2017).

Glutelin is initially synthesized as a precursor polypeptide with a molecular weight of 51–57 kDa. This precursor undergoes enzymatic cleavage to produce the α and β subunits, which then form large, insoluble aggregates through disulphide linkages, contributing to the low solubility and limited functional properties of native glutelin (Amagliani et al., 2017).

Prolamin, the least soluble fraction in rice, is insoluble in water but soluble in alcohol-based solutions such as 60–70% ethanol. Isoelectric Focusing analysis typically shows five bands between pI 6.0 and 6.5 (Padhye & Salunkhe, 1979).

Overall, glutelin and prolamin dominate the rice protein profile but offer limited solubility due to their structural and chemical characteristics. Glutelin, in particular, contains high levels of glutamic and aspartic acids, which lower its pI (pI ~4.5) and contribute to the poor solubility of RPI near neutral pH (Agboola et al., 2005). In contrast, albumin and globulin, though present in smaller quantities, exhibit greater water solubility and better functional attributes, making them suitable for applications requiring foaming, emulsification, or solubility under mild conditions (Amagliani et al., 2017).

Nutritionally, rice proteins compare favourably to other plant sources. Han et al. (2015) reported that rice protein contains 41.1–41.7 g of essential amino acids per 100 g of protein, closely matching the 41.2 g in soy protein isolate. Albumin has the highest lysine content among rice proteins, followed by glutelin and globulin, whereas prolamin is notably deficient in lysine.

Due to its high albumin content, rice bran typically has higher lysine levels than brown or milled rice (Day, 2013).

Recent developments in extraction techniques, including enzymatic hydrolysis and alkaline treatment followed by isoelectric precipitation (Amagliani et al., 2017), have allowed for more efficient recovery and enhanced purity of rice proteins, as well as better retention of essential amino acids. Such advancements improve not only the nutritional profile but also downstream applications of RPI in food formulations.

2.1.2 Physicochemical and functional properties

The use of proteins as food ingredients depends largely dependent on how they behave and interact within food systems during preparation, processing, storage, and consumption. Their performance is determined by interactions among protein molecules and with other components and water within the food matrix, which together shape the overall structure and behaviour of the product (Amagliani et al., 2017).

2.1.2.1 Solubility

Solubility is a key determinant of how proteins behave in systems such as emulsions, foams, and gels. The solubility of RPI, however, is relatively low near neutral pH, which is a major limitation in applications requiring water dispersion. The poor solubility is linked to the prevalence of glutelins, which make up approximately 80% of the total rice protein content (Amagliani et al., 2017). Glutelins form extensive intermolecular interactions and hydrophobic aggregates under native conditions, thereby reducing water compatibility (Amagliani et al., 2017; Hamada, 1997). However, extreme pH conditions can break these aggregates, improving solubility (Amagliani et al., 2017). For example, enzymatic hydrolysis breaks peptide bonds, exposing more polar groups and increasing hydrophilicity (Ji et al., 2024). Similarly, ultrasonication and pH shifting have been reported to disrupt protein aggregates and improve aqueous dispersion (Yang et al., 2023).

2.1.2.2 Water-holding and oil-holding capacity

Water-holding capacity (WHC) and oil-holding capacity (OHC) are vital in the formulation of products like sausages, bakery items, and spreads (Amagliani et al., 2017). WHC refers to the ability of a protein to retain moisture during processing and storage, contributing to mouthfeel and juiciness. RPI's WHC has been reported to be 2.5–3.5 g/g protein, depending on processing methods and protein integrity (Agboola et al., 2005; Amagliani et al., 2017).

Enzymatically modified rice proteins demonstrate even higher WHC due to enhanced surface area and porosity (Amagliani et al., 2017).

OHC, on the other hand, is influenced by the hydrophobic regions of the protein and its ability to bind lipids (Amagliani et al., 2017). RPI exhibits moderate OHC (1.5–2.0 g oil/g protein), which is sufficient for applications in emulsified meats and bakery goods (Amagliani et al., 2017). Enhanced OHC has been observed when rice proteins are partially denatured or blended with more amphiphilic proteins like pea or soy, supporting their role in fat-rich matrices (Chrastil, 1992).

2.1.2.3 Emulsifying properties

The emulsifying behaviour of proteins is governed by multiple factors, many of which also influence foaming properties. Protein emulsification is commonly assessed using indices such as emulsifying activity, and emulsion stability. The emulsifying activity index (EAI) refers to the ability of proteins to adsorb rapidly at the oil–water interface and form emulsions, whereas the emulsifying stability index (ESI) measures the ability of the protein to maintain the stability of the formed emulsion over time. (Amagliani et al., 2017).

Emulsifying properties are essential in multiphase systems such as salad dressings, protein-fortified beverages, and dairy alternatives. RPI generally exhibits lower emulsification performance than animal proteins, largely due to its compact structure and poor solubility at neutral pH (Padhye & Salunkhe, 1979). However, functional enhancement is possible. For example, enzymatic hydrolysis increases molecular flexibility and allows rice protein to more readily adsorb at oil–water interfaces, forming stable films (Padhye & Salunkhe, 1979; Yang et al., 2023).

2.1.2.4 Foaming capacity

Foaming capacity refers to the ability of proteins to stabilize interfacial area per unit of weight or concentration (Amagliani et al., 2017). This property is influenced by solubility, surface activity, and the ability of the protein to form cohesive films. Unmodified RPI has limited foamability due to its dense molecular structure. Nevertheless, enzymatic and physical modifications, such as limited hydrolysis or high-intensity ultrasonication, can enhance foaming properties. Agboola et al. (2005) demonstrated that enzymatically treated rice proteins form finer and more stable foams, owing to increased surface flexibility and reduced aggregation. These improvements support the use of RPI in bakery products like cakes, muffins, and mousses, particularly when animal proteins need to be excluded.

2.1.2.5 Thermal stability

Thermal stability is another significant attribute that affects protein functionality during cooking or processing. Differential scanning calorimetry (DSC) studies reveal denaturation temperatures for rice protein fractions ranging from 70 to 85°C (Bechtel & Juliano, 1980). Denaturation exposes reactive groups, allowing for increased hydration, improved WHC, and potential cross-linking. While mild heating promotes functional improvements, excessive heating beyond optimal thresholds may result in aggregation and irreversible insolubility, reducing the protein's usability in emulsified or solubilized formats (Amagliani et al., 2017; Yang et al., 2023).

An additional aspect of RPI functionality involves its interaction with other food matrix components. Rice proteins can form synergistic networks with polysaccharides (e.g., starch, methylcellulose) and other proteins (e.g., soy, pea), resulting in improved structure and stability in composite systems. Ji et al. (2024)investigated RPI–starch gels and found that ultrasound-treated rice protein enhanced gel strength and reduced phase separation, suggesting that controlled modification not only improves functionality but also opens new applications in high-protein, gluten-free systems.

2.1.2.6 Gelation

Gelation is one of the most critical functional attributes of proteins in food systems, particularly in products where texture, moisture retention, and structural integrity are desired (Yang et al., 2023). While many animal-derived proteins, such as gelatine and egg white proteins, are renowned for their strong gel-forming ability, plant-based proteins like RPI often exhibit comparatively weaker gelation behaviour (Amagliani et al., 2017). Nonetheless, the gelation potential of RPI can be significantly influenced by factors such as concentration, temperature, pH, ionic strength, and the presence of synergistic ingredients (Ji et al., 2024; Yang et al., 2023).

Native rice proteins exhibit limited gelation capacity, primarily due to their compact globular structure and low molecular flexibility (Amagliani et al., 2017). Glutelins, the dominant protein fraction in RPI, are responsible for its limited but modifiable gelation ability (Adebiyi et al., 2009). Upon heating, these proteins undergo denaturation, which involves the unfolding of the tertiary structure and exposure of internal hydrophobic residues (Yang et al., 2023). This process facilitates aggregation through hydrophobic interactions, hydrogen bonding, and disulphide bond formation, which are essential for gel network formation. However, the degree of aggregation and the quality of the resulting gel are highly sensitive to thermal and environmental conditions (Amagliani et al., 2017).

Heat-induced gelation of RPI is generally initiated at denaturation temperatures between 70°C and 85°C (Yang et al., 2023). At these temperatures, the glutelin fraction becomes reactive and starts to interact with neighbouring protein molecules (Ogawa et al., 1987). Controlled heat treatment can lead to the formation of a fine-stranded gel network, whereas excessive heating may cause irreversible aggregation, phase separation, and the development of brittle or coarse gel structures. Moreover, RPI often requires relatively high protein concentrations (typically above 20% (w/w)) to form self-supporting gels, which may limit its direct application in low-protein formulations (Yang et al., 2023).

Various strategies have been explored to enhance the gel-forming ability of RPI. Enzymatic hydrolysis, for instance, is a well-established method that improves gelation by partially breaking down protein chains, thereby increasing flexibility and the number of reactive sites. Proteolytic enzymes such as papain, trypsin, and alkaline protease have been commonly employed for this purpose, with alkaline protease showing particularly high effectiveness due to its extensive hydrolysis capacity and ability to disrupt hydrophobic regions, thereby significantly improving the solubility and functional properties of RPI (Yang et al., 2023). Incorporating hydrocolloids, such as carrageenan, methylcellulose, or modified starches, has also proven effective in improving RPI gelation (Ji et al., 2024). These polysaccharides can act synergistically by binding water, stabilizing protein–protein interactions, or contributing to the overall gel matrix via phase separation and network reinforcement. Ji et al. (2024) reported that RPI–starch composite gels, particularly when treated with ultrasound, showed improved gel uniformity and increased elasticity compared to RPI alone.

pH also plays a critical role in RPI gelation. Near the isoelectric point of rice protein (~pH 4.5), electrostatic repulsion between protein molecules is minimized, promoting aggregation and gel network formation (Amagliani et al., 2017). However, for food applications at neutral pH (pH 6.5–7.5), functional enhancement through physical or enzymatic modification is often necessary to achieve desirable gel texture and stability (Yang et al., 2023).

Physical processing methods such as ultrasonication and high-pressure processing have been increasingly investigated for their potential to enhance the gelation behaviour of plant proteins. Ultrasound treatment, for example, can disrupt protein aggregates, improve solubility, and increase exposure of reactive groups. These changes promote better protein dispersion and alignment during heating, leading to stronger and more homogeneous gel matrices (Ji et al., 2024).

The final properties of RPI-based gels depend on a complex interplay between intrinsic protein characteristics and extrinsic processing conditions (Yang et al., 2023). Factors such as heating rate, ionic strength, and the presence of divalent salts (e.g., Ca2+) can modulate gel firmness, elasticity, and water-holding capacity (Amagliani et al., 2017). Blending RPI with other proteins like pea or soy can also produce composite gels with improved mechanical strength and reduced brittleness, thus expanding its use in plant-based sausages, dairy analogues, and structured emulsions (Ji et al., 2024).

2.1.3 Food applications

RPI has gained considerable attention in the food industry due to its nutritional adequacy, hypoallergenic nature, and favourable functional properties. Its application spans across various sectors including bakery, beverages, meat analogues, dairy alternatives, sports nutrition, and gluten-free formulations. While RPI is less functionally potent in some aspects compared to soy or whey proteins, its advantages in digestibility, flavour neutrality, and consumer perception position it as a promising ingredient in clean-label and allergen-sensitive product development (Amagliani et al., 2017; Yang et al., 2023).

In the bakery industry, RPI is used to enhance nutritional content and improve the texture of gluten-free and high-protein baked goods. Its ability to retain water and fat supports desirable crumb structure and softness in gluten-free breads, muffins, and protein-enriched cookies (Amagliani et al., 2017). Moreover, rice protein's mild flavour and light colour make it an attractive option compared to other plant proteins, which may impart bitterness or colour instability. The moderate foaming ability of RPI also lends itself to volume expansion and structural integrity during baking.

In the beverage sector, RPI is incorporated into protein shakes, dairy-free smoothies, and plant-based milks due to its digestibility and hypoallergenic profile. However, its poor solubility at neutral pH poses a technical challenge in aqueous systems, leading to sedimentation or phase separation. To address this, enzymatically hydrolyzed rice protein or nano-emulsified forms have been explored to improve suspension stability (Yang et al., 2023). These modified RPI forms offer better dispersion and sensory acceptability in ready-to-drink beverages and fortified water.

RPI also plays an emerging role in meat analogues and vegetarian sausages. While its gelation ability is limited in native form, it can serve as a valuable structural component when combined with starches (Ji et al., 2024). Its high thermal stability allows it to withstand cooking without excessive denaturation, and its mild flavour ensures that target seasonings are not masked. Moreover, the elastic and cohesive textures achieved through protein–starch or protein–hydrocolloid blends support the formulation of convincing meat alternatives.

In dairy alternative applications, RPI is incorporated into yogurt-style products, protein-fortified plant milks, and non-dairy creams. While soy and almond dominate this market, rice protein offers a hypoallergenic and clean-label option for consumers with sensitivities to nuts or legumes. Formulations often blend RPI with other stabilizers such as carrageenan, xanthan gum, guar gum, or modified starches (Sethi et al., 2016), to counteract its limited emulsifying and thickening capacities (Agboola et al., 2005). Still, the use of enzymatically modified or ultrasonicated rice protein has shown promise in improving viscosity and creaminess in dairy-free systems (Yang et al., 2023).

The sports nutrition and health food sectors have shown increasing interest in RPI due to its rich amino acid profile and suitability for vegan and hypoallergenic markets. Rice protein is often included in protein bars, powders, and ready-to-drink beverages. Although it has a slightly lower biological value than whey or casein, studies show that a combination of rice and pea protein can achieve a protein efficiency ratio similar to animal-based products (Amagliani et al., 2017). Additionally, rice protein is less likely to cause gastrointestinal discomfort compared to dairy proteins, making it suitable for sensitive populations such as children, the elderly, or individuals with irritable bowel conditions.

2.2 Faba bean protein isolate (FBPI)

As the global demand for sustainable, plant-based protein sources rises, the search for alternatives to dominant crops like soy and pea has intensified. One promising candidate is the faba bean (*Vicia faba L.*), a pulse crop that combines strong agronomic resilience with excellent nutritional properties. Despite its long-standing cultivation in parts of Asia, Africa, and the Mediterranean, the faba bean remains underexploited in Western agriculture and food systems (Martineau-Côté et al., 2022). This underutilization stands in contrast to its adaptability, faba beans thrive in a variety of environments, including cooler boreal regions, making them an attractive option for expanding production in temperate climates like Canada (Martineau-Côté et al., 2022; Oomah et al., 2011)

Faba beans possess a favourable nutritional profile. Their seeds are rich in protein, starch, and dietary fibre, and contain only minimal fat (Martineau-Côté et al., 2022). In fact, faba beans generally exhibit higher protein levels than many commonly consumed legumes, such as peas, lentils, and chickpeas (Raikos et al., 2014). However, it contains various anti-nutritional factors (ANFs), including tannins, phytic acid, oxalates, lectins, and enzyme inhibitors, which can interfere with nutrient absorption and digestion (Martineau-Côté et al., 2022).

From an agronomic perspective, faba beans contribute positively to sustainable farming systems. They are effective nitrogen fixers, reducing the need for synthetic fertilizers and the associated risk of environmental degradation such as waterway eutrophication (Martineau-Côté et al., 2022).

2.2.1 Chemical composition

Faba bean protein isolate (FBPI), derived from *Vicia faba L.*, has emerged as a promising plant-based protein source due to its high protein content, favourable amino acid profile, and increasing market acceptance in alternative protein applications (Martineau-Côté et al., 2022). The composition of FBPI is influenced by several factors including cultivar, growing conditions, and the method of extraction and isolation. Typically, FBPI contains 85–90% protein on a dry matter basis, with the remainder comprising carbohydrates, ash, fat, and fibre (Vioque et al., 2012).

The primary storage proteins in faba bean are globulins, which make up approximately 60–70% of the total protein content, with vicilin (7S) and legumin (11S) being the two dominant subunits. Albumins, which represent about 10–20% of the total protein fraction, contribute significantly to the solubility and enzymatic activity of FBPI (Vioque et al., 2012). These proteins possess balanced amino acid profiles, particularly rich in lysine, leucine and arginine, though sulphur-containing amino acids such as methionine and cysteine are present in lower concentrations (Martineau-Côté et al., 2022). Legumin and vicilin, the primary storage proteins, have a unique structure that allows them to dissociate into their respective subunits under certain conditions, such as changes in pH or the presence of salts. This structural flexibility is one reason for the functional versatility of faba bean protein isolate in various food applications (Martineau-Côté et al., 2022).

In addition to globulins, FBPI contains smaller amounts of other protein fractions, such as albumins, which are water-soluble and contribute to the solubility of the proteins, and glutelins, which are more insoluble and contribute to the structural integrity of the protein network (Karaca et al., 2011; Martineau-Côté et al., 2022). These protein components, though present in lesser amounts, play supportive roles in the overall functionality of the isolate.

From a nutritional standpoint, FBPI is superior to many other legume-based isolates. It exhibits a high biological value and is considered complete in most essential amino acids, with the exception of the limiting sulphur amino acids. According to Vioque et al. (2012) , FBPI contains up to 9% arginine and 8% lysine relative to total amino acid content, making it especially suitable for muscle-building, sports nutrition, and children's diets.

In addition to protein fractions, FBPI may contain residual amounts of starch, non-starch polysaccharides (such as cellulose and hemicellulose), and minor quantities of antinutritional compounds like tannins, vicine, and convicine (Shi et al., 2017). These compounds can influence digestibility and nutritional value. However, most of these antinutritional factors are significantly reduced during the protein extraction process, especially when techniques such as isoelectric precipitation or membrane filtration are used (Badjona et al., 2025).

The presence of natural bioactive compounds in FBPI, such as phenolic compounds and peptides with antioxidant or antimicrobial activity, also enhances its value in functional foods (Badjona et al., 2024a). These bioactives, although minor in quantity, contribute to the emerging interest in FBPI as both a nutritive and health-promoting ingredient.

2.2.2 Physicochemical and functional properties

FBPI exhibits a diverse range of physicochemical and functional properties that make it suitable for a variety of food applications. These properties are primarily influenced by the molecular structure of the proteins, extraction method, degree of purity, and environmental factors such as pH, ionic strength, and temperature (Badjona et al., 2024a). Among legume proteins, FBPI is recognized for its excellent emulsifying, foaming, and water-binding capabilities, making it a competitive alternative to soy protein isolate in many food systems (Vioque et al., 2012).

2.2.2.1 Solubility

Solubility is one of the foundational physicochemical properties of FBPI and plays a pivotal role in determining its performance in food systems (Badjona et al., 2024a). FBPI generally demonstrates low solubility at its isoelectric point (~pH 4.5), but solubility improves significantly under acidic or alkaline conditions. The globulin-rich nature of FBPI contributes to its limited solubility at neutral pH, although modifications such as pH shifting, ultrasonication, or enzymatic hydrolysis have been shown to increase solubility and dispersion in aqueous environments (Badjona et al., 2024a).

2.2.2.2 Water-holding and oil-holding capacity

Water-holding capacity (WHC) and oil-holding capacity (OHC) are critical for texture, shelf stability and juiciness in meat analogues and bakery products (Badjona et al., 2024a). FBPI has demonstrated WHC values of over 1.7 g water/g protein in some preparations (Raikos et al., 2014), indicating strong interactions between water molecules and protein matrices (Badjona et al., 2024a). Likewise, FBPI exhibits a high OHC (~6.12 g oil/g protein)(Eckert et al., 2019), outperforming other plant proteins such as soy and lupin. This enhanced ability to retain oil within the protein matrix makes FBPI particularly suitable for use in fat-rich formulations, where maintaining emulsion stability and desirable texture is essential (Badjona et al., 2024a).

2.2.2.3 Emulsifying properties

FBPI has an amphiphilic nature, owing to hydrophilic and hydrophobic amino acid residues enabling the formation and stabilization of oil-in-water emulsions. However, the emulsifying capacity of FBPI is strongly influenced by its solubility, particularly at neutral pH. This limitation is largely attributed to FBPI’s compact globular structure, which reduces protein unfolding and surface activity (Badjona et al., 2024a; Karaca et al., 2011). For instance, emulsions formed at pH 7 tend to have large droplet sizes, while under acidic conditions, the solubility of FBPI is enhanced and smaller oil droplet sizes can be achieved (Badjona et al., 2024a).

Despite its amphiphilic nature, the emulsifying activity index (EAI) and emulsion stability index (ESI) are generally lower than those of other legume proteins such as pea, lentil, and chickpea. For instance, EAI values of FBPI range from 27–36.4 m^2/g**,** with stability times around 40–48 minutes**,** figures that are lower than those reported for pea and lupin proteins (Badjona et al., 2024a; Yang et al., 2018). However, when compared to RPI, FBPI exhibits relatively better emulsifying properties under native conditions. RPI tends to show lower emulsification performance due to its compact globular structure and poor solubility at neutral pH, which limit its ability to adsorb at oil–water interfaces and form stable emulsions (Padhye & Salunkhe, 1979; Yang et al., 2023).

2.2.2.4 Foaming capacity

FBPI also exhibits strong foaming capacity and foam stability, which are essential for aerated products such as whipped toppings and mousse-like formulations (Badjona et al., 2024a). These functional traits are enhanced when FBPI is partially denatured, as protein unfolding increases surface activity and the ability to entrap air.

However, FBPI has shown limited foaming capacity across different pH levels. For example, foaming capacity values of 31.2% at pH 5 and 66.7% at pH 7 have been reported (Badjona et al., 2024a; Eckert et al., 2019), both of which are considerably lower than those observed in other plant proteins isolates such as pea and lentil (approximately 200 and 400% respectively). One of the primary reasons for this inferior foaming performance is the low solubility of FBPI, which impairs protein dispersion and adsorption at the air-water interface (Badjona et al., 2024a).

Foams produced from FBPI exhibited a multimodal bubble size distribution, characterized by large, distorted polyhedral structures with thin, poorly defined lamellae (Badjona et al., 2024a; Martínez-Velasco et al., 2018); characteristics that highlight the limited ability of FBPI proteins to maintain a stable foam structure over time.

2.2.2.5 Thermal stability

FBPI primarily consists of the storage proteins legumin (11S) and vicilin (7S), which play a central role in determining its thermal and functional behaviour in food applications. These proteins are stabilized in their native state through hydrogen bonding, electrostatic, and hydrophobic interactions. Upon heating, denaturation occurs, often irreversibly, resulting in structural unfolding that affects protein solubility, gelation, and emulsification properties (Badjona et al., 2024a).

The thermal stability of FBPI can be evaluated using differential scanning calorimetry (DSC), which reveals distinct denaturation temperatures corresponding to its main protein fractions (Badjona et al., 2024a). Typically, FBPI exhibits two endothermic transitions: one around 90°C (associated with vicilin) and another near 100°C (attributed to legumin), particularly when analyzed in 0.5 M NaCl solutions (Arntfield et al., 1986; Badjona et al., 2024a). These denaturation peaks reflect the unfolding of globular protein domains and are sensitive to environmental factors such as pH and ionic strength.

The denaturation temperature (Td) of FBPI varies depending on the protein isolation method. For instance, FBPI obtained through alkaline-isoelectric precipitation tends to show a lower Td (~85°C) compared to micellized FBPI, which retains a higher Td (~90°C) (Arntfield et al., 1986). This difference is due to the harsh conditions of alkaline-isoelectric processes, which can disrupt native protein structures more significantly than the milder micellization method (Arntfield et al., 1986; Badjona et al., 2024a).

Further research indicates that the legumin-rich (11S) fraction of FBPI denatures at approximately 95.4°C, while the vicilin-rich (7S) fraction exhibits a lower denaturation temperature of around 83.8°C (Kimura et al., 2008). These findings align with thermal profiles observed in typical FBPI samples, where the onset of denaturation is about 83°C, with peak transitions near 94°C (Badjona et al., 2024a; Nivala et al., 2017).

In addition to extraction methods, pH also significantly influences the thermal behaviour of FBPI. Extremely acidic ($pH < 2.5$) or alkaline ($pH > 11.5$) conditions are known to reduce the denaturation temperature and enthalpy, thereby compromising thermal stability (Arntfield et al., 1986; Badjona et al., 2024a).

2.2.2.6 Gelation

The gelation capacity of FBPI is one of its most important functional traits, especially in the development of plant-based meat, dairy alternatives, and other texture-critical food systems (Martineau-Côté et al., 2022). Gelation refers to the formation of a three-dimensional protein network capable of entrapping water and providing structural rigidity to food products. FBPI, although a plant-derived protein, exhibits promising gelation behaviour under heat-induced conditions, particularly when formulated at high concentrations and under controlled pH environments (Langton et al., 2020).

The primary proteins responsible for gel formation in FBPI are vicilin (7S) and legumin (11S) globulins (Vogelsang-O'Dwyer M, 2020). These proteins begin to denature upon heating within a typical temperature range of 75°C to 95°C (Arntfield et al., 1986; Kimura et al., 2008), which results in the exposure of hydrophobic groups and sulfhydryl residues, facilitating protein aggregation and network formation. Upon heating, partial unfolding of these globular proteins enhances intermolecular interactions, including hydrogen bonding and disulphide bridge formation (Vogelsang-O'Dwyer M, 2020).

However, FBPI does not always form strong, self-supporting gels under all conditions. Its gelation behaviour is highly influenced by environmental factors such as pH, ionic strength, and protein concentration (Langton et al., 2020). For instance, near the pI (pH ~4.5), proteins aggregate more readily due to reduced electrostatic repulsion. Conversely, at neutral pH (~7.0), electrostatic repulsion may limit the density of the gel network, often requiring additional structuring agents to support stable gel matrices (Langton et al., 2020).

Ionic strength also plays a critical role in modulating protein solubility and, consequently, gelation. According to Langton et al. (2020) , the addition of 2% NaCl (0.34 M) further modifies gel characteristics. At pH 5, NaCl induces a dual-network structure with coarser protein aggregates surrounded by a fine-grained matrix, linked to changes in solubility, particularly of the 7S globulins. At pH 7, however, the addition of NaCl tends to disrupt network integrity, yielding more open and heterogeneous structures and reducing mechanical strength.

Experimentally, a minimum protein concentration of 12% w/w has been reported as necessary for FBPI to form self-supporting gels (Vogelsang-O'Dwyer M, 2020), highlighting the importance of protein density in achieving gelation. However, the final gel characteristics are ultimately the result of a complex interplay between protein concentration, ionic environment, and pH.

To further improve the gelation properties of FBPI, several enhancement strategies have been explored, including enzymatic modification, physical treatments such as ultrasonication, and the incorporation of hydrocolloids. Badjona et al. (2025) demonstrated that ultrasound-treated FBPI formed stronger and more elastic gels due to improved protein dispersion and increased surface reactivity. Similarly, combining FBPI with gelling polysaccharides such as starches has shown to improve water retention, gel strength, and structural integrity (Nilsson et al., 2023)

2.2.3 Food applications

FBPI is increasingly being used in a wide range of food applications due to its favourable nutritional profile, functionality, and sustainable production potential. As a clean-label, non-GMO, and allergen-friendly protein source, FBPI aligns well with consumer trends toward plant-based diets, minimal processing, and high-protein functional foods. Its application areas include meat analogues, dairy alternatives, gluten-free bakery products, protein beverages, and functional snacks (Martineau-Côté et al., 2022).

One of the most prominent uses of FBPI is in the development of plant-based meat analogues such as burgers, sausages, and meatballs. Its high protein content and gelation ability allow it to provide structure and textural integrity to meat substitutes. When combined with hydrocolloids or starches, FBPI forms elastic, fibrous matrices that mimic the chewiness and moisture retention of conventional meat products (Guerrero et al., 2025). Kantanen et al. (2022) demonstrated that increasing the proportion of FBPI in the mixture improved textural parameters such as hardness, chewiness, and cutting strength. However, results from Ferawati et al. (2021) indicated that higher protein levels might reduce hardness, suggesting that extrusion conditions, particularly moisture and temperature, strongly influence final product attributes

FBPI has also shown promise in cereal-based applications, particularly in bakery and pasta products (Thomsen et al., 2025). When used as a flour or isolate, FBPI enhances protein content and contributes additional fibre and minerals. For instance, breads incorporating fermented FBPI had higher digestibility and a reduced glycaemic index (Coda et al., 2017), while pasta fortified with FBPI exhibited increased elasticity, fracturability, and nutrient density (Petitot et al., 2010). The suitability of FBPI in gluten-free baking has also been explored, with fermented faba bean flour improving volume, softness, and nutritional value (Sozer et al., 2019).

In beverage systems, FBPI can function as an effective emulsifier, forming stable oil-in-water emulsions under specific pH and salt conditions (Thomsen et al., 2025). Gumus et al. (2017) found that FBPI formed the smallest droplet sizes at neutral pH among tested legumes, although emulsion stability was sensitive to salt concentration and storage conditions. Moreover, enzymatic hydrolysis of FBPI has been used to improve emulsion and oxidative stability, particularly at moderate degrees of hydrolysis (Liu et al., 2019). FBPI-stabilized emulsions have also been developed for high-protein functional drinks (Nawaz et al., 2021), showing stability after homogenization and UHT processing under certain protein concentrations.

Despite its potential, challenges remain for FBPI's widespread use. Off-flavours, often linked to antinutritional compounds like tannins and vicine/convicine, as well as beany volatiles, can affect consumer acceptance. Processing methods and selective breeding are being explored to mitigate these issues. In addition, strategies such as enzymatic or physical modification are under investigation to further enhance FBPI's functionality and scalability in industrial applications (Thomsen et al., 2025)

2.3 Food polysaccharides

Food polysaccharides are essential biopolymers with diverse applications in the food industry due to their functional properties like water retention, gel formation, and structural stabilization. They form the backbone of many food products, particularly in systems requiring texture modification and viscosity enhancement. These biopolymers are composed of long chains of monosaccharide units and interact with other food components such as proteins and lipids, playing a pivotal role in forming gels and emulsions. They are used widely in food products to improve mouthfeel, extend shelf life, and control moisture retention. Polysaccharides not only contribute to the texture but also offer the possibility of creating healthier, more sustainable food alternatives, particularly as consumer interest in plant-based and low-fat products increases (Ye et al., 2024).

In this section, three types of polysaccharides frequently used in plant-based protein gels and structured foods are reviewed: methylcellulose, modified potato starch, and modified maize starch. This literature review also discusses their specific applications in plant-based food systems, with a focus on their role in gel formation.

2.3.1 Methylcellulose

Methylcellulose is a chemically modified, non-ionic polysaccharide derived from cellulose. It is one of the most useful polymers in the food industry due to its stability across a wide range of pH levels and in the presence of salts. Its primary feature is thermoreversible gelation, meaning it forms gels when heated and returns to a sol state upon cooling. This unique property makes it valuable in food applications, particularly in plant-based and low-fat food formulations. It also has a great stability across a wide range of pH levels and in the presence of salts. Methylcellulose is also widely used in creating meat analogues and as a stabilizer in processed foods to enhance texture, retain moisture, and provide structural integrity (Peñaranda & Garrido, 2024).

Methylcellulose is capable of forming strong, stable gels due to the substitution of hydroxyl groups (-OH) with methyl groups (-CH3) on the cellulose backbone (Peñaranda & Garrido, 2024). This modification enhances its gel-forming functionality, allowing it to create a three-dimensional fibrillar network when heated through intermolecular interactions, including hydrogen bonds and van der Waals forces (Bakhsh et al., 2021).

Earlier studies suggested that methylcellulose gelation was driven primarily by hydrophobic interactions between polymer chains. However, more recent research using advanced imaging techniques including Cryogenic Transmission Electron Microscopy (cryo-TEM), Small-Angle Neutron Scattering (SANS), Small-Angle X-ray Scattering (SAX), and rheology has revealed that upon heating, methylcellulose self-assembles into thin, stiff fibrils (Bonetti, 2023). These fibrils, approximately 15–20 nm in diameter, form an interconnected 3D gel network composed of both crystalline and amorphous domains. The gelation typically occurs when methylcellulose is heated to temperatures between 55 °C and 65 °C for 10 to 30 minutes, depending on molecular weight and concentration (Knarr & Bayer, 2014)The length of these fibrils increases with molecular weight, although the diameter remains relatively constant. As a result, lower molecular weight methylcellulose tends to form shorter fibrils, leading to weaker gels (Bonetti, 2023).

According to Coughlin et al. (2021), an alternative mechanism often discussed for methylcellulose gelation is viscoelastic phase separation. In this process, methylcellulose exhibits lower critical solution temperature behaviour, which triggers the formation of interconnected, polymer-rich regions during phase separation. These regions become kinetically trapped due to polymer chain entanglements, creating a stable structure. Gelation occurs when these entangled domains extend throughout the entire matrix. This process is believed to involve multiple stages. For instance, at low and high concentrations, clear gels form, either through aggregation of hydrophobic domains or via crystallization, while at elevated temperatures, however, gelation results from phase separation, where polymer-dense microdomains disrupt complete phase demixing and produce a turbid gel.

2.3.1.1 Functional role of methylcellulose in food systems, particularly as a gelling agent

In food applications, methylcellulose serves as a thickener, emulsifier, stabilizer, and most notably, a gelling agent in plant-based meat formulations (Peh et al., 2024). Upon heating to 50–60°C, methylcellulose undergoes hydrophobic interactions that lead to the formation of a three-dimensional gel network (Peh et al., 2024). Gelation is strongly concentration-dependent, typically requires a minimum concentration of 1.0–1.5% (w/w), although more consistent and stable gels are observed at concentrations above 2% (w/w) (Knarr & Bayer, 2014). This thermogelation provides structural rigidity and juiciness in high-moisture meat analogues during cooking. This thermogelation provides structural rigidity and juiciness in high-moisture meat analogues during cooking and also helps to reduce water and oil loss, thereby enhancing cooking yield and textural quality (Peh et al., 2024).

One major advantage of methylcellulose is that it can gel and return to a sol state reversibly, which means it does not require chemical crosslinkers, making it less toxic and more cost-effective (Nasatto et al., 2015). However, since the gelation is based on physical rather than chemical bonds, methylcellulose hydrogels are not as strong or stable as chemically crosslinked ones. They tend to break down in water over time, with up to 50% dissolving after just one day and almost all of it dissolving within a month. Because of this, when stronger and longer-lasting gels are needed, chemical crosslinking might be necessary to improve stability and mechanical strength (Bonetti, 2023)

The performance of methylcellulose in food systems is influenced by several factors, including molecular weight, degree of substitution, concentration, and the presence of other ingredients such as salts and proteins (Nasatto et al., 2015). Higher molecular weight variants tend to form stronger gels, while low molecular weight methylcellulose may struggle to form strong networks due to fewer interaction points. (Nasatto et al., 2015).

Methylcellulose, approved under the food additive code E461 in Europe, is widely used in the food industry for its multifunctional properties. It is particularly valuable in plant-based food formulations as an emulsifier, thickener, gelling agent, and texturizer. As a derivative of cellulose, methylcellulose is non-digestible, non-toxic, and hypoallergenic, making it suitable for clean-label and allergen-free products (Nasatto et al., 2015).

In meat analogues, methylcellulose is particularly valued for its ability to bind ingredients, reduce oil absorption, and deliver a juicy, meat-like texture. Its thermo-reversible gelation not only helps maintain product structure during cooking but also reduces moisture and oil losses (Peh et al., 2024). This functionality enhances cooking yield and texture, which are critical for consumer acceptance. Kyriakopoulou et al. (2021) reported that methylcellulose contributes to emulsion stability and helps minimize oil and water separation in plant-based meat substitutes (Peh et al., 2024). Further supporting its practical relevance, Bakhsh et al. (2021) evaluated methylcellulose concentrations from 0 to 4% in soy protein-based patties and found that a 3% inclusion effectively minimized water and cooking losses. This concentration also produced textural properties, such as cohesiveness and springiness, comparable to those of traditional beef patties, likely due to enhanced binding throughout the matrix.

Beyond meat analogues, methylcellulose plays an important role in plant-based bakery and sauce systems. In baked goods, it improves volume, crumb structure, and moisture retention, and is especially beneficial in gluten-free formulations, where it replicates some of the viscoelastic properties typically provided by gluten (Nasatto et al., 2015). In sauces, dressings, and creams, methylcellulose supports viscosity control and, emulsion stabilization and enables the reduction or replacement of fat and eggs, making it highly suitable for low-fat and vegan product development (Nasatto et al., 2015).

2.3.2 Modified starch from potato

Potato is a widely cultivated and versatile crop valued for both fresh consumption and processed food applications. The chemical makeup of potatoes, including starch, non-starch polysaccharides, simple sugars, and organic and inorganic compounds plays a key role in determining the quality, texture, and nutritional value of various potato-based products (Dupuis & Liu, 2019).Among these components, starch is the most abundant carbohydrate in potatoes and is of major economic importance due to its wide range of food and industrial uses (Ellis et al., 1998). In the human diet, starch serves as a primary energy source, contributing significantly to global nutrition (Dupuis & Liu, 2019).

Despite its natural utility, native potato starch often lacks the necessary stability for industrial applications, particularly under conditions involving high temperature, low pH, or mechanical stress. To overcome these limitations, starch is frequently modified using physical, chemical or enzymatic methods**,** which allows the starch to gain new functional properties. These modifications can involve introducing new chemical groups or changing the molecular structure of starch granules (Feng et al., 2020). Common types of modified starches include esterified, carboxymethylated, pregelatinized, and acetylated starches (Feng et al., 2020), as well as those produced through acid hydrolysis, cross-linking, substitution, oxidation, or by forming amylose–lipid complexes (Koksel et al., 2023).

PenBind® 850, the modified potato starch (PS) used in this study, is a food-grade starch product manufactured via chemical cross-linking (CLM), most likely using monosodium phosphate (MSP) (Rohima et al., 2025). Cross-linking with MSP introduces phosphate groups into the starch granule structure, displacing hydroxyl groups and forming phosphate bridges. These modifications strengthen intermolecular bonds within the starch, thereby reducing water uptake and improving thermal and mechanical stability (Kaur et al., 2012).

Cross-linking introduces covalent phosphate bridges between hydroxyl groups of amylose and amylopectin, restricting granule swelling and leaching during gelatinization while improving the granule's internal structure. This modification also reduces solubility and syneresis by creating a denser, more stable network (Rohima et al., 2025). According to the same study, cross-linked starches demonstrated lower moisture content due to enhanced water evaporation during drying, and higher structural integrity due to phosphate bond formation. The substitution of hydroxyl groups by phosphate limits the availability of binding sites for water, thereby reducing water absorption and granule expansion during heating. These effects are advantageous in food systems that require high thermal and freeze–thaw stability (Dupuis & Liu, 2019; Sweedman et al., 2013).

2.3.2.1 Functional properties

Modified potato starch particularly when cross-linked using phosphate-containing agents such as monosodium phosphate (MSP), exhibits reduced swelling power and solubility due to restricted chain mobility and increased internal bonding (Kaur et al., 2006). This reduced mobility results from the formation of phosphate bridges, where hydroxyl groups on amylose and amylopectin chains are substituted by phosphate groups. These substitutions establish covalent crosslinks that are stronger and more thermally stable than the hydrogen bonds found in native starch, thereby creating a tighter, more rigid molecular structure that limits water penetration and suppresses granule swelling (Rohima et al., 2025).

According to Rohima et al. (2025), as phosphate content and crosslink density increase, water absorption capacity further declines because fewer free hydroxyl groups are available to bind water. This structural compactness also leads to lower solubility, as the modified starch becomes more resistant to breakdown during thermal processing. Additionally, the crosslinked matrix offers better resistance to syneresis during cooling and freeze–thaw cycles by restricting amylose leaching, which minimizes water separation and enhances the stability of starch-based gels at low temperatures.

Despite these limitations in water interaction, cross-linked starch contributes significantly to gel firmness by acting as a structural filler within protein matrices. It reinforces the network and reduces phase separation, supporting the development of fibrous, cohesive gel structures during thermal processing (Yang et al., 2019). Moreover, in mixed protein–starch systems, charged residues on proteins can interact via charge dipole mechanisms with starch-bound phosphate groups, stabilizing the network and inhibiting starch retrogradation

These properties are particularly beneficial in applications like plant-based meat analogues, where moisture retention, structural integrity, and thermal stability are essential to achieving a desirable meat-like texture and shelf-life performance.

2.3.3 Modified waxy maize starch

One notable type of starch is waxy maize starch, derived from maize and composed almost entirely of amylopectin, a highly branched polysaccharide. This unique composition gives waxy maize starch distinct structural and functional properties that are valuable in food, pharmaceutical, and industrial applications (Liu et al., 2021). Compared to normal or high-amylose maize starches, waxy maize starch has a higher degree of branching and greater molecular weight, which significantly influence its behaviour during processing (Bertoft, 2013). The amylopectin in waxy maize starch consists of various chain types (A-, B-, and C-chains), each differing in length and function, collectively governing the starch's swelling, gelling, and flow properties under thermal or mechanical treatment (Liu et al., 2021; Singh et al., 2006).

In food applications, waxy maize starch is used to enhance texture, form gels, reduce syneresis, and stabilize products such as yogurts, sauces, pie fillings, and baby food (Šárka & Dvořáček, 2017). Waxy starches also find use in specialized formulations, including fat replacers (Mohammadi, 2012). However, despite its favourable native characteristics, waxy maize starch is still limited in industrial processing due to its sensitivity to shear, heat, and acid (Rodríguez-Hernández et al., 2006). To address these limitations, chemical modification techniques such as cross-linking, esterification, etherification, oxidation, and acid hydrolysis are routinely employed to improve its structural integrity, thermal stability, and digestibility profile (Han & BeMiller, 2007; Sweedman et al., 2013).

One such modification is hydroxypropyl distarch phosphate, which is both cross-linked and hydroxypropyl-substituted. This dual modification enhances thermal stability, reduces retrogradation, and improves water-holding capacity and gel strength (Šárka & Dvořáček, 2017).

FIRM-TEX®, the modified waxy maize starch (MS) used in this study, is a food-grade starch produced through chemical cross-linking and classified as hydroxypropyl distarch phosphate. Cross-linking is typically achieved using phosphate-based reagents, while hydroxypropyl groups are introduced via etherification reactions (Punia et al., 2024). This dual modification enhances the structural integrity of the starch during heating and shearing, particularly in high-moisture, protein-rich food systems such as plant-based meats (Pietrasik, 1999).

2.3.3.1 Functional properties

The functional benefits of modified waxy maize starch stem from its dual modification, which includes cross-linking and hydroxypropylation. Cross-linking, typically achieved through phosphate-based reagents, introduces covalent bridges between starch chains, enhancing granule rigidity and reducing the likelihood of rupture during gelatinization. Meanwhile, hydroxypropyl substitution improves hydration and prevents retrogradation by creating steric hindrance. thereby improving freeze–thaw stability and extending shelf life (Singh et al., 2007)

These combined modifications result in starch pastes that are more viscous, stable, and resistant to breakdown during extended heating, agitation, and acidic conditions, compared to their unmodified counterparts (Singh et al., 2007). Consequently, dual-modified starches are widely employed in processed foods such as salad dressings, canned goods, frozen meals, and puddings due to their superior stability and functional versatility (Singh et al., 2007; Wurzburg, 1986)

Chemical modifications alter the starch structure by forming permanent bonds with hydroxyl groups, which not only strengthen internal interactions but also modify the hydrophilicity or hydrophobicity of the polymer. In particular, cross-linking introduces lateral links that can reduce the porosity of the gel matrix. This decreased porosity affects important properties such as solubility, swelling behaviour, and reactivity, all of which influence the final texture and stability of food products (Włodarczyk-Stasiak et al., 2017).

In protein-rich gel systems, like plant-based sausages, MS enhances the gel structure by forming compact, less porous networks that improve water-holding capacity (WHC) and textural integrity (Włodarczyk-Stasiak et al., 2017). This functionality is especially valuable in plant-based meat formulations, where challenges like phase separation and moisture loss often compromise product quality (Kyriakopoulou et al., 2021).

Modified waxy maize starch is increasingly used as fat substitutes in the formulation of low-fat meat products, including sausages, nuggets, patties, and ham due to restricted swelling, form denser, more elastic gels (Włodarczyk-Stasiak et al., 2017).For instance, Mohammadi (2012) demonstrated that modified waxy maize starch could effectively replace fat in beef sausages, with an optimal inclusion level of 2%, without negatively affecting product quality.

2.4 Protein-polysaccharide interactions

Protein–polysaccharide interactions are critical in determining the structure, stability, and textural characteristics of many food systems (Pang et al., 2022). Among various processing techniques, thermal processing, often involving heat or hydrothermal conditions, is one of the most widely applied methods in the food industry (Wang et al., 2021). It induces structural and functional changes in both polysaccharides and protein. For instance, in the presence of excess water, heat disrupts the semi-crystalline structure of starch granules, promoting the leaching of amylose and amylopectin molecules (Ai & Jane, 2015). Simultaneously, proteins may undergo unfolding, exposing hydrophobic and thiol groups, which can lead to protein aggregation through intermolecular interactions (Wang & Guo, 2019).

In recent years, the interplay between starch and protein during thermal treatment has garnered growing interest, particularly for its role in modulating the textural and rheological behaviour of composite systems (Wang et al., 2021). Ghumman et al. (2016) demonstrated that the addition of albumins and globulins to starch systems reduced both the storage modulus (G′) and loss modulus (G″) during heating and cooling, with globulins exerting a more pronounced effect. This reduction likely results from competitive interactions between proteins and starch molecules that alter the gelatinization and network formation behaviour of starch (Wang et al., 2021).

At the molecular level, proteins contain numerous hydrophilic groups, such as amide, hydroxyl, carboxyl, and thiol, that are capable of interacting with starch chains (Wang et al., 2021). These interactions are governed not by a single mechanism but rather by a combined influence of various covalent and non-covalent forces, including electrostatic interactions, hydrogen bonding, van der Waals forces, hydrophobic effects, and covalent linkages (Jenkins et al., 1987; Wang et al., 2021). For example, studies on lentil protein–starch composite gels confirm the presence of both hydrogen bonding and covalent linkages (Joshi et al., 2014). The dominant interaction type depends on factors such as protein concentration, the molecular structure of the polymers, and specific processing conditions (Wang et al., 2021). Furthermore, these molecular-scale associations can evolve into larger, ordered structures during thermal processing, leading to the formation of three-dimensional gel networks that define the macroscopic properties of composite systems (Dupuis & Liu, 2019). Interestingly, the presence of protein can also influence nutritional outcomes, such as resistant starch formation. Lu et al. (2016) found that adding potato protein to potato starch increased resistant starch content, possibly by limiting enzymatic hydrolysis, whereas Escarpa et al. (1997) reported that incorporating bovine serum albumin had the opposite effect, reducing resistant starch formation.

Thermal processing can promote molecular-scale assemblies between starch and protein chains, which evolve into larger-scale ordered structures that critically influence the macroscopic properties of the system (Wang et al., 2021). On a microscale, these interactions can result in the formation of three-dimensional gel networks composed of both starch and protein (Wang et al., 2021).

According to Bühler et al. (2022), in the development of starch–protein-based food products, such as plant-based meat analogues, dry powders containing plant proteins and starch are commonly hydrated to create a biopolymer mixture. Under specific conditions, these mixtures tend to separate into two distinct phases due to thermodynamic incompatibility, especially at lower concentrations. This phase separation results in one phase becoming enriched in starch and the other in protein. Even at relatively low levels, such as 1% carbohydrate and 5% protein, phase separation has been observed. However, formulations used in meat analogues typically contain much higher concentrations, often exceeding 30% protein and 2% carbohydrate. At these levels, the system generally becomes multiphase with minimal molecular integration.

Instead of forming a homogeneous matrix, the components in such dense systems interact primarily through physical means, with steric hindrance and competitive water binding significantly influencing gelation behaviour. For instance, the presence of protein can raise the onset temperature for starch gelatinization and simultaneously reduce paste viscosity.

2.5 Egg white proteins

Comprising the majority of a whole egg by volume, egg white accounts for roughly 58%, while the yolk and shell represent about 31% and 11%, respectively. Its composition is predominantly water (88%), followed by proteins (10.5%) and minor amounts of carbohydrates (0.5%), minerals (0.8%), and fats (0.2%) (Campbell et al., 2003; Razi et al., 2023).

Egg white is a complex biological fluid made up of various proteins, each with specific structural and functional roles that are critical to its performance in food systems. These proteins contribute to the unique foaming, gelling, and emulsifying abilities of egg white (Chang et al., 2018)., which are widely exploited in food formulation and processing. The primary proteins found in egg white include ovalbumin, ovotransferrin, ovomucoid, ovomucin, lysozyme, globulins, and avidin (Razi et al., 2023). Although they differ in abundance and molecular characteristics, these components often act synergistically to deliver the desired techno-functional attributes in products such as baked goods, meringues, and emulsions.

Ovalbumin is the most prevalent protein in egg white, making up over half of its total protein content (Razi et al., 2023). It plays a critical role in defining the isoelectric point of egg white (around pH 4.5) (Huntington & Stein, 2001). Ovalbumin is particularly valued for its excellent thermal responsiveness and ability to form gels. Under heating, it denatures and aggregates into either linear or compact structures, resulting in gels that vary in clarity, from transparent to opaque *d*epending on processing conditions like pH, salt concentration, and protein content (Croguennec et al., 2007). Its surface-active nature also enables foam formation, as it rapidly unfolds and adsorbs at air–water interfaces. Additionally, ovalbumin contributes to emulsification, particularly under acidic conditions, where its increased flexibility and surface hydrophobicity improve emulsion stability (Razi et al., 2023; Weijers et al., 2006)

Ovotransferrin accounts for around 12% of egg white proteins and is known for its antimicrobial action and ability to bind iron (Razi et al., 2023). Although it is not effective in foam production by itself, its synergistic interaction with lysozyme greatly enhances foam stability (Alleoni & Antunes, 2004). As the least thermally stable egg white protein, it forms weak and coarse gels when heated alone, but co-gelation with other proteins can improve its network formation (Razi et al., 2023) . Ovotransferrin also exhibits emulsifying activity, particularly due to its capacity to adapt its conformation and stabilize oil–water interfaces across a wide range of pH and ionic strengths (He et al., 2021).

Ovomucoid is a heat-stable glycoprotein, rich in carbohydrates, but does not significantly contribute to gelation due to its inability to self-aggregate during heating (Yuno-Ohta et al., 2016). However, it exhibits trypsin inhibitory activity and contributes to foam stability when combined with other proteins like lysozyme (Jin et al., 2023). Alone, it does not produce foam, but it can support long-lasting foam structures in complex protein systems. Its contribution is more stabilizing than structure-forming (Razi et al., 2023).

Although present in smaller quantities (1.5–3.5%), ovomucin is largely responsible for the thick, gel-like texture of raw egg white due to its viscous and elastic properties (Razi et al., 2023). It is ineffective at generating foam on its own but serves as a strong foam stabilizer when combined with lysozyme or globulins (Johnson & Zabik, 1981). Its polymeric nature and ability to interact through non-covalent bonds contribute to gel elasticity and viscosity (Offengenden & Wu, 2013). Moreover, ovomucin supports emulsifying activity due to its high surface hydrophobicity (Razi et al., 2023).

Lysozyme is notable for its antimicrobial action and its role in stabilizing foams through electrostatic interactions with ovomucin (Razi et al., 2023). This interaction is essential in forming a protective interfacial film during whipping, which prevents drainage and collapse (Zhao et al., 2021). Lysozyme cannot form heat-induced gels by itself unless modified chemically (e.g., with thiol reagents). However, upon partial unfolding over time, its emulsifying and foaming properties can be enhanced (Sheng et al., 2016). Its contribution is crucial in foam film strength and shelf-life extension of aerated products (Razi et al., 2023).

Globulins, particularly the G2 and G3 types, constitute around 4% of egg white proteins and are strongly associated with foam formation (Razi et al., 2023). Their foaming ability is often enhanced by interactions with ovomucin and lysozyme, leading to stable, voluminous foam structures (Campbell et al., 2003). While their role in gelation is limited, they are effective surface-active agents in whipped or aerated systems (Razi et al., 2023).

Though present in trace amounts, avidin plays a minor structural role in food systems. It is mainly recognized for its antimicrobial activity through biotin binding, which limits microbial growth by depriving organisms of an essential nutrient (Razi et al., 2023). It does not participate in foaming or gelation but may contribute indirectly to food preservation. Some studies also suggest that peptides derived from avidin may exhibit antioxidant or bioactive effects (Abeyrathne et al., 2018).

2.5.1 Heat-induced gelation and aggregation behaviour of egg white proteins

Egg white proteins are highly sensitive to heat (Jiang et al., 2024). Upon thermal treatment, they undergo denaturation, which alters their native structure and often leads to unwanted aggregation; ultimately compromising their functional qualities (Liu et al., 2023). The characteristics of the aggregates formed are strongly temperature-dependent, with different heating conditions promoting the development of either soluble or insoluble aggregates (Jiang et al., 2024).

The nature of the aggregates formed is highly dependent on the temperature and duration of heating. At moderate temperatures (58–70 °C), the proteins form soluble aggregates, stabilized mainly by hydrophobic interactions and hydrogen bonds (Mine et al., 1990). In contrast, heating at higher temperatures (80–95 °C) promotes more extensive denaturation and the formation of insoluble aggregates through covalent disulphide bonds and hydrophobic clustering (Croguennec et al., 2002; Croguennec et al., 2007) .These insoluble aggregates are typically associated with stronger, but more brittle and less water-retentive gels.

As Alleoni (2006) explains, heat-induced gelation involves two main stages. First, thermal energy induces conformational changes in the protein structure, resulting in partial unfolding. This leads to increased molecular mobility and the formation of an elastic network. In the second stage, the denatured proteins interact and aggregate, driven by sulfhydryl–disulphide interchange and hydrophobic interactions. If aggregation occurs too rapidly or in an unregulated manner, it can result in disordered protein clusters with poor gel uniformity and reduced water retention, ultimately leading to excessive syneresis.

Thus, the heat-induced gelation of egg white proteins is governed by a delicate balance between denaturation and aggregation kinetics, which in turn determine the mechanical strength, elasticity, and microstructure of the resulting gels.

2.5.2 Formation and structure of egg white gels

Egg white protein gels are a type of hydrogel (Razi et al., 2023), formed through heat-induced denaturation and aggregation (Campbell et al., 2003). This process is driven by non-covalent interactions such as electrostatic forces, hydrophobic interactions, and hydrogen bonds, as well as disulphide cross-linking between protein molecules (Nojima & Iyoda, 2018). According to Razi et al. (2023), egg white begins to lose its fluidity around 60°C, with two major thermal transitions: ovotransferrin denaturation at 60–65°C and ovalbumin at 80–85°C. Ovotransferrin is particularly important in the early stages of coagulation (Croguennec et al., 2002). As heating continues, proteins unfold and form high molecular weight soluble aggregates through intermolecular β-sheet interactions, eventually creating a stable gel network through sulfhydryl-disulphide exchanges (Razi et al., 2023). Gel strength typically increases upon cooling, as hydrogen bonds further stabilize the network (Tang et al., 1994).

Despite their wide use, heat-induced egg white protein gels can suffer from limited mechanical strength, which restricts their applications in more advanced or engineered food structures (Campbell et al., 2003). This limitation arises primarily from the formation of heterogeneous and coarse protein networks during thermal denaturation and aggregation. Upon heating, proteins such as ovalbumin unfold and form new disulphide and hydrophobic interactions. However, under suboptimal conditions (e.g., near the isoelectric point or without structuring agents), these interactions can result in irregular network formation with weak junction zones, large pores, and poor cohesiveness (Campbell et al., 2003; Croguennec et al., 2002; Mine, 1996). Such structures have reduced elasticity and lower water-holding capacity, ultimately compromising their mechanical integrity and functional performance in complex food matrices (Croguennec et al., 2002)

2.5.3 Physicochemical factors that influence egg white gelation

Among the various factors affecting egg white protein gelation, pH is particularly influential. Supporting this, Raikos et al. (2007a) observed that at pH 8, egg white proteins exhibited higher gelation temperatures and improved cohesion compared to more acidic conditions (pH 5 and 2). Similarly, Khemakhem et al. (2019) observed that hardness and elasticity were lowest near the isoelectric point (pH 4.5), while cohesiveness and adhesiveness were greater than those formed at pH 6.5. At the isoelectric point, protein-protein interactions dominated, leading to increased aggregation and the formation of weaker gels with larger pores. In contrast, at higher pH levels, such as pH 7, protein-solvent interactions were more pronounced, and the gel structure was characterized by ordered linear polymers due to the repulsive electrostatic forces that limited random aggregation (Razi et al., 2023).

In addition to pH, salts and hydrocolloids or polysaccharides can significantly modify egg white gelation (Razi et al., 2023). The presence of NaCl, for example, can influence protein unfolding and aggregation behaviour, often increasing gelation temperature at high pH (Raikos et al., 2007b). Polysaccharides such as xanthan gum, gellan gum, carrageenan, and hydroxypropylmethylcellulose (HPMC) also interact with proteins through electrostatic or steric mechanisms, altering gel firmness and microstructure (Razi et al., 2023).

Souza et al. (2018) noted that weak interactions between xanthan gum and egg proteins often result in softer gels. This limited interaction is attributed to weak attractive forces between the molecules, which compromises the structural integrity of the resulting gel. Babaei et al. (2019) found that adding gellan gum increased gel hardness at acidic pH (4.0) but had little effect at neutral pH. However, it improved water retention in both cases, likely due to the hydrophilic properties of gellan gum which act by binding water. Similarly, observed firmer gels at pH 3.0 in egg white/HPMC gels attributed to the formation of oppositely charged aggregates. At neutral pH, however, phase separation weakened the gel due to electrostatic repulsion between negatively charged biopolymers (Razi et al., 2023)

Moreover, Zhang et al. (2019) found that moderate xanthan gum levels (0.06% (w/w)) improved gel hardness, springiness, and cohesiveness, but higher concentrations disrupted protein-protein interactions due to increased viscosity. The same study showed that gel whiteness peaked at 0.06% (w/w), correlating with a denser protein–polysaccharide matrix. Razi et al. (2018) also demonstrated that basil seed gum enhanced egg white gel properties by increasing WHC, hardness, and elasticity.

The effect of xanthan gum at concentrations of 0–0.18 % (w/w) on egg white gel properties has also been explored. Research by Zhang et al. (2019) found that hardness, springiness, and cohesiveness increased at 0.06% (w/w) xanthan gum but declined at higher concentrations. The rise in viscosity at elevated xanthan gum levels likely disrupted protein aggregate interactions. The peak in gel whiteness observed at 0.06% (w/w) xanthan gum was associated with a denser gel structure and enhanced protein-polysaccharide and protein-protein interactions. Similarly, Razi et al. (2018) reported that adding basil seed gum to egg white gels enhanced viscosity, water-holding capacity, elastic modulus, and hardness.

2.5.4 Food applications

Egg white proteins are well known for their exceptional functional properties, namely gelling, foaming, and emulsifying, which make them valuable ingredients in the dairy, meat, and bakery sectors (Razi et al., 2023). As gelling agents, egg white proteins have demonstrated significant potential in improving the texture and structural integrity of various food systems. For instance, Jitesh et al. (2011) incorporated 3% (w/w) egg white protein into lizardfish surimi and observed a notable enhancement in gel strength during frozen storage at –20 °C. Similarly, Hajidun et al. (2013) investigated the impact of egg white protein on common carp surimi and found that increasing egg white protein concentration improved textural attributes such as hardness, cohesiveness, adhesiveness, and elasticity, although a slight decrease in whiteness was noted.

Egg white proteins have also been extensively evaluated in meat emulsions for their impact on texture and binding in protein-based gels. According to Carballo et al. (1995), while studies have shown that egg white does not significantly affect water-binding capacity or reduce cooking and purge loss, its influence on texture is well established. Egg white protein has been shown to increase hardness, chewiness, and penetration force in sausage-type products, contributing to a firmer and more cohesive matrix. This texturizing effect appears to be linear and independent of other variables such as fat or starch content, indicating that egg white acts primarily through protein-protein network formation. However, findings across the literature have been mixed, some reports suggest that egg white can interfere with gelation in surimi systems or dilute protein concentration, especially in formulations with low-quality myofibrillar proteins. On the other hand, under optimized conditions, egg white has been found to enhance the texture of high-quality surimi and actomyosin gels.

Beyond traditional applications, egg white proteins are gaining attention in the development of plant-based meat analogues, especially for gluten-free and clean-label formulations (Razi et al., 2023). While gluten and hydrocolloids like xanthan gum are often used to enhance texture in vegetarian sausages, they are unsuitable for consumers with gluten intolerance or those seeking simpler ingredient lists. To address this, Zhao et al. (2022) evaluated the use of two commercial egg white proteins (P110 and M200) at concentrations ranging from 0% to 10% (w/w) as replacements for gluten and xanthan gum in vegetarian sausage formulations. P110 represents a regular spray-dried egg white powder (EWP), while M200 is a high gel strength variant that has undergone a proprietary alkali treatment to enhance its functional properties. The results showed that both egg white proteins significantly improved textural properties, bringing them closer to those of commercial meat sausages. Among the two, M200 demonstrated superior gelation, attributed to its more ordered protein structure and higher α-helix content, indicating enhanced tyrosine-related restructuring. Despite the improvements in texture, the addition of egg white proteins had no significant effect on colour, product length, or cooking-related weight loss.

In dessert applications, egg white proteins have been used to enhance the gelation of custard-based products (Razi et al., 2023). Tong et al. (2020) investigated the effects of egg white albumen and heat treatment on the structure and allergenicity of egg custard. Their study found that heating altered the tertiary structure of egg white albumen, reducing its allergenic potential while simultaneously improving its digestibility and contributing to the overall gel matrix.

2.6 Conclusions

The literature reviewed in this chapter highlights the distinct compositional and functional properties of rice protein isolate (RPI) and faba bean protein isolate (FBPI), both of which exhibit limitations in solubility, emulsification, water-holding capacity, and especially heat-induced gelation when used as single ingredients. Although FBPI shows comparatively stronger gelling potential than RPI, both proteins require high concentrations, favourable pH conditions, or structural modification to form stable networks. The incorporation of food polysaccharides, particularly methylcellulose, modified potato starch, and modified waxy maize starch has been shown to enhance viscosity, water retention, and structural integrity in composite systems, while egg white proteins contribute well-defined thermal gelation and strong intermolecular networks. However, despite numerous studies examining individual ingredients or binary combinations, limited research has explored how FBPI or RPI behave within complex, multi-component matrices containing both egg white and hydrocolloids under controlled heat-induced conditions. Moreover, gaps remain regarding how these interactions translate into the development of structured food products such as plant-based or vegetarian sausages. These gaps directly lead to the research objectives of this thesis, which aim to characterise the gelation behaviour of FBPI and RPI, examine their interactions with egg white and hydrocolloids, and apply these insights to the development of meat free sausage analogues with improved textural and physicochemical attributes.

Chapter 3. Heat induced gelation behaviour of FBPI and RPI with varying levels of with egg white powder

3.1 Introduction

The growing interest in plant-based protein sources stems from concerns about environmental sustainability, human health, and the need to diversify protein supplies in food systems. Among these alternative proteins, legume- and cereal-based protein isolates have gained particular prominence due to their high protein content and relatively favourable functional properties. Faba bean protein isolate (FBPI) and rice protein isolate (RPI), in particular, have attracted attention for their potential to serve as versatile ingredients in formulated foods. However, many plant protein isolates exhibit limited gelation capacity on their own, often producing weaker networks or coarser structures than animal proteins (Karabulut et al., 2024; Su et al., 2015). As a result, combining plant proteins with a strongly gelling protein, such as egg white (EW), has emerged as a promising strategy to enhance the textural and functional performance of plant-protein-based gels (Bai et al., 2022; Zhao et al., 2024)

In plant–animal protein mixtures, egg white typically contributes robust gel-forming capacity due to the presence of ovalbumin and other globular proteins known to form extensive disulphide-linked networks (Su et al., 2015). In contrast, proteins extracted from faba beans or rice often rely on synergistic interactions to achieve desirable rheological and textural characteristics. Such interactions can improve cohesiveness, water-holding capacity (WHC), and elasticity, especially when optimized in terms of pH, protein ratio, and processing conditions (Hamed et al., 2024; Pramualkijja et al., 2022). Despite this potential, limited research has focused on how FBPI and RPI perform in composite systems with EW across different mixing ratios, especially in heat-induced gelation processes at neutral pH.

Consequently, this chapter is structured in three main steps to systematically evaluate the gelation behaviour and functional enhancement of FBPI and RPI, both individually and in combination with egg white powder (EWP). In the first step, the minimum gelation concentration (MGC) of FBPI and RPI was determined at their native pH levels. This step aimed to establish a baseline of the intrinsic gelation capacity of each protein isolate without pH adjustment, offering insight into their natural functionality under heat-induced gelation conditions.

In the second step, a concentration at or slightly below the determined MGC (e.g., 12% for FBPI and 15% (w/w) for RPI) was selected and both protein dispersions were adjusted to a standardized pH of 7 to enable direct comparison under identical conditions. Despite the absence of gel formation at 15% (w/w) RPI, this concentration was selected for further analysis to better understand how the protein behaves under neutral conditions. Specifically, it allowed the evaluation RPI's response to stress and deformation in terms of structural and rheological properties, even in the absence of gelation, a topic that, to the best of my knowledge, remains scarcely studied.

Moreover, since this chapter aimed to evaluate the impact of egg white addition on RPI systems, using a sub-gelling concentration provides an opportunity to test whether functional ingredients like egg white can promote gelation through synergistic interactions.

In the third step, egg white (EW) was incorporated into both FBPI and RPI systems in varying proportions at pH 7 to investigate the functional impact of EW addition under controlled conditions and to explore how plant–animal protein interactions influence gel formation and quality. Egg white proteins are well documented for their strong gelation capacity, typically forming self-supporting gels at concentrations as low as 8–10% w/w due to the presence of ovalbumin and ovotransferrin, which readily unfold and aggregate under heat to form cohesive, elastic networks (Razi et al., 2023; Zayas, 1997). As such, egg white was utilized in this study as a functional benchmark and positive control to enhance the gelation properties of FBPI and RPI, both of which exhibit limited independent gel-forming ability at similar concentrations.

To comprehensively characterize the systems, rheological analyses were performed to elucidate how frequency and strain affect the viscoelastic behaviour of the composite gels, highlighting the transition from predominantly elastic to viscous responses under increasing deformation. These results were complemented by texture analysis, including measurements of hardness, cohesiveness, and springiness, to quantify the mechanical integrity and firmness of the resulting gels. Furthermore, water-holding capacity (WHC) measurements and scanning electron microscopy (SEM) provided structural insight into the internal gel network, including pore size, matrix compactness, and overall homogeneity. Together, these methods help clarify how the inclusion of egg white alters the gelation properties of plant-based proteins and offers a viable strategy for enhancing gel formation and structural performance.

3.2 Material and Methods

3.2.1 Materials

Unflavoured powders of faba bean protein isolate (**FBPI;** 85% protein, 5.4% fat, 3.8% carbohydrate) and rice protein isolate (**RPI;** 80% protein, 5.3% fat, 6.3% carbohydrate) were purchased from NZPROTEIN® (Auckland, New Zealand); while egg white powder (**EWP;** 85% protein, 0.2% fat, <2.0% carbohydrate) was purchased from Keto Store NZ Ltd (New Zealand).

All other chemicals and reagents, including sodium azide (NaN_3), hydrochloric acid (HCl) and sodium hydroxide (NaOH), were of analytical grade and purchased from Sigma Co., Ltd. (St. Louis, MO USA). Canola oil used for rheological tests was purchased from Woolworths's supermarket (Auckland, New Zealand).

3.2.2 Determination of minimum gelation concentration (MGC) of FBPI and RPI

Dispersions of FBPI and RPI 15% (w/w) were prepared by dispersing each protein powder in deionized water. The mixtures were magnetically stirred at 500 rpm (IKA® C-MAG HS7, Germany) at room temperature for 24 hours to ensure proper dispersion and dissolution. The native pH of each dispersion was measured in triplicate using a calibrated pH meter (Accumet AB150, Fisher Scientific), which was calibrated with pH 4 and pH 7 buffer solutions, yielding pH values of 6.49 for RPI and 7.09 for FBPI. The average pH value was recorded within 24 hours of preparation.

Subsequently, these protein dispersions were diluted with deionized water to achieve concentrations of 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, and 15% (w/w), and each sample was mixed thoroughly using a stirring plate (IKA® C-MAG HS7, Germany) at 500 rpm for 30 minutes

Samples (5 mL) of FBPI and RPI dispersions at each concentration between 5% and 15% (w/w) were placed in test tubes and heated in a water bath (T100, Grant Instruments, UK) at 95°C. The samples required approximately 8 minutes to reach the target temperature (from room temperature), after which they were held at 95 °C for 30 minutes. The samples were then immediately cooled in an ice water bath consisting of crushed ice and water to promote rapid cooling. Samples remained in this bath for approximately 40 minutes, and then stored at 4°C for approximately 12 hours. After equilibration at room temperature for 2 hours, the heat-treated samples were visually assessed for both gel formation and gel strength by inverting the test tubes. The lowest concentration at which the sample remained intact in the inverted tube was considered the minimum gelation concentration (Agboola et al., 2005).

3.2.3 Preparation of FBPI and RPI stock dispersions

Stock dispersions of 12% (w/w) FBPI, 15% (w/w) RPI, 12% (w/w) EW, and 15% (w/w) EW were prepared by dissolving the powder in deionized water containing 0.02% (w/w) sodium azide to prevent microbial growth. The mixtures were magnetically stirred at 500 rpm using a stirring plate (IKA® C-MAG HS7, Germany) at room temperature for 24 hours to ensure complete hydration. All stock dispersions were then stored at 4°C and used within one week.

3.2.4 Preparation of composite dispersions (FBPI-EW and RPI-EW)

Composite dispersions of FBPI-EW and RPI-EW were prepared by mixing FBPI (12% (w/w)) and EW (12% (w/w)) in ratios of 100:0, 60:40, 50:50, 40:60, and 0:100. Similarly, RPI (15% (w/w)) stock dispersion was mixed with EW (15% (w/w)) dispersion in the same ratios. The mixtures were stirred at room temperature for 1 hour to ensure proper dispersion and dissolution. Subsequently, the pH was adjusted to 7.0 using 1 M NaOH and 1 M HCl, followed by slow stirring at 300 rpm on a stirring plate (IKA® C-MAG HS7, Germany) for an additional 2 hours. A pH meter (Accumet AB150, Fisher Scientific) was used to measure the pH of all dispersions.

3.2.5 Preparation of composite gels

Thirty grams of the composite FBPI/EW and RPI/EW dispersions were transferred into plastic containers and sealed with plastic caps. The gels were formed by heating the solutions in a water bath (IKA® T100, Grant Instruments, UK) set to 95°C. The dispersions required approximately 13 minutes to reach the target temperature, after which they were held at 95 °C for 30 minutes to allow gel formation. Immediately after heating, the containers were transferred to an ice–water bath, prepared using crushed ice and cold water to facilitate rapid cooling. Samples remained in this bath for approximately 1 hour until reaching room temperature. The gels were stored at 4 °C for 12 hours to allow the maturation of gels (Zhao et al., 2024).

3.2.6 Rheological testing

The rheological properties of composite gels (FBPI/EW and RPI//EW) were analyzed using a DHR-3 dynamic rheometer (TA Instruments Trios Version 4.3.1.39215-Waters LLC, New Castle, Delaware, USA) taken into account the protocol described by Chen et al. (2020) .

The samples of composite gels were loaded between 40 mm parallel steel Peltier plate with a gap of 1 mm. To avoid water loss, an appropriate amount of oil was added to the edge of the sample before the test began. After allowing the sample to reach equilibrium for 120 seconds, an amplitude sweep was conducted at 25°C over a strain range of 0.1–1000% at a frequency of 1 Hz. Following a 2-minute resting phase, a frequency sweep was performed at 25°C over a range of 0.01–10 Hz with a constant strain amplitude of 1.0%. The storage modulus (G′) and loss modulus (G″) were recorded throughout the entire test.

3.2.7 Water holding capacity (WHC)

WHC was determined following the procedure outlined by Ji et al. (2024) with slight modifications. Approximately 10 g of each composite gel was weighed and placed into 15 mL centrifuge tubes. These samples were then centrifuged at 10,000 x g for 20 minutes (Centrifuge Sigma 6-16KS, Germany). After centrifugation, excess water in the centrifuge tubes was removed by inverting them and keeping them in that position for one hour.

WHC (%) was determined by calculating the ratio of the remaining sample weight (after centrifugation) compared to the original sample weight.

WHC (%) = (Weight after centrifugation / Initial weight) × 100

3.2.8 Texture analysis

The textural properties of FBPI/EW and RPI/EW composite gels at different ratios were measured by compression test using, a modified version of the method based on Zhao et al. (2024). . After equilibrating at room temperature for 2 hours, gels were cut into cylinders with a diameter of 20 mm and a height of 10 mm. Each gel was compressed using a texture analyzer (TA-XT Plus, Stable Micro Systems Ltd., Godalming, UK) equipped with a P/35 mm cylindrical aluminum probe. The test parameters were set as follows: pre-test and test speeds at 1.0 mm/s, post-test speed at 5.0 mm/s, trigger force at 5.0 g, and deformation at 50%, with a data acquisition rate of 100 points.

Exponent Software (Version 6.1.15.0, Stable Micro Systems Ltd., UK) was used to generate force-time curves and calculate texture attributes, including hardness, cohesiveness and springiness.

3.2.9 Scanning electron microscopy (SEM)

The microstructure of the FBPI/EW and RPI/EW composite gels were analysed by SEM, using the protocols described by Cao et al. (2025), Zhao et al. (2024) and Chen et al. (2020). Each sample was cut into 10 mm × 10 mm pieces, placed in a plastic container, covered with aluminum foil, and frozen at −20 °C for approximately 2 days**.** The frozen gels were then freeze-dried using a Labconco FreeZone 6 freeze dryer set to around −52 °C and a vacuum pressure of ~0.140 mbar for 3 days**,** with continuous monitoring of both temperature and vacuum. Once completely dried, the chamber temperature was increased to 25 °C (under vacuum) until the samples reached equilibrium. Subsequently, the dried samples were mounted on SEM stubs using double-sided carbon tape**,** and finally placed in the microscope chamber for imaging. The freeze-dried samples were examined under a Hitachi TM3030 Plus **(**Tokyo, Japan**)** tabletop SEM at an accelerating voltage of 5 keV**,** using a mixed-signal detection system at 100× and 300× magnifications.

3.2.10 Statistical Analysis

All samples were prepared and measured in at least duplicate, and results are presented as mean ± standard deviation (SD). Statistical analysis was conducted using SPSS Statistics 26.0 (IBM, USA). One-way analysis of variance (ANOVA) and Tukey's HSD test was performed to identify specific group differences at a significance level of $p < 0.05$.

3.3 Results and Discussion

3.3.1 Determining the minimum gelation concentration of FBPI and RPI via visual observation

The minimum gelation concentration (MGC), defined as the lowest protein concentration required to form a gel under specific conditions, was determined to be 12% w/w for FBPI, aligning with findings reported by Ehsanzamir (2018). Gelation was observed within the 12–15% (w/w) concentration range, with a self-supporting gel forming at 12% (w/w) and above. At concentrations below this threshold, the dispersions remained predominantly liquid, as illustrated in Figure 3.1. Similar trends were reported by Kamani et al. (2024) , who observed no gel formation at ≤6% (w/w) but achieved firm gels at 12% (w/w). Meanwhile, Nivala et al. (2021) reported an MGC of 10% (w/w) for FBPI under acidic and heat-treated conditions, highlighting the influence of environmental factors. Kamani et al. (2024) also emphasized that gelation behaviour may be influenced not only by protein concentration but also by the presence of non-protein components such as starches and fibre, which can modify gel network formation.

According to Ehsanzamir (2018), heat-induced gelation of FBPI is primarily driven by protein denaturation, which promotes the formation of disulphide bonds and exposes hydrophobic residues, reinforcing gel strength. Continued heating enhances protein–protein interactions and aggregation, further facilitating gel network development.

In contrast, attempts to determine the MGC for RPI revealed no gel formation within the tested range of 5% to 15% (w/w) as shown in Figure 3.1, indicating its limited ability to form a self-supporting gel on its own. Literature indicates that RPI typically requires a much higher concentration, around 20–25% (w/w) for gelation under standard heat treatment (Nguyen, 2025). This higher concentration requirement is often attributed to RPI's low solubility, structural rigidity, and the presence of antinutritional factors, all of which hinder the development of a cohesive gel network. Jayaprakash et al. (2022) further emphasized that RPI's gelation behaviour is highly dependent on environmental factors such as pH, ionic strength, and the addition of stabilizers. In this context, incorporating functional agents like egg white powder presents a promising strategy to enhance RPI's gelation capacity. These additives can compensate for RPI's inherent limitations by improving intermolecular interactions and promoting network formation, thus enabling gelation at lower protein concentrations.

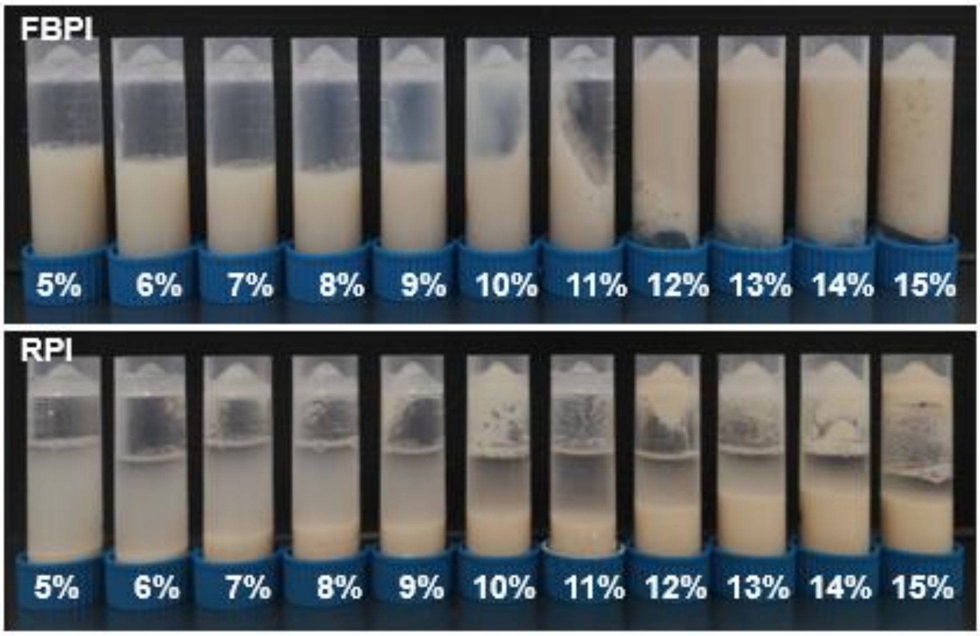
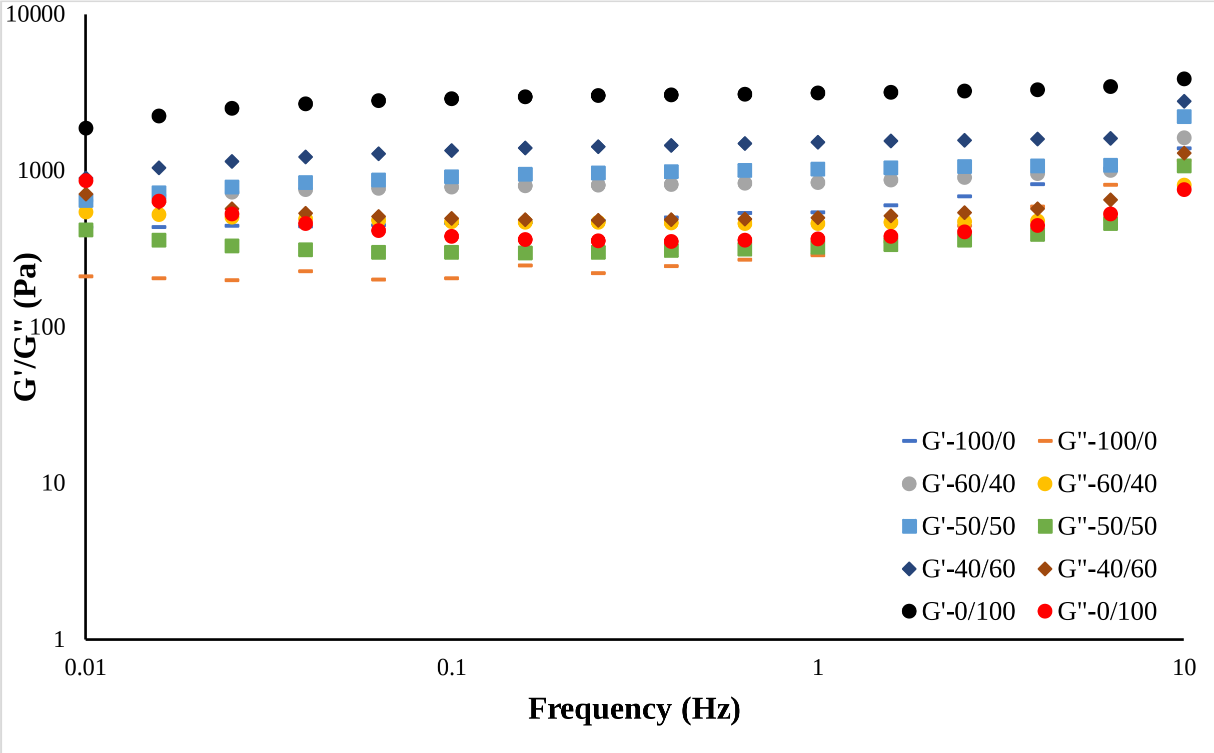


Figure 3.1 Visual appearance of heat-induced gels of FBPI (top) and RPI (bottom) at various concentrations (5% to 15% (w/w)) formed at 95°C for 30 minutes

3.3.2 Rheological analysis

The structural stability of FBPI/EW and RPI/EW composite gels at total concentrations of 12% and 15% (w/w), respectively, was investigated to assess their viscoelastic properties and determine the influence of protein ratio on the gel network's ability to withstand deformation across different frequencies. The storage modulus (G′) and loss modulus (G″) of the composite gels as a function of frequency are shown in Figure 3.2.



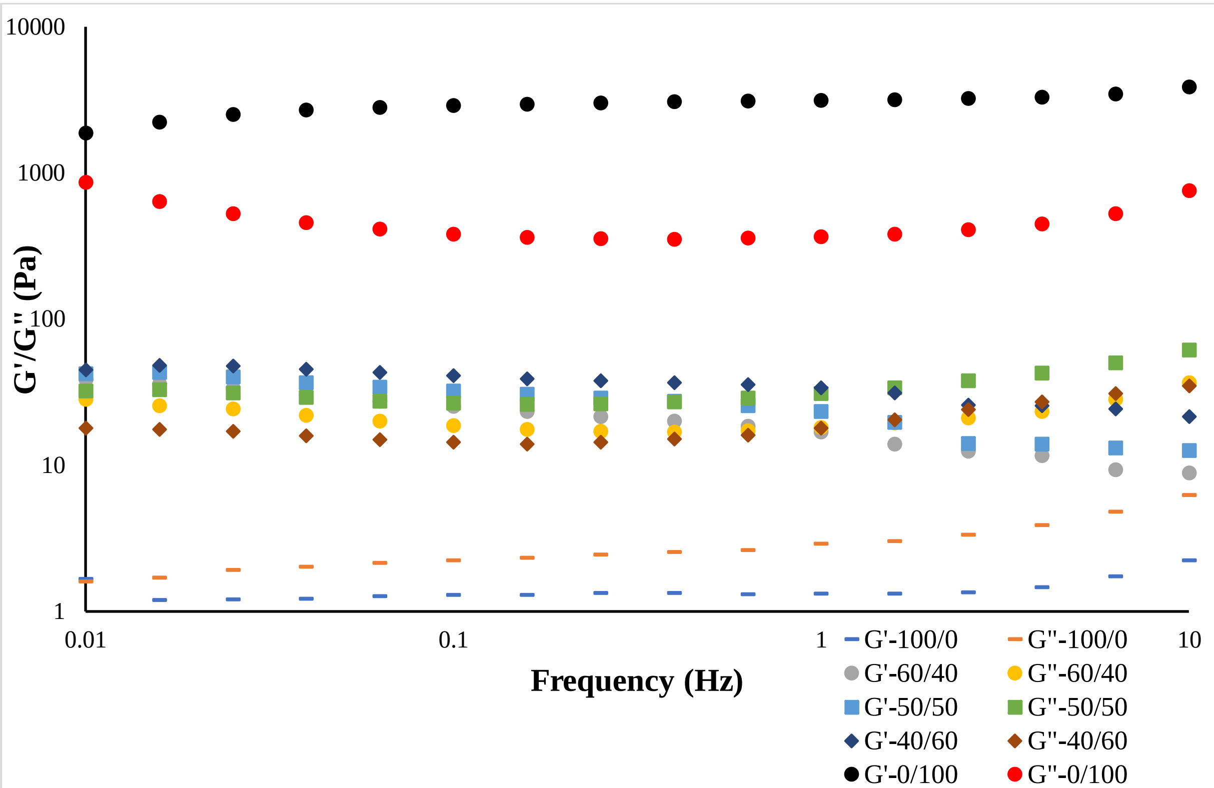


Figure 3.2 Storage modulus (G') and loss modulus (G'') from frequency sweep test of FBPI-EW (top) and RPI/EW (bottom) composite gels at different ratios (100/0, 60/40, 50/50, 40/60, and 0/100)

According to the data, the G’ and G’’ of FBPI/EW composite gels were frequency-dependent, both increasing with frequency. This frequency dependence may be attributed to the enhanced molecular mobility of solid particles at higher frequencies (Su et al., 2015). Additionally, G’ remained consistently higher than G’’ across all gel mixtures, indicating the formation of gel-like network structure in FBPI-EW mixtures at various ratios. Similar trends have been observed in soybean protein isolate/egg white gels (Su et al., 2015).

To further assess the viscoelastic behaviour of the composite gels, the loss factor (tan δ = G″/G′) was analyzed at 1 Hz (Table 3.1). For pure egg white, tan δ was 0.12, indicating a strong gel with predominantly solid-like properties and good ability to recover after deformation (Bai et al., 2022). However, as the proportion of FBPI increased, tan δ also increased, indicating the formation of weaker gels, likely due to the increased contribution of G″ modulus.

Similarly, the frequency sweep results for RPI/EW composite gels at different ratios revealed that G′ remained higher than G″ at higher EW proportions (40/60 and 0/100), reinforcing their gel-like nature. However, as the proportion of RPI increased, G″ exceeded G′ at lower frequencies, indicating a transition toward more viscous-dominated behaviour. Notably, the 100/0 (pure RPI) system exhibited the lowest G′ values and a crossover of G″ over G′, consistent with the absence of gel formation observed in TPA analysis. This behaviour is characteristic of weakly structured protein networks with insufficient intermolecular interactions to sustain elasticity across deformation rates (Zhang, 2025)

In contrast, the mixed ratios (60/40, 50/50, and 40/60) displayed intermediate viscoelastic properties, with an evident transition from viscous-dominated to elastic-dominated behaviour as EW content increased. This trend is aligned with the frequency sweep results of FBPI/EW composite gels, confirming that the addition of egg white (EW) enhances the structural integrity of RPI-based gels and, improves their ability to resist deformation. The observed trends are consistent with previous studies on protein-based gel systems, where the incorporation of EW has been shown to reinforce gel networks by enhancing intermolecular interactions (Bai et al., 2022; Su et al., 2015).

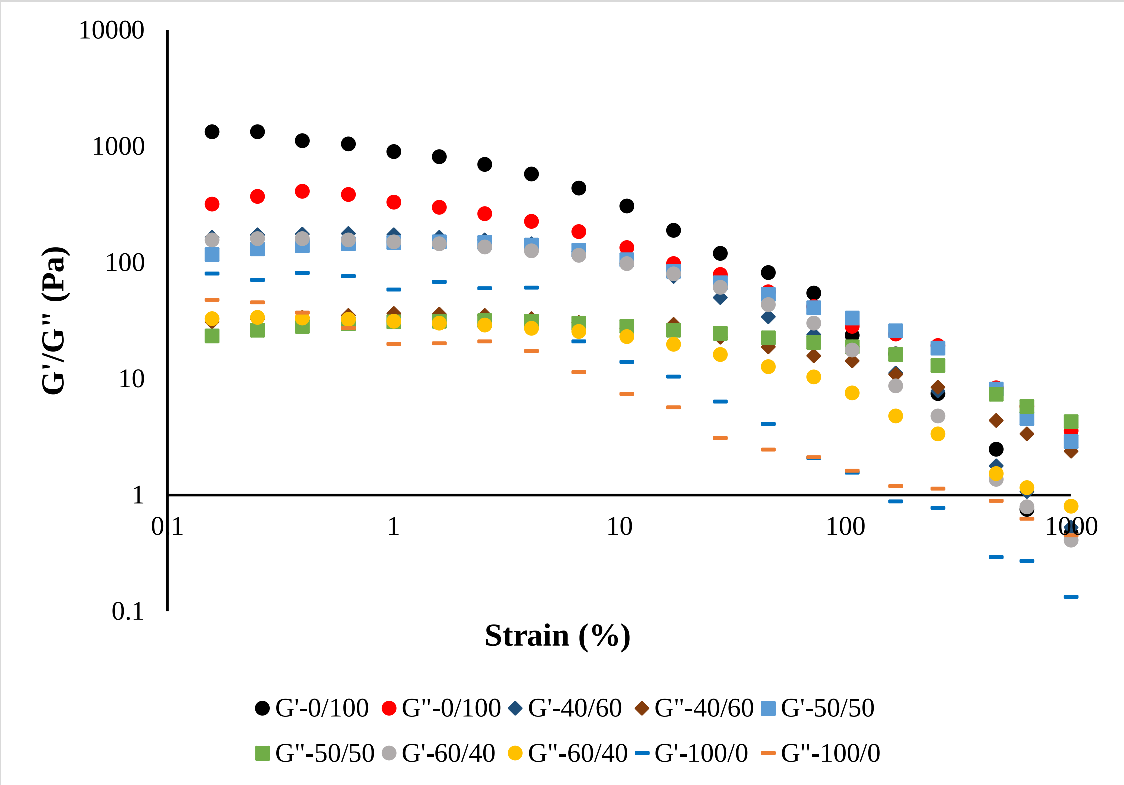
Additionally, as the EW ratio increased in the composite gels, the loss factor (tan δ) decreased, indicating a shift from a more dissipative, viscous-dominated structure (RPI-rich) to a more elastic, deformation-resistant gel (EW-rich).

Table 3.1 Tan δ values at 1 Hz for FBPI/EW and RPI/EW composite gels at varying protein ratios

Plant-based protein/EW ratio	FBP/EW (Tan (δ))	RPI/EW (Tan (δ))
100/0 (Pure plant protein)	0.60 ± 0.31[a]	2.21 ± 0.07[a]
60/40	0.54 ± 0.18[a]	1.08 ± 0.07[b]
50/50	0.33 ± 0.002[b]	1.33 ± 0.13[c]
40/60	0.32 ± 0.03[b]	0.53 ± 0.05[d]
0/100 (pure EW)	0.12± 0.02[c]	0.12± 0.02[e]

Values within the same column with different superscript letters indicate significant differences ($p < 0.05$).

Viscoelastic materials exhibit characteristics of both solids and liquids, responding differently to applied stress. A viscoelastic solid undergoes deformation at a controlled rate until reaching a stable shape, and upon stress removal, it gradually recovers its original form. Conversely, a viscoelastic liquid continues to deform as long as stress is applied and only partially regains its initial structure once the stress is removed (Badjona et al., 2025). The amplitude sweep results for FBPI/EW and RPI/EW composite gels at various ratios, are illustrated in Figure 3.3.



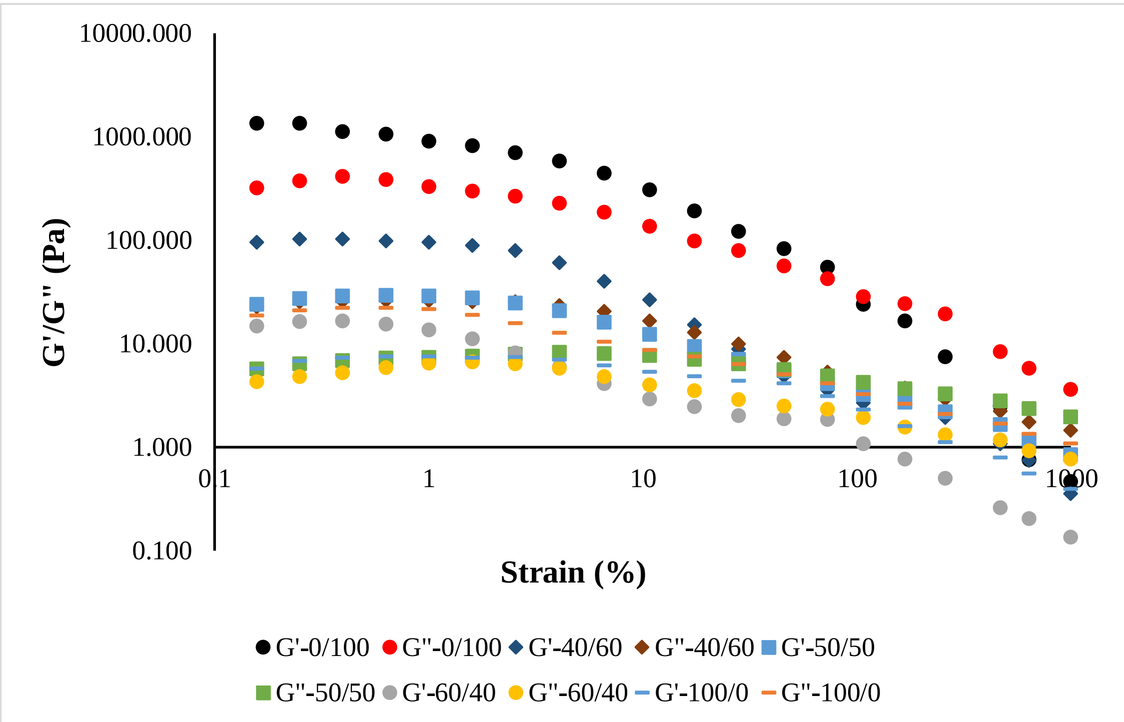


Figure 3.3 Storage modulus (G') and loss modulus (G'') from amplitude sweep test of FBPI-EW (top) and RPI/EW (bottom) composite gels at different ratios (100/0, 60/40, 50/50, 40/60, and 0/100)

Within the linear viscoelastic region (LVR) (0.1% to 10% strain), the FBPI/EW gels deformed elastically, characterized by G′ exceeding the G″, indicating the gel-like nature of samples. Beyond this range, G′ declines more rapidly than G″, signifying structural breakdown and the transition to non-linear behaviour. A similar trend was reported by Badjona et al. (2025) for FBPI dispersions at 12, 15 and 20% (w/w) concentrations being G′ remained higher than G″ across the strain range of 0.1 to 10%.

Furthermore, within the LVR (0.1% to 10% strain) the G' modulus decreased with the addition of FBPI**.** This behaviour can be attributed to the formation of globulin aggregates from FBPI**,** which disrupt the egg white protein network, leading to a weaker gel structure (Kuang et al., 2023).

The crossover point (G′ = G″)**,** marking the transition from solid-like to liquid-like behaviour (Badjona et al., 2025), occurred at lower strain values in pure FBPI gels**,** indicating reduced gel strength compared to pure egg white gels (See Table 3.2). However, in mixed systems, as FBPI concentration increased (e.g., 60/40), the crossover point shifted to higher strain values, indicating an enhanced gel strength compared to the EW-dominant ratio (40/60).

Regarding the RPI/EW composite gels, the results showed that the LVR was extended with the increasing EW content in the composite gels. For instance, the 0/100 (pure EW) gel exhibited the highest G' value**,** indicating strong elasticity and a well-structured network, with a broad LVR and delayed onset of structural breakdown. As the proportion of RPI increased**,** G' progressively decreased, and the LVR shortened**,** indicating a weaker gel structure with lower mechanical stability, particularly in the 50/50 and 60/40 samples, which displayed an earlier transition to non-linear behaviour.

The 100/0 (pure RPI) sample lacked a well-defined LVR, with G" predominating over G', indicating a predominantly viscous response and confirming the absence of gelation, as also observed in texture analysis. These findings align with those of Pramualkijja et al. (2022), who reported that egg white solutions exhibited a significant increase in G' upon the addition of collagen tripeptide hydrolysate, whereas whey protein solutions only showed a modest increase**,** reinforcing the superior gelation properties of EW over other proteins. Additionally, their study found that egg white solutions resisted increasing strain better than whey protein solutions, a result similar to the trend observed in the RPI/EW gels**,** where EW-rich samples exhibit greater structural resilience, whereas RPI-dominant formulations show earlier structural breakdown.

Table 3.2 Tan δ at the crossover point for FBPI/EW and RPI/EW composite gels at varying protein ratios

Plant-based protein/EW ratio	FBP/EW (Tan (δ))	Crossover Strain (%)	RPI/EW (Tan (δ))	Crossover Strain (%)
100/0 (Pure plant protein)	1.31 ± 0.41[a]	164.32 ± 201.57[a]	3.08 ± 0.82[a]	0.10 ± 0.00[a]
60/40	1.08 ± 0.09[b]	404.47 ± 182.96[b]	1.34 ± 0.33[b]	9.34 ± 6.36[b]
50/50	1.13 ± 0.13[c]	515.45 ± 381.45[c]	1.09 ± 0.13[b]	137.60 ± 219.43[c]
40/60	1.34 ± 0.29[d]	242.39 ± 149.11[d]	1.14 ± 0.11[b]	33.93 ± 26.40[d]
0/100 (Pure EW)	1.09 ± 0.06[e]	84.55 ± 67.04[e]	1.09 ± 0.06[b]	84.55 ± 67.04[e]

Values within the same column with different superscript letters indicate significant differences ($p < 0.05$).

3.3.3 Water holding capacity (WHC)

The WHC results of FBPI/EW and RPI/EW composite gels at different ratios are presented in Table 3.3. The FBPI gel had a lower WHC compared to the pure EW gel; however, as the EW ratio increased in the composite gels, WHC also improved, reaching its peak at a 50/50 ratio. Similar trends were reported by Su et al. (2015) in soybean protein/EW composite gels, where the highest WHC was recorded at an equal ratio, surpassing the WHC of individual soybean protein or EW gels at the same total protein concentration. This suggests a synergistic interaction between the two proteins, leading to improved WHC compared to their individual forms.

Beyond the 50/50 ratio, EW content became predominant, and the WHC declined. Zhao et al. (2024) suggested that the addition of EW facilitated gel formation to a certain extent and enhanced protein cross-linking, leading to a denser gel network and improved water retention. However, as EW content became predominant, the gels appeared rougher and more fragile, making them more susceptible to structural disruption during centrifugation, which led to water release and a subsequent decrease in WHC.

In contrast to the FBPI/EW results, the WHC of RPI/EW composite gels benefited significantly from the addition of EW. Gels with higher EW ratios (40/60 and 50/50) exhibited the highest WHC values of 73.58% and 63.31%, respectively ($p < 0.05$), reflecting the positive impact of EW on water retention in these gels. These results align with previous studies on hybrid protein, gels such as that of (Lian et al., 2025), who investigated combinations of rice protein and EW protein. Their study demonstrated synergistic effects between these heterologous proteins, leading to improved gel strength and WHC. They reported that while a 5:5 rice protein/EW protein gel lacked sufficient mechanical strength, rice protein/EW protein gels with higher EW proportions (2:8 and 3:7) exhibited significantly enhanced gel strength and WHC. The study also reinforces the role of EW protein in improving gel network integrity by enhancing water retention and gel structure in hybrid protein systems.

Table 3.3 Water holding capacity (WHC, %) of composite gels prepared from different FBPI/EW and RPI/EW ratios

Plant-based protein/EW ratio	FBP/EW	RPI/EW
100/0 (Pure plant protein)	88.71 ± 0.17^{a}	**No gel**
60/40	92.62 ± 0.31^{b}	61.66 ± 0.30^{a}
50/50	96.63 ± 0.22^{c}	63.31 ± 0.18^{b}
40/60	93.21 ± 0.15^{d}	73.58 ± 0.17^{c}
0/100 (Pure EW)	90.31 ± 0.13^{e}	

Values within the same column with different superscript letters indicate significant differences ($p < 0.05$).

3.3.4 Texture analysis

Texture plays a crucial role in assessing the fundamental gel properties of food gels and significantly impacts overall food quality (Zhao et al., 2024). Common parameters used to analyze texture include hardness, cohesiveness, and springiness. The variations in these parameters for among FBPI/EW and RPI/EW composite gels at different ratios are presented in Table 3.4.

Table 3.4 Textural parameters (hardness, springiness, and cohesiveness) of FBPI/EW and RPI/EW composite gels at varying protein ratios (100/0, 60/40, 50/50, 40/60, 0/100)

Plant-based protein/EW ratio	Hardness (N)		Springiness (mm)		Cohesiveness	
	FBPI/EW	RPI/EW	FBPI/EW	RPI/EW	FBPI/EW	RPI/EW
100/0 (Pure plant protein)	134.30 ± 15.16^{a}	No gel	0.750 ± 0.006^{a}	No gel	0.382 ± 0.001^{a}	No gel
60/40	197.96 ± 42.46^{b}	112.28 ± 7.45^{a}	0.756 ± 0.019^{a}	0.724 ± 0.017^{a}	0.417 ± 0.000^{b}	0.292 ± 0.001^{a}
50/50	358.76 ± 56.59^{c}	275.29 ± 15.77^{b}	0.766 ± 0.003^{a}	0.774 ± 0.010^{b}	0.494 ± 0.001^{c}	0.482 ± 0.001^{b}
40/60	474.69 ± 21.41^{d}	357.54 ± 43.92^{c}	0.829 ± 0.003^{b}	0.794 ± 0.026^{c}	0.470 ± 0.001^{d}	0.515 ± 0.076^{c}
0/100 (Pure EW)	545.89 ± 102.54^{e}		0.823 ± 0.004^{b}		0.541 ± 0.001^{e}	

Values within the same column with different superscript letters indicate significant differences ($p < 0.05$).

As the EW ratio increased, gel hardness also increased, reaching its highest value at the pure EW ratio (0/100) among all composite gels. Similar findings were reported by Su et al. (2015) in their study on soybean protein/EW protein composite gels, where they observed a linear increase in gel hardness was observed with the addition of egg white, indicating no synergistic effect. They attributed this to the high hardness of EW gels, as ovalbumin, the most abundant protein in EW and the primary contributor to its gelling properties, exhibits peak gel hardness around pH 6.5. Therefore, since these experiments were carried out at pH 7, it can be suggested that EW played a dominant role in enhancing the hardness of the composite gels under these experimental conditions. Moreover, both G′ and G″ increased with higher EW content, further supporting the observed trend in hardness.

Notably, the hardness of FBPI/EW composite gels was higher than that of RPI/EW mixtures. This result can be attributed to the fact that FBPI and EW both possess gel-forming properties, whereas RPI does not exhibit gel-forming characteristics on its own. Similarly, Zhao et al. (2024) observed gel formation in both components of pea protein isolate (PPI)/EW composite gels. They reported that when the proportion of EW was lower than PPI, EW was dispersed within the PPI gel matrix, enhancing gel hardness. In the same way, when PPI was present in lower proportion than EW, it was incorporated into the EW gel network, also contributing to increased hardness.

Regarding cohesiveness and springiness, both parameters generally increased with the proportion of EW in RPI/EW and FBPI/EW composite gels. However, this trend was not consistent across all ratios. Notably, the 40/60 FBPI/EW sample exhibited slightly lower cohesiveness than the 50/50 sample, indicating that the relationship was not strictly linear. Despite this, FBPI/EW gels still demonstrated higher cohesiveness than RPI/EW gels at most ratios. Interestingly, in FBPI/EW composite gels, springiness at the 40/60 FBPI/EW ratio was slightly higher than that of pure EW. Lin et al. (2017) attributed this to the ability of EW protein to integrate into plant-based gel networks, enhancing gel properties and increasing springiness. However, when EW protein is no longer embedded within plant-based protein molecules and instead forms a gel independently, its structural characteristics changed (Zhao et al., 2024). EW protein is a cohesive protein, and upon heating, its molecules randomly aggregate into large protein clusters (Gharbi & Labbafi, 2018). Gels formed by such large aggregates tend to be opaque and less elastic (Zhao et al., 2024), explaining the reduction in springiness observed in pure EW gels compared to composite ones.

3.3.5 Microstructure analysis

Figures 3.4 and 3.5 present the micrographs of FBPI/EW and RPI/EW composite gels at 100× and 300× magnifications, accompanied by their respective digital photographs. Marked differences in appearance were observed depending on the protein source and composition. When FBPI predominated (ratios of 100:0 or 60:40), the gels exhibited uneven, porous, and creamy-white surfaces. Increasing the EW ratio produced smoother, glossier gels. In the RPI/EW composite gels, all gels with different ratios exhibited a grainy texture and**;** high RPI ratios yielded opaque gels with a creamy off-white hue**,** more opaque than pure EW gel but slightly translucent in thinner regions.

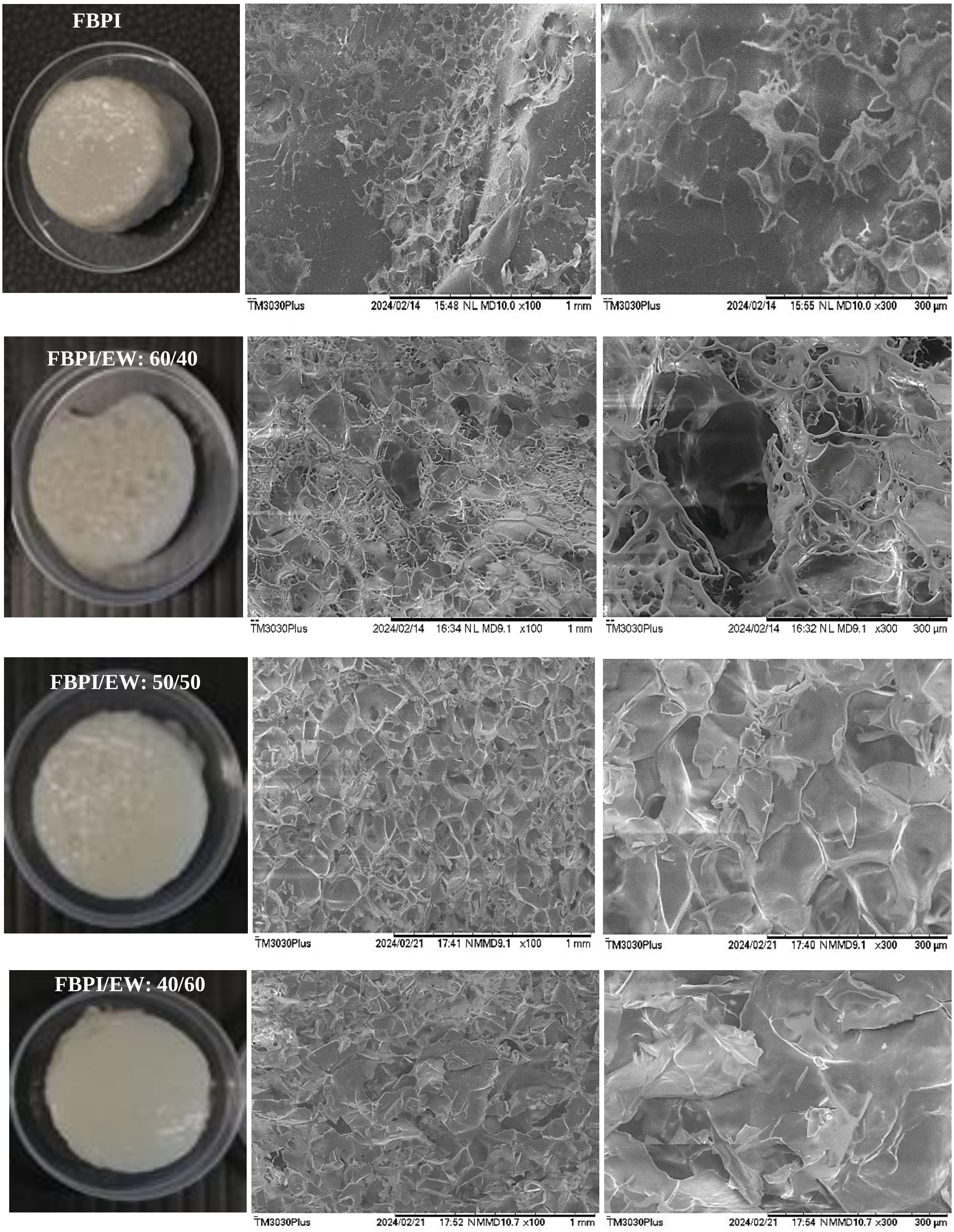


Figure 3.4 Visual appearance and microstructural (SEM) images (100x with a 1-mm scale bar, and 300× with a 300-μm scale bar) of FBPI/EW composite gels at different ratios.

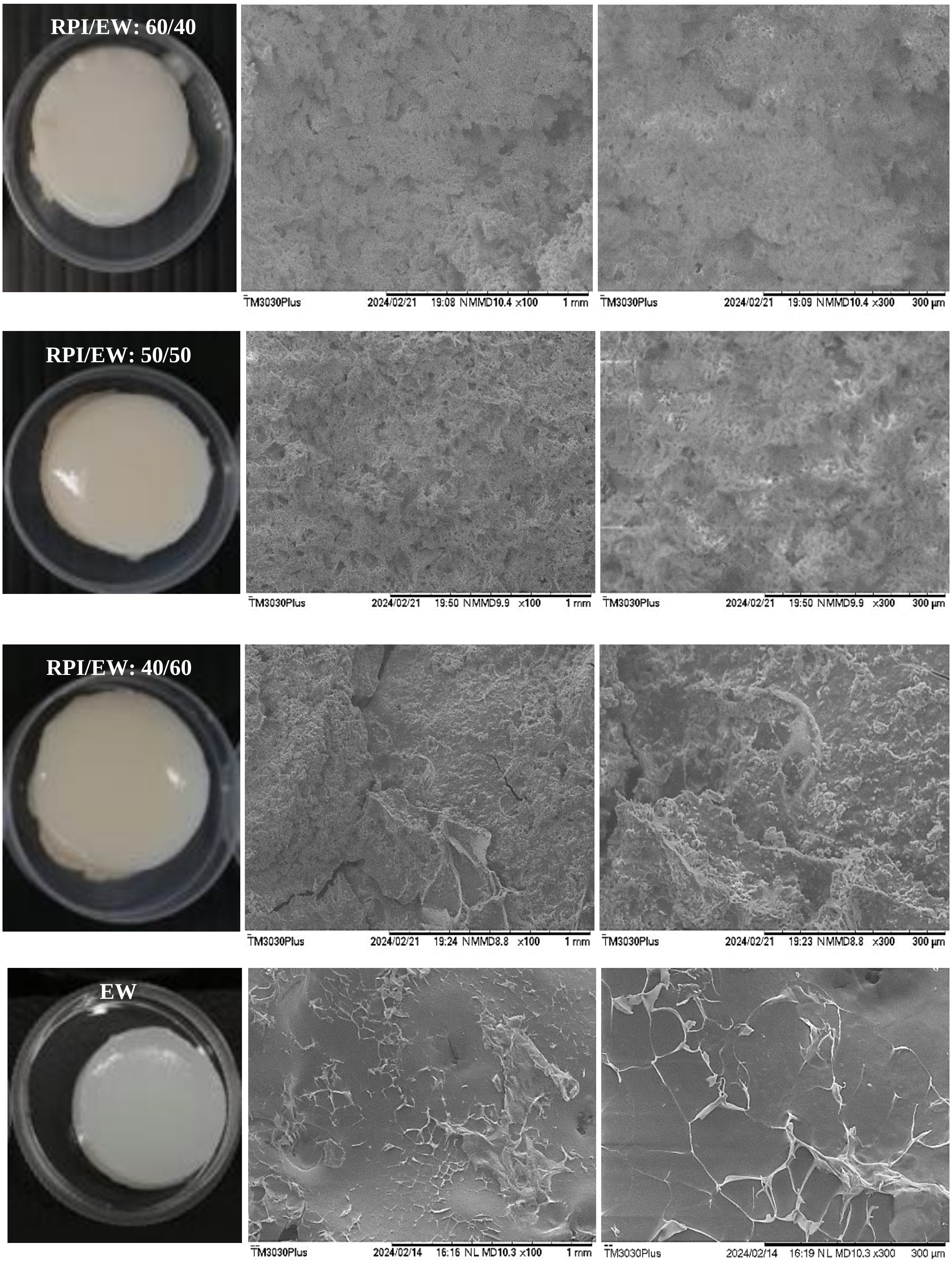


Figure 3.5 Visual appearance and microstructural (SEM) images (100x with a 1-mm scale bar, and 300× with a 300-μm scale bar) of RPI/EW composite gels at different ratios, including pure EW as a reference.

The SEM images revealed that increasing the EW fraction improved the formation of cohesive, well-defined protein networks, yielding more homogeneous and cohesive structures with finer pores and reduced phase separation, whereas higher FBPI levels resulted in comparatively coarser microstructures with larger pore sizes. Similar morphological features have been reported in soybean protein isolate (SPI) and EW composite gels, where adding more EW increased homogeneity by reducing large aggregates and improving protein-protein interactions, thus enhancing springiness and WHC**.** However, when the SPI/EW ratio fell below 1:1, the network structure became rougher and formed larger particles due to faster protein aggregation and a more heterogeneous EW network at pH 7.0 (Su et al., 2015).

Likewise, Hamed et al. (2024) observed that FBPI particles displayed angular edges and spherical portions at 500× magnification, reflecting extensive protein aggregation and partially globular configurations.

Moreover, Vogelsang-O'Dwyer et al. (2020) noted that spray-dried FBPIs typically appear smooth and shrunken, underscoring how different processing conditions (e.g., drying methods) can alter protein microstructure.

This limited self-gelling tendency in FBPI is consistent with broader evidence on plant protein isolates (Gulzar et al., 2024), whereby synergy with a strongly gelling protein (such as EW) is crucial for developing robust networks. Indeed, Su et al. (2015) demonstrated that EW proteins readily aggregate at neutral pH, forming heterogeneous matrices with high hardness but lower water-retention ability, a property that complements plant isolates by improving overall gel structure.

In RPI/ EW composite gels, a similar pattern was observed in which increasing the EW proportion led to more uniform protein networks, while higher RPI content resulted in irregular microstructures characterized by somewhat spongy areas, lacking clearly defined pore networks and presenting less dense matrices. Such heterogeneity is often attributed to the limited gelation capacity of rice proteins alone (Cheng et al., 2024), as noted for other plant-derived isolates (e.g., soybean) which form coarser and less elastic gels when not combined with complementary gelling agents (Zhao et al., 2015). The capacity of EW to form a stable, interconnected matrix via disulphide bond formation and other intermolecular forces (Zhao et al., 2016) appears to compensate for RPI's lower cohesive strength, consistent with observations in soybean/EW mixtures whose gel networks improve substantially when EW is present at moderate to high ratios (Su et al., 2015).

3.4 Conclusions

The gelation behaviour of FBPI and RPI under heat treatment was significantly influenced by their intrinsic properties and by the incorporation of EW powder at various ratios. The native pH of the protein dispersions also influenced their minimum gelation concentration (MGC). FBPI dispersions exhibited a pH close to neutral (pH 7.09), favouring greater electrostatic repulsion, improved solubility, and enhanced unfolding during heating, conditions that facilitate network formation and enable gelation at concentrations as low as 12% w/w (Badjona et al., 2024b; Langton et al., 2020). In contrast, RPI, with a natural pH of 6.49, failed to form a cohesive gel even at concentrations up to 15% (w/w), highlighting its limited independent gelation ability when used alone under the tested conditions. Previous studies have shown that rice proteins form gels more effectively under acidic conditions, where reduced solubility facilitates their incorporation into the protein network, whereas higher solubility at neutral or slightly alkaline pH results in a smaller proportion of protein participating in the gel structure (Kaspchak et al., 2020). Furthermore, at neutral pH, the predominance of α-helical structures in RPI has been reported to weaken intermolecular interactions, inhibit aggregation, and consequently limit cohesive gel formation (Ellepola et al., 2006; Kaspchak et al., 2020).

When combined with EW, both FBPI/EW and RPI/EW systems exhibited viscoelastic characteristics typical of mixed protein gels, although the structural contribution varied with blend ratio. The incorporation of EWP into FBPI dispersions promoted the formation of softer networks, reflecting interactions that modulate the strong intermolecular associations normally established by EW proteins. In RPI/EW composites, mechanical reinforcement became evident only at higher EW proportions, indicating that the limited network-forming capacity of RPI can be compensated by the gelling ability of ovalbumin and other EW proteins, which dominate structural development under heat.

Rheological analysis revealed that both FBPI/EW and RPI/EW composite gels exhibited typical viscoelastic behaviour, with storage modulus (G′) exceeding loss modulus (G″) across the frequency sweep for EW-rich systems. However, the addition of FBPI to EW dispersions increased the loss factor (tan δ), indicating the formation of weaker gels, whereas in RPI/EW composites, the enhancement of elastic behaviour was only observed with higher EW proportions. This confirmed that egg white proteins, rich in ovalbumin and capable of extensive network formation, effectively improved the mechanical stability of plant-based protein gels.

Texture analysis showed that hardness, springiness, and cohesiveness of the composite gels significantly increased with higher EW proportions. Notably, FBPI/EW gels exhibited higher hardness values compared to RPI/EW gels at equivalent ratios, due attributed to the inherent gelling capacity of FBPI, whereas RPI largely relied on EW to form stable structures. Water-holding capacity (WHC) measurements supported these findings, with maximum WHC values observed near the 50/50 ratios for both protein systems. The presence of egg white protein facilitated denser gel networks capable of retaining more water, although excessive EW content slightly compromised WHC due to increased fragility and susceptibility to water loss under centrifugal force.

Microstructural analysis using SEM further revealed that increasing the proportion of EW led to more homogeneous and cohesive gel matrices, characterized by finer pore structures and reduced phase separation. In contrast, gels with higher plant protein content exhibited coarser, more porous networks, reflecting limited protein-protein interactions. Particularly, FBPI-dominant gels displayed larger, irregular pores, while RPI-rich gels showed spongy, less compact structures, consistent with the poor gelation ability observed for RPI.

In summary, the incorporation of EW powder markedly enhanced the gelation performance, textural properties, water retention, and microstructural homogeneity of both FBPI and RPI gels. While FBPI benefited from synergistic interactions with EW proteins to strengthen its network, RPI required the addition of EW to compensate for its intrinsic structural deficiencies for independent gelation. These results highlight the potential of EW powder as a functional co-gelling agent to create composite protein systems with improved quality attributes, offering promising strategies for the development of plant-based and hybrid food products. Future studies could explore the molecular-level interactions between plant and animal proteins within the gel matrix to better understand the mechanisms driving the observed improvements in gelation and textural properties.

Chapter 4. Effect of modified starches and methylcellulose on the heat-induced gelation of FBPI and RPI

4.1 Introduction

The growing consumer demand for sustainable, health-conscious, and ethically produced foods has driven rapid innovation in the development of plant-based meat alternatives (Carhuancho-Colca et al., 2024). This shift has led to increased utilization of plant-derived proteins from legumes, cereals, grains, and tubers in food formulation.

Among these, faba bean protein isolate (FBPI) and rice protein isolate (RPI) have gained attention for their promising nutritional profiles and functional properties. FBPI is known for its high protein content, strong gelation capacity, and favourable water-holding properties (Badjona et al., 2024a), while RPI offers advantages such as hypo allergenicity and a neutral sensory profile, making it particularly suitable for diverse plant-based applications (Agboola et al., 2005).

One of the main challenges in developing plant-based meat analogues is achieving desirable texture and structural integrity that closely mimic those of animal-derived meats. To overcome this, formulations commonly incorporate functional ingredients beyond the protein base, such as binders, water, fats, colorants, and flavourings**,** which work collectively to replicate the appearance, taste, and mouthfeel of conventional meat products (Peñaranda & Garrido, 2024). Among these, hydrocolloids, such as starches and gums, are widely utilized in the food industry to enhance texture and improve the structural integrity of various food systems. Starch, a polysaccharide naturally present in plants, is one of the most commonly used carbohydrate-based hydrocolloids due to its functional versatility and cost-effectiveness. In addition to being affordable, starches provide multiple technological benefits, including water binding, thickening, gelling, emulsion stabilization, and improving adhesion in processed foods (Aktaş & Gençcelep, 2006). These attributes make starches especially popular in emulsified meat products, where they help optimize texture and reduce formulation costs.

To broaden their applications, many commercial starches undergo physical or chemical modification, resulting in enhanced functional properties that native starches cannot always provide (Bashir & Aggarwal, 2019). In particular, chemically modified starches derived from waxy maize and potato are frequently used in plant-based and meat analogue systems to enhance viscosity, water retention, and binding performance. In this study, cross-linked waxy maize starch provides improved resistance to shear and heat during processing, while chemically modified potato starch enhances water binding, freeze-thaw stability and the ability to enhance texture without overpowering flavour (Zhao et al., 2023).

Regarding concentration, previous research has investigated the optimal levels of modified starches in sausage-type systems. For instance, Pietrasik (1999) examined the role of phosphate-modified potato starches in meat emulsions, finding that while they improved yield, levels exceeding 3% (w/w) could lead to undesirable softness in texture. However, industry reports and formulation trials have suggested that in low-meat sausages or plant-based analogues, starch can be included up to around 10% (w/w), though this is often considered the upper limit to avoid excessive softness or poor mouthfeel.

Further supporting this, Liu et al. (2008) investigated the use of heat-stable amylase-modified potato starch, as a fat replacer in reduced-fat sausages. The starch used was partially hydrolysed by amylase, followed by a heat stabilization treatment to improve its thermal stability. Their results showed that adding 2% or 4% (w/w) modified potato starch significantly reduced energy content and improved tenderness, while maintaining hardness similar to full-fat controls.

Similarly, Mohammadi (2012) evaluated the functional impact of chemically modified cross-linked waxy maize starch in reduced-fat beef sausages. Cross-linking enhances the starch's resistance to shear, acid, and thermal processing, resulting in improved functional stability (Punia et al., 2024). These chemical modifications enhance key properties such as water-binding capacity, swelling control, and gel firmness, all of which are critical in compensating for the reduced fat content. In composite food systems such as reduced-fat sausages, fat plays an essential role in contributing to structure, mouthfeel, and juiciness. By mimicking fat functionality, modified waxy maize starch helps to retain moisture, stabilize the protein-starch matrix, and prevent syneresis during cooking and storage. The study showed that inclusion levels between 1% and 3.25% (w/w) of modified waxy maize starch improved water retention, reduced cooking loss, and enhanced sensory acceptance, while a formulation using 2% (w/w) modified waxy maize starch and 5% (w/w) wheat flour resulted in a 34.9% energy reduction, preserving desirable texture and flavour attributes.

In addition to starches, methylcellulose plays a critical role in plant-based formulations due to its distinctive thermal gelation properties. Unlike many other hydrocolloids, methylcellulose forms a gel upon heating and melts when cooled (Singh et al., 2024), a behaviour that helps maintain product shape during cooking while providing a juicy, meat-like mouthfeel. Because of these functional advantages, methylcellulose is commonly used in plant-based burgers and sausages (Wei et al., 2024). Studies and patents have reported effective application ranges between 0.2% and 4.0% (w/w) (Wei et al., 2024), with optimal concentrations typically between 0.5% and 1.5% to improve structure and product stability (Bakhsh et al., 2021).

Based on this context, the present study systematically investigated the effects of incorporating modified waxy maize starch (MS; 2% and 5% (w/w)), modified potato starch (PS; 5% and 10% (w/w)), and methylcellulose (Me; 0.8% and 1.6% (w/w)) into FBPI (12% (w/w)) and RPI (15% (w/w)) dispersions. The heat-induced gels were characterized through rheological measurements (frequency and amplitude sweeps), water-holding capacity (WHC) analysis, microstructural examination using scanning electron microscopy (SEM) and evaluation of textural attributes.

The modified starches used in this study, derived from waxy maize (MS; FIRM-TEX®) and potato (PS; PenBind® 850), are both classified as E1442 (Incorporated, 2021, 2024), indicating they are cross-linked starches produced via phosphorylation (Sweedman et al., 2013). MS, is widely applied in thermally processed meat and meat analogue products due to its enhanced water-binding capacity, thermal and shear stability, and ability to contribute to gel strength without excessive viscosity (Incorporated, 2024; Mohammadi, 2012). In contrast, PS, offers low viscosity, reversible gelation, and a clean sensory profile, making it suitable for applications requiring soft, moist textures and improved water retention (Incorporated, 2021). Both starches were selected following technical recommendations from the ingredient supplier Hawkins Watts (New Zealand), and their concentration levels were determined based on supplier guidance and prior research findings (Liu et al., 2008; Mohammadi, 2012; Punia et al., 2024).

Commercial methylcellulose (Me; Methocel™ A4C) is a chemically modified cellulose derivative, produced by the etherification of cellulose with methyl groups, which imparts thermoreversible gelation properties (Peñaranda & Garrido, 2024), which mimics the thermal setting properties of animal proteins. The selected levels of 0.8% and 1.6% (w/w) were selected based on supplier recommendations and fall within the typical effective usage range reported for plant-based applications (Wei et al., 2024).

4.2 Material and Methods

4.2.4 Materials

Unflavoured powders of faba bean protein isolate (**FBPI;** 85% protein, 5.4% fat and 3.8% carbohydrate) and rice protein isolate (**RPI;** 80% protein, 5.3% fat, and 6.3% carbohydrate) were purchased from NZPROTEIN® (Auckland, New Zealand).

Commercial methylcellulose (**Me;** Methocel™ A4C; pH of a 2% aqueous solution: 5.0–8.0) and two commercial modified food starches; modified waxy maize starch (**MS;** FIRM-TEX®; pH of a 20% w/w slurry: 4.5–7.0) and modified potato starch (**PS;** PenBind® 850; pH 5.5–8.0) were kindly provided by Hawkins Watts, New Zealand. Technical specification sheets for these ingredients are included in Appendices A1 and A2.

All other chemicals and reagents such as sodium azide (NaN3), hydrochloric acid (HCl) and sodium hydroxide (NaOH), were of analytical grade and purchased from Sigma Co., Ltd. (St. Louis, MO USA). Canola oil used for rheological test was purchased from a local supermarket (Woolworths, Auckland, New Zealand).

4.2.2 Preparation of stock dispersions

A 24% (w/w) stock dispersion of FBPI was prepared by dispersing the FBPI powder in deionized water containing 0.02% (w/w) sodium azide to prevent microbial growth. The mixture was magnetically stirred at 500 rpm at room temperature for 24 hours to ensure complete hydration. Similarly, a 30% (w/w) stock dispersion of RPI was prepared following the same procedure. Both FBPI and RPI stock dispersions were then stored at 4°C and used within one week.

Methylcellulose (3.2% (w/w)) dispersions were prepared following the hot-cold technique described by Alamprese and Mariotti (2015). The methylcellulose powder was gradually dispersed into hot deionized water (90°C) approximately half volume of the total required, and stirred at 150rpm for 15 minutes by using a food blender (Thermomix TM5, Vorwerk, Germany). Then, the remining volume was completed by adding cold water (5°C) and thoroughly mixed for another 15 minutes or until a smooth dispersion was obtained. The solution was then kept in the fridge 24 hours to ensure its full hydration. Afterward, the Me dispersion (3.2% (w/w)) was diluted with deionized water to achieve a final concentration of 1.6% w/w with continuous stirring at 300 rpm for 2 hours (IKA® C-MAG HS7, Germany)

To prepare dispersions of PS 20% (w/w) and modified starch from maize 10% (w/w), each starch was respectively dissolved in deionized water at room temperature and stirred continuously at 300 rpm for 24h (IKA® C-MAG HS7, Germany) to ensure complete hydration. In addition, sodium azide (0.02% (w/w)) was added to each dispersion to inhibit bacterial growth.

The 20% (w/w) PS dispersion was then diluted with deionized water to obtain 10% and 5% (w/w) dispersions. Similarly, the 10% (w/w) modified waxy maize starch dispersion was diluted to 5% and 2% (w/w) concentrations. Each dispersion was thoroughly stirred continuously at 300 rpm for 2 hours (IKA® C-MAG HS7, Germany).

After complete hydration, all dispersions were adjusted to pH 7 using 1 M HCl and 2 M NaOH. After the initial pH adjustment, the dispersions were stirred at 500 rpm on a stirring plate (IKA® C-MAG HS7, Germany) for an additional 2 hours, with further pH adjustments made as necessary. A pH meter (Accumet AB150, Fisher Scientific) was used to measure the pH of all dispersions.

4.2.3 Preparation of composite dispersions

Composite dispersions of FBPI (12% (w/w), 30 g total) were prepared by mixing equal amounts of a 24% (w/w) FBPI stock dispersion with varying concentrations of functional additives. For samples containing 2% and 5% (w/w) MS, 15g of 4% and 10% (w/w) MS dispersions were used, respectively. To prepare FBPI samples containing 5% and 10% (w/w), 15g of the stock FBPI dispersion was mixed with 15g of 10% and 20% (w/w) PS dispersions respectively.

For Me incorporation, FBPI dispersions with 0.8% and 1.6% (w/w) Me were obtained by combining the 15 g of FBPI stock dispersion with 15g of 1.6% and 3.2% (w/w) Me dispersions, respectively. All mixtures were stirred continuously at 300 rpm for 24 hours at room temperature using a magnetic stirrer (IKA® C-MAG HS7, Germany) to ensure complete hydration.

Following the same protocol, RPI composite dispersions (15% (w/w)) were prepared with 2% and 5% (w/w) MS, 5% and 10% (w/w) PS**,** and 0.8% and 1.6% (w/w) Me by mixing with the corresponding concentrations of each additive dispersion.

After complete hydration, the pH of all dispersions was measured using a pH meter (Accumet AB150, Fisher Scientific) and adjusted to pH7. Table 4.1 summarizes the formulations of the samples used in the preparation of composite gels, including control samples.

Table 4.1 Formulations of samples used for preparing composite gels by mixing plant protein isolates (FBPI or RPI; 12% (w/w)) with MS (2% and 5% (w/w)), PS (5% and 10% (w/w)), or Me 0.8% and 1.6% (w/w)). Control samples containing only protein isolates, MS, PS, or Me were also included.

Sample	FBPI or RPI (% w/w)	MS (%w/w)	Sample	FBPI or RPI (% w/w)	PS (%w/w)	Sample	FBPI or RPI (%w/w)	Me (%w/w)
FBPI 12%/MS 2%	12	2	FBPI 12%/PS 5%	12	5	FBPI 12%/Me 0.8%	12	0.8
FBPI 12%/MS 5%	12	5	FBPI 12%/PS 10%	12	10	FBPI 12%/Me 1.6%	12	1.6
RPI 15%/MS 2%	15	2	RPI 15%/PS 5%	15	5	RPI 15%/Me 0.8%	15	0.8
RPI 15%/MS 5%	15	5	RPI 15%/PS 10%	15	10	RPI 15%/Me 1.6%	15	1.6
FBPI 12% (control)	12	-	FBPI 12% (control)	12	-	FBPI 12% (control)	12	-
RPI 15% (control)	15	-	RPI 15% (control)	12	-	RPI 15% (control)	15	-
MS 2% (control)	-	2	PS 5% (control)	-	5	Me 0.8% (control)	-	0.8
MS 5% (control)	-	5	PS 10% (control)	-	10	Me 1.6% (control)	-	1.6

4.2.4 Preparation of composite gels

Composite plant protein-modified starches (MS or PS) gels were prepared based on the procedure described by Ji et al. (2024), with slight modifications. The plant protein–modified starch dispersions were first incubated at 65°C for 20 minutes in an incubator (Thermoline Scientific©) to promote uniform hydration and prevent sedimentation of the starch. This was followed by heating at 95 °C for 30 minutes in a temperature-controlled water bath (IKA® T100, Grant Instruments, UK). After heating, samples were immediately cooled in an ice-water bath to room temperature. The resulting gels were stored at 4 °C for 12 hours to allow full gel maturation (Zhao et al., 2024).

For the preparation of plant protein–Me composite gels, the same procedure was used, with the exception that the dispersions were incubated at a lower temperature of 50 °C for 20 minutes, before undergoing the same heating and cooling steps. As noted by Yi et al. (2022), holding the mixture at around 50 °C promotes proper methylcellulose dispersion and improves its thermal responsiveness during the subsequent heating cycle.

4.2.5 Rheological testing

The rheological properties of the composite gels formulated with plant-based proteins and either modified starches or methylcellulose, were analyzed using a DHR-3 dynamic rheometer DHR-3 rheometer (TA Instruments Trios Version 4.3.1.39215-Waters LLC, New Castle, Delaware, USA) following the method described in Chapter 3, Section 3.2.6 and based on the protocol described by Chen et al. (2020).

4.2.6 Water holding capacity (WHC)

The water-holding capacity was determined following the procedure outlined by Ji et al. (2024) with slight modifications as described in Chapter 3, Section 3.2.7.

4.2.7 Texture analysis

To analyze the textural properties (hardness, cohesiveness, and springiness of the composite gels formulated with plant-based proteins and either modified starches or methylcellulose, were measured by compression test using a modified version of the method described by Zhao et al. (2024), as described previously in Chapter 3, Section 3.2.8.

Since the gels were not firm enough to be cut, the measurements were taken directly in the plastic container with the probe height adjusted to 60 mm. The measurements were conducted only on samples that remained intact and did not collapse when the container was inverted.

4.2.8 Scanning electron microscopy (SEM)

The microstructures of all composite gels were analysed by SEM, using the same method described in Chapter 3, Section 3.2.9.

4.2.9 Statistical Analysis

All samples were prepared and measured in at least duplicate, and results are presented as mean ± standard deviation (SD). Statistical analysis was conducted using SPSS Statistics 26.0 (IBM, USA). One-way analysis of variance (ANOVA) and Tukey's HSD test was performed to identify specific group differences at a significance level of $p < 0.05$.

4.3 Results and Discussion

Depending on the sample formulations used in this study, gelation was either induced or did not occur, as influenced by the type and concentration of the ingredients. Some formulations formed stable gels, while others remained in a liquid state without developing a stable gel network. Table 4.2 summarizes whether gelation occurred, based on these formulation differences. Further details are discussed in subsequent sections including the microstructure analysis.

Table 4.2 Summary of gel formation outcomes for formulations containing FBPI or RPI combined with combined with MS, PS, or Me including control samples.

Sample (% w/w)	FBPI or RPI (%w/w)	MS (% w/w)	Gelation
FBPI 12%/MS 2%	12	2	Yes
FBPI 12%/MS 5%	12	5	Yes
RPI 15%/MS 2%	15	2	Yes
RPI 15%/MS 5%	15	5	Yes
FBPI 12% (control)	12		Yes
RPI 15% (control)	15		No
MS 2% (control)		2	No
MS 5% (control)		5	No

Sample (% w/w)	FBPI or RPI (% w/w)	PS (% w/w)	Gelation
FBPI 12%/PS 5%	12	5	Yes
FBPI 12%/PS 10%	12	10	Yes
RPI 15%/PS 5%	15	5	No
RPI 15%/PS 10%	15	10	Yes
FBPI 12% (control)	12		Yes
RPI 15% (control)	15		No
PS 5% (control)		5	No
PS 10% (control)		10	Yes

Sample (% w/w)	FBPI or RPI (% w/w)	Me (% w/w)	Gelation
FBPI 12%/Me 0.8%	12	0.8	No
FBPI 12%/Me 1.6%	12	1.6	Yes
RPI 15%/Me 0.8%	15	0.8	No
RPI 15%/Me 1.6%	15	1.6	No
FBPI 12% (control)	12		Yes
RPI 15% (control)	15		No
Me 0.8% (control)		0.8	No
Me 1.6% (control)		1.6	No

4.3.1 Rheological analysis

4.3.1.1 Frequency sweep analysis of FBPI-Me and RPI-Me composite gels

The rheological behaviour of FBPI and RPI composite gels, (blended mixtures with modified starches and methylcellulose) was evaluated through frequency and amplitude sweeps to assess their viscoelastic properties.

The storage modulus (G′) and loss modulus (G″) of the plant protein-based-Me composite gels (FBPI–Me and RPI–Me) as a function of frequency are shown in Figure 4.1. The results indicated that the FBPI-Me composite gel at Me 1.6% (w/w) exhibited the highest G′ modulus, consistently exceeding G″ across the frequency range**,** confirming a dominant elastic (gel-like) behaviour. This result might be attributed to a strong molecular interaction between FBPI and Me, leading to a stable viscoelastic solid. For the FBPI-Me 0.8% (w/w) mixture, G' was greater than G″ at lower frequencies, but at higher frequencies (~6 Hz and above), an inversion occurred where G″>G′, indicating a transition towards more liquid-like behaviour at elevated oscillation frequencies. This suggest that the gel network is weaker and more susceptible to frequency-dependent structural relaxation**.** The Me control samples (Me at 0.8% and 1.6% (w/w)) consistently exhibited low G′ and G**″** values, with G″ remaining higher than G′ at low frequencies (~0.39 Hz and 1.58 Hz and above, respectively), indicating that methylcellulose alone at these concentrations does not form a strong gel network. These results are in agreement with prior rheological studies indicating that Me at low concentrations and room temperature behaves as an entangled polymer solution, rather than forming a well-structured gel (Desbrières et al., 2000).

According to Niemczyk-Soczynska et al. (2019), the viscoelastic behaviour of Me solutions is highly dependent on concentration**.** During heating, Me undergoes physical crosslinking via hydrophobic interactions among polymer chains, leading to an increase in G′ modulus. The authors observed a gelation process more pronounced at higher concentrations (e.g., 6.75% and 10% (w/w)), where G′ exhibited a sigmoidal increase, reaching higher maximum values compared to lower concentrations such as 1%**.** As a result, higher Me concentrations produced stiffer hydrogels**,** directly affecting the mechanical strength and structure of the final gel.

In contrast, the RPI–Me mixtures at both 0.8% and 1.6% (w/w) Me concentrations displayed predominantly viscous behaviour, as indicated by G″ consistently exceeding G′ across the entire frequency range. This dominance of the loss modulus indicated a more liquid-like response**,** characteristic of an unstable or weakly structured gel network**.** Furthermore, as previously established, RPI at 15% (w/w) alone lacks the ability to form a gel**,** and the incorporation of Me at these concentrations did not sufficiently enhance the structural integrity of the system to achieve stable gel formation.

At low frequencies (~0.01 to 0.1 Hz), the differences in G′ between the two formulations were less pronounced, indicating that at lower deformations, the structures behave similarly.

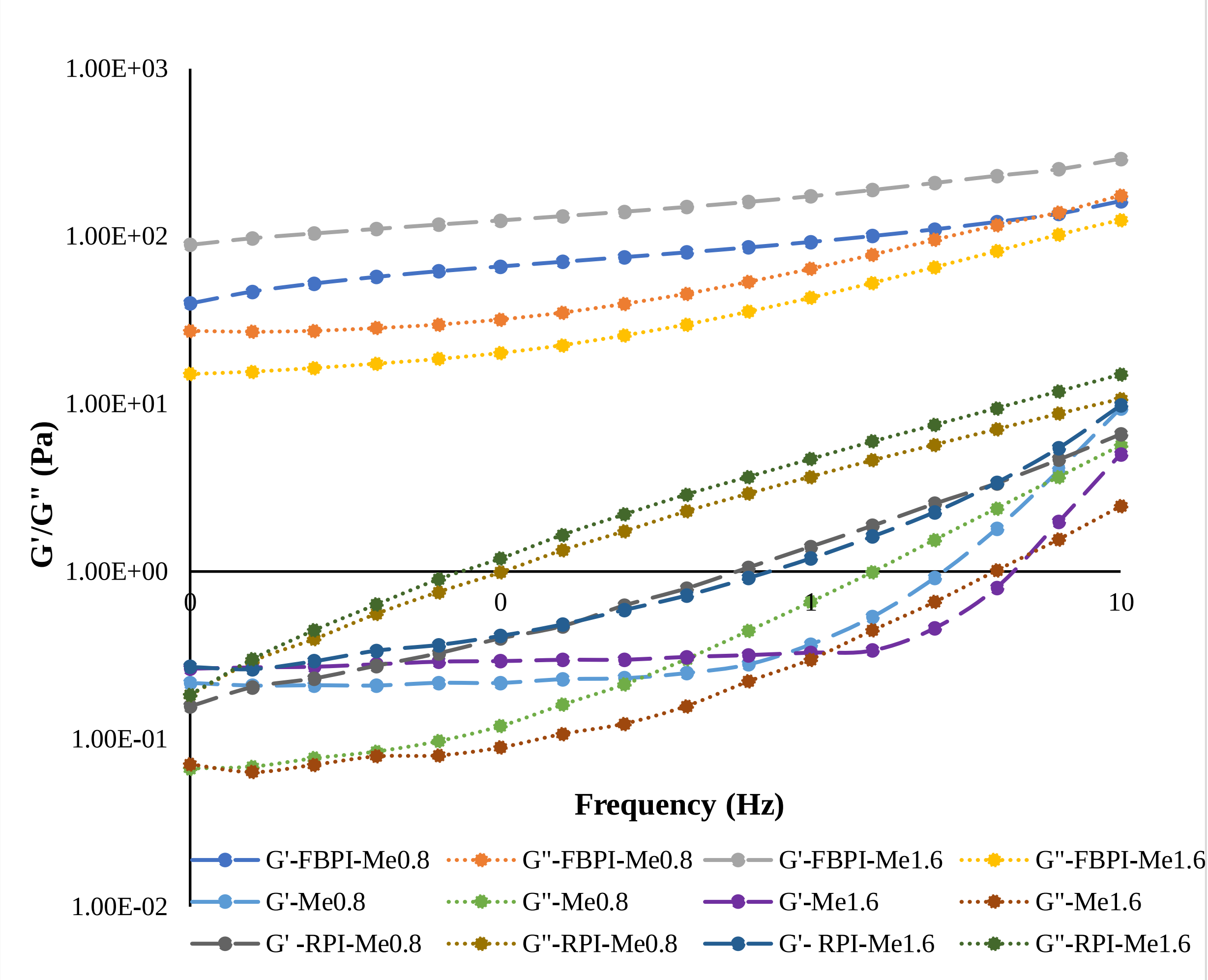


Figure 4.1 Storage modulus (G′) and loss modulus (G″) from frequency sweep tests of FBPI–Me and RPI–Me composite gels containing 0.8% and 1.6% (w/w) Me

4.3.1.2 Frequency sweep analysis of composite gels made from FBPI or RPI with modified starches

The frequency sweep analysis of plant-based protein gels formulated with modified starches, i.e., waxy maize starch (MS) at 2% and 5% (w/w), and potato starch (PS) at 5% and 10% (w/w), revealed predominantly elastic behaviour across all samples, as indicated by the G′ consistently exceeding the G″ throughout the entire frequency range (Figure 4.2 and 4.3). An increase in starch concentration led to higher G′ values for both starch types, reflecting improved gel strength and structural integrity. Among all formulations, the FBPI–PS 10% (w/w) gel exhibited the highest G′ values, suggesting the formation of a particularly robust and well-organized gel network. This enhanced gelation may be attributed to the starch-to-protein ratio which plays a crucial role in defining the final gel characteristics. Higher protein content in starch–protein composite systems has been associated with increased pasting temperatures and reduced gel firmness, likely due to changes in water distribution and limited starch granule swelling (Ribotta et al., 2007). In contrast, the superior performance of the FBPI–PS 10% (w/w) gel suggests that a more balanced ratio facilitated favourable starch–protein interactions, resulting in a synergistically reinforced viscoelastic network (Nilsson et al., 2023).

While the FBPI–MS gels also maintained G' > G", they displayed lower G' values compared to their PS counterparts, implying a comparatively weaker gel structure.

Conversely, control samples containing only modified starches (MS and PS) exhibited more pronounced frequency dependence and significantly lower G' and G" values, although G' remained greater than G" across the frequency range. This behaviour confirms that, under the given conditions, starch alone forms a weaker and less cross-linked gel network. These observations are consistent with previous research on potato starch -whey protein fibril mixed gels indicating that protein incorporation can strengthen starch gels by forming additional cross-links within the gel matrix, thereby leading to higher G' values (Chen et al., 2020). In addition, previous studies on MS have also shown that the addition of additional structuring agents, such as proteins, can develop a strong viscoelastic network, with higher G’ modulus and yield stress (Vu Dang et al., 2009).

On the other hand, the viscoelastic behaviour of RPI composite gels in combination with different concentrations of MS and PS showed that G' consistently exceeded G" in all tested samples, indicating a more elastic than viscous behaviour, a fundamental characteristic of gel-like structures (Vu Dang et al., 2009). However, gel strength varied among formulations. Specifically, RPI–MS at 5% (w/w) exhibited the highest G' values, suggesting the formation of a more rigid gel network. This enhanced gel strength may be attributed to the properties of cross-linked waxy maize starch (MS), which is nearly free of amylose and rich in amylopectin. Its ability to swell under heat and resist mechanical breakdown contributes to greater water-binding capacity and reinforces the protein matrix through the presence of swollen, deformable granules, ultimately supporting a more elastic, solid-like structure (Vu Dang et al., 2009).

Following this, RPI–PS at 10% (w/w) and RPI–MS at 2% (w/w) displayed moderate viscoelastic properties, indicating that higher concentrations of PS are required to induce a gel structure comparable to that achieved with lower concentrations of MS. In contrast, RPI-MP at 5% (w/w) did not form a gel, despite showing G' > G". The result indicates that the sample behaved more like a weak viscoelastic dispersion rather than a structured gel, even though the system exhibited some elasticity. This result may be attributed either to the PS concentration being below the threshold required to form a stable gel matrix and promote a continuous protein–starch network capable of resisting deformation, or to the potential destabilizing effect of large protein aggregates. Previous studies have shown that PS gels can be weaken upon the incorporation of protein isolates under neutral pH conditions (Chen et al., 2020). It has been suggested that large protein particles, such as fibril aggregates formed at higher concentrations may reduce the contact zones between swollen starch granules, thereby disrupting their packed structure and leading to lower viscoelastic moduli (Chen et al., 2020).

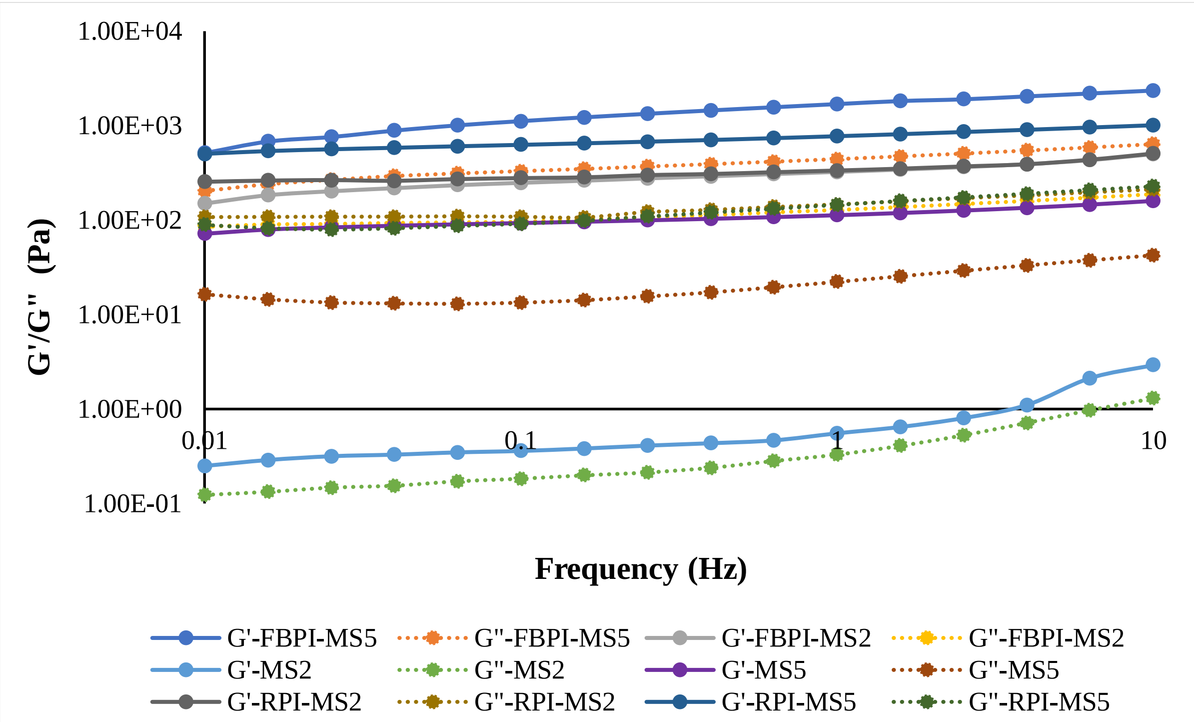


Figure 4.2 Storage modulus (G′) and loss modulus (G″) from frequency sweep tests of FBPI–MS and RPI–MS composite gels containing 2% and 5% (w/w) MS

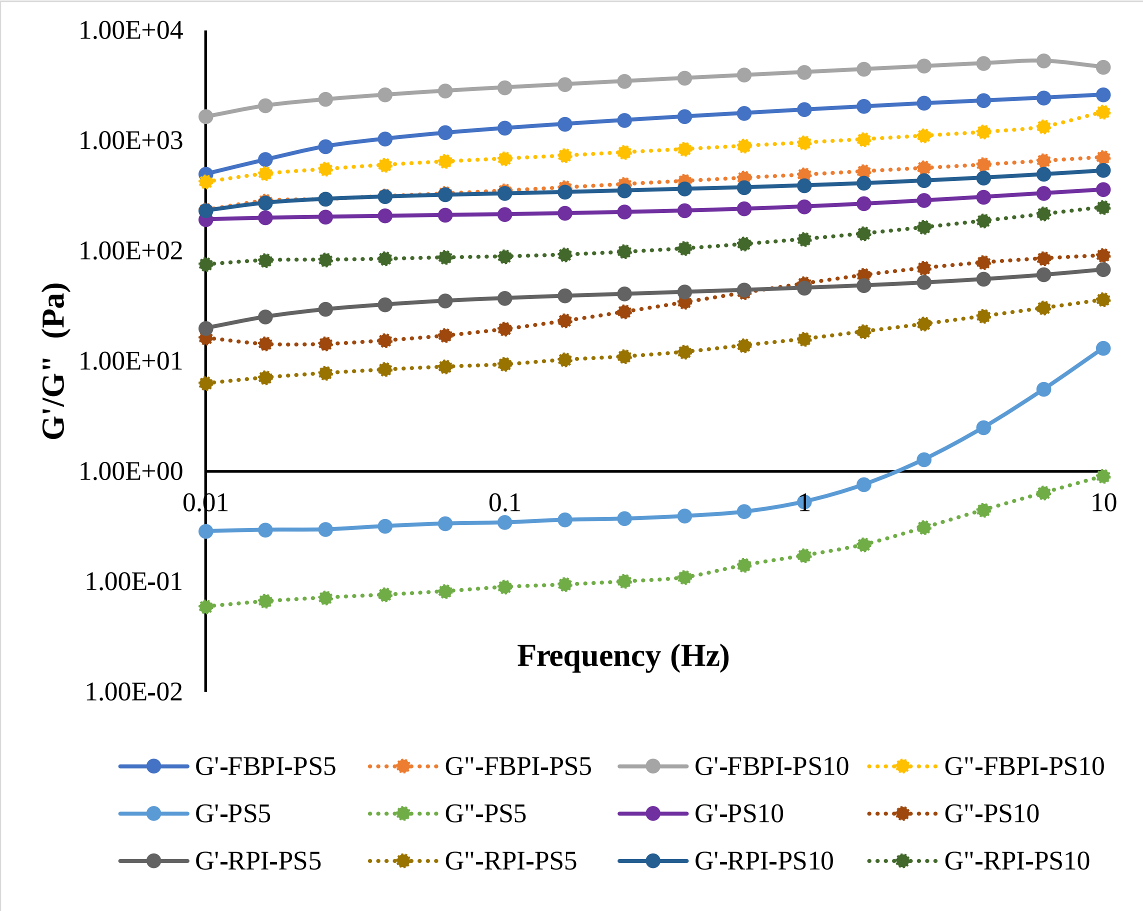


Figure 4.3 Storage modulus (G′) and loss modulus (G″) from frequency sweep tests of FBPI–PS and RPI–PS composite gels containing 5% and 10% (w/w) PS

To further assess the viscoelastic behaviour of the composite gels, the loss factor (tan δ = G″/G′) was analyzed at 1 Hz (Table 4.3). According to rheological principles, a tan δ value less than 1 indicates that the material exhibits predominantly elastic behaviour, characteristic of solid-like materials. Conversely, a tan δ value greater than 1 suggests that the material behaves more like a viscous liquid (Bakhsh et al., 2021).

In FBPI-Me composite samples, increasing the Me concentration from 0.8% to 1.6% significantly decreased tan δ from 0.70 to 0.25, indicating enhanced gel elasticity and the formation of a more solid-like network. This trend was not observed in RPI-based gels, where tan δ values were markedly higher, 2.74 (0.8% (w/w)) and 4.17 (1.6% (w/w)), both exceeding 1, suggesting that RPI–Me composite samples formed weak, highly viscous networks. The hydrocolloid-only Me samples also showed elevated tan δ values (>0.9), suggesting that Me alone does not form a strong gel, and its improved performance in the FBPI composites may be attributed to protein–hydrocolloid interactions.

In FBPI–MS composite gels, tan δ remained low (0.26 at 2% (w/w) and 0.48 at 5% (w/w)), confirming solid-like, elastic behaviour.

In RPI–MS composite samples, tan δ dropped from 0.54 at 2% (w/w) to 0.18 at 5% (w/w), indicating a concentration-dependent reinforcement of the gel matrix. Interestingly, the hydrocolloid-only MS control samples had similar or slightly higher tan δ values (~0.54 and 0.20), corroborating the idea that starch incorporation especially in RPI systems enhanced gel cohesion at higher MS levels.

FBPI–PS composite gels maintained low tan δ values at both concentrations (0.26 and 0.23), again reflecting a strong, elastic network. RPI–PS composite gels also showed decreasing tan δ with increasing starch concentration (0.54 at 5% (w/w) and 0.40 at 10% (w/w)), but remained less elastic than their FBPI–PS equivalents. The PS-only controls exhibited intermediate tan δ values (0.32–0.39), showing that PS alone has some inherent gel-forming ability, which is substantially improved when combined with FBPI.

Table 4.3 Tan δ values at 1 Hz for plant-based proteins-hydrocolloids composite gels

Hydrocolloid (type and concentration (% w/w)**)**	**Composite FBPI sample (tan (δ)**	**Composite RPI sample (tan (δ)**	**Hydrocolloid only sample (tan (δ)**
0.8% Me	0.70 ± 0.11^{a}	2.74 ± 1.14^{a}	1.82 ± 0.68^{a}
1.6% Me	0.25 ± 0.13^{b}	4.17 ± 1.50^{a}	0.98 ± 0.61^{a}
2% MS	0.26 ± 0.06^{b}	0.54 ± 0.40^{b}	0.54 ± 0.37^{a}
5% MS	0.48 ± 0.29^{b}	0.18 ± 0.01^{b}	0.20 ± 0.01^{a}
5% PS	0.26 ± 0.02^{b}	0.54 ± 0.29^{b}	0.32 ± 0.26^{a}
10% PS	0.23 ± 0.01^{b}	0.40 ± 0.11^{b}	0.39 ± 0.27^{a}

Values within the same column with different superscript letters indicate significant differences ($p < 0.05$).

4.3.1.3 Amplitude sweep analysis of FBPI-Me and RPI-Me composite gels

The observations of the structural integrity of the composite gels under increasing deformation**,** showed that all FBPI–Me composite samples exhibited predominantly elastic character, with G′ consistently higher than (G″ throughout the linear viscoelastic region (LVR, <10% strain). The gel containing 1.6% (w/w) Me and FBPI showed the highest G′ values, indicating a more rigid and well-structured gel network capable of withstanding higher strains before structural breakdown. Both composite gels, FBPI–Me at 0.8% and 1.6% (w/w), exhibited a progressive loss of structure with increasing strain, as evidenced by the decline in G′ values.

These results were consistent with previous rheological measurements on the instability of methylcellulose networks under the same deformation conditions (Yang et al., 2020)**.** Samples containing Me alone at both concentrations did not display a clear LVR, and G″ remained greater than G′ at all strains levels, indicating predominantly viscous behaviour rather than gel formation**.** These rheological characteristics are consistent with existing literature, which reports that methylcellulose at low concentrations functions as a viscosity modifier rather than a gel-forming agent, unless reinforced by other structuring components such as proteins or polysaccharides (Ji et al., 2022).

For both RPI-Me composite gels (0.8% and 1.6% (w/w) Me), the loss modulus (G") remained higher than the storage modulus (G') across the entire strain range, indicating that these formulations exhibit predominantly viscous behaviour.

At low strain values, both samples exhibited an initial plateau, suggesting a weak network capable of resisting deformation to some extent. However, as strain increased, G’ declined more sharply than G", particularly in RPI-Me 0.8% (w/w) sample, indicating early structural breakdown and greater susceptibility to deformation. In contrast, the RPI-Me at 1.6% (w/w) sample showed a more gradual decline in G' modulus, suggesting that increasing the methylcellulose concentration slightly enhanced the stability of the RPI network and delayed complete structural collapse under strain. However, the predominance of G" over G' throughout the measurement indicates that additional structuring agents or higher polymer concentrations may be necessary to enhance the gelation properties of RPI-Me systems.

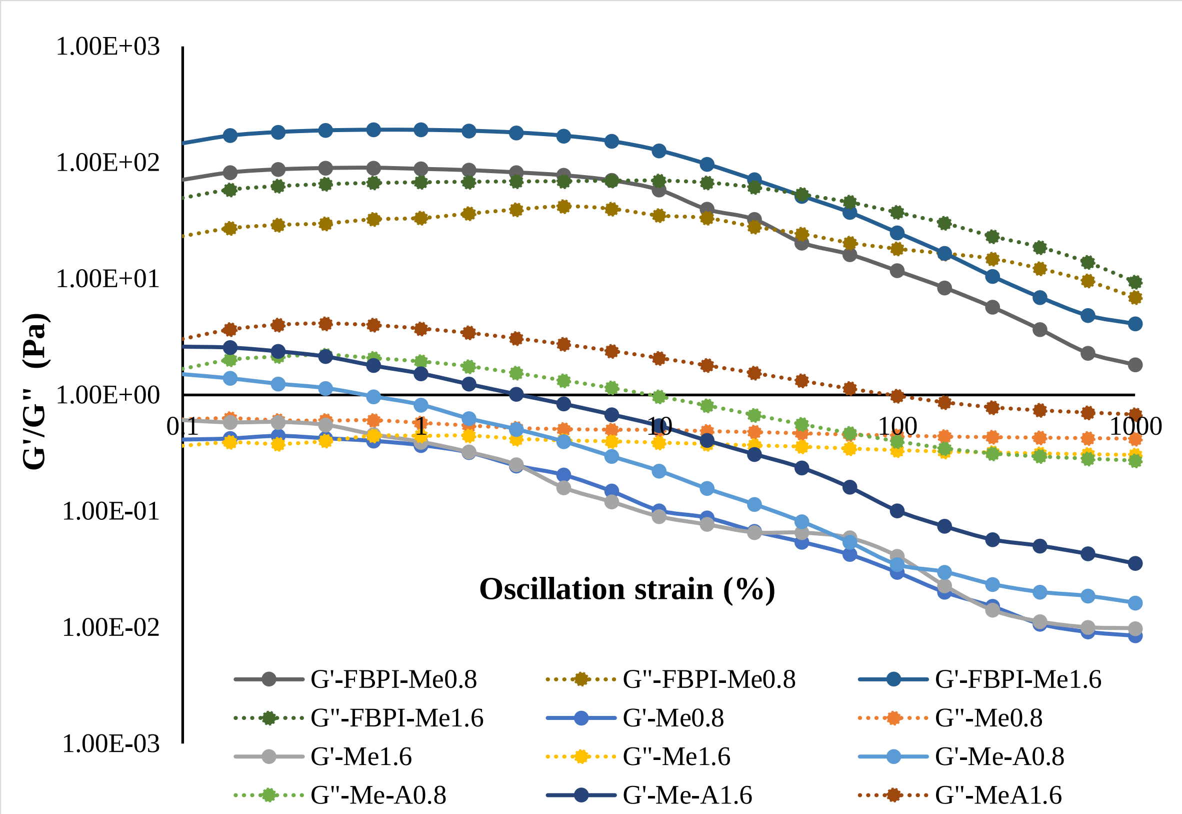


Figure 4.4 Storage modulus (G′) and loss modulus (G″) from amplitude sweep tests of FBPI–Me and RPI–Me composite gels containing 0.8% and 1.6% (w/w) Me

4.3.1.4 Amplitude sweep analysis of composite gels made from FBPI or RPI with modified starches

As shown in Figures 4.5 and 4.6, for the FBPI-based composite gels formulated with modified starches (MS and PS), G' was greater than the G" across all formulations within the LVR, indicating the formation of predominantly elastic gel networks. Among the tested samples, when the strain was 0.1–1%, the G′ modulus of the FBPI–PS 10% (w/w) composite gel remained stable with strain, and exhibited the largest LVR, followed by FBPI–MS 5% (w/w). This indicates that higher concentrations of both starches, PS and MS significantly enhanced the gel's resistance to deformation and promoted a more rigid and interconnected network. This behaviour is consistent with previous reports suggesting that increasing the concentration of PS enhances the gelation properties of globular proteins (Chen et al., 2020). This improvement is likely associated with a lower protein-to-starch ratio, which facilitates synergistic increases in the gel's G′. Such enhancements may result from mechanisms such as phase separation or protein network percolation, both of which contribute to reinforcing the gel structure (Chen et al., 2020).

Once the strain was greater than approximately 1.6% and exceeded the LVR, G′ decreased with increasing strain, while G″ initially rose slightly and then decreased abruptly. The FBPI-PS 5% (w/w) sample showed an earlier decline in G', followed by FBPI-MS 2% (w/w), indicating a less cohesive network, which is more susceptible to structural breakdown under applied stress. Nevertheless, increasing the MS concentration from 2% to 5% (w/w) resulted in higher G' values, reinforcing the notion that starch concentration is a critical factor in enhancing gel strength in starch–protein composite systems (Nilsson et al., 2023).

The analysis of the control gels, consisting solely of modified starches, MS and PS demonstrated weaker viscoelastic behaviour compared to the composite protein-starch systems. Across all samples, G′ was consistently higher than G″ throughout the low-strain region, indicating a dominant elastic character. However, the absolute values of both G′ and G″ were significantly lower than those observed in the protein-containing gels. Among the starch controls, the 10% (w/w) PS sample exhibited the highest G′ values, suggesting a modest ability to form a more cohesive and elastic structure compared to its lower concentration (5% (w/w)) or MS. The G′ values of the 5% and 2% (w/w) MS samples were notably lower, showing limited resistance to deformation. Furthermore, all control samples exhibited a gradual reduction in G′ as strain increased, which is a typical pattern indicating structural degradation and weak gel formation, behaviour commonly associated with modified starches (Wen et al., 2020). As supported by recent findings (Liu et al., 2021) once modified starches surpass their LVR, the integrity of their internal network begins to deteriorate irreversibly. This is likely due to a loss of intermolecular interactions and a decline in mechanical strength. In the transition region, the starch network enters a dynamic phase where structural junctions are both forming and breaking. With increasing deformation, the main starch chains start to unravel, while some of the longer branched chains may temporarily entangle under shear. Initially, the rate of network formation may balance the rate of network breakdown; however, as strain continues to rise, junction loss surpasses formation, leading to a decrease in chain entanglements and ultimately causing the gel network to collapse. This progression culminates at the yield point, where G′ and G″ intersect, signifying the transition from solid-like to fluid-like behaviour.

Interestingly, the results of RPI-starch composite gels under increasing strain revealed that formulations with MS exhibited superior structural integrity. Specifically, the RPI–MS 5% (w/w) gel showed the highest G′ values within the LVR, indicative of a robust and stable gel network. In contrast, RPI-PS at 10% (w/w) displayed slightly lower G' values, showing a less elastic gel compared to RPI-MS at 5% (w/w).

RPI-PS at 5% (w/w) showed the weakest structure, with G' values significantly lower than the other gels and a more pronounced drop at higher strains, confirming its inability to form a stable gel. In addition, its rapid decline in G' and the crossover of G' and G" at lower strains also indicated early structural breakdown, reinforcing that this concentration (PS5% (w/w)) was below the gelation threshold required for network formation. The differences in gel breakdown behaviour indicated that MS-based gels (RPI-MS5% (w/w), RPI-MS 2% (w/w)) were more resistant to deformation, whereas PS-based gels required a higher concentration (10% (w/w)) to achieve a comparable network strength.

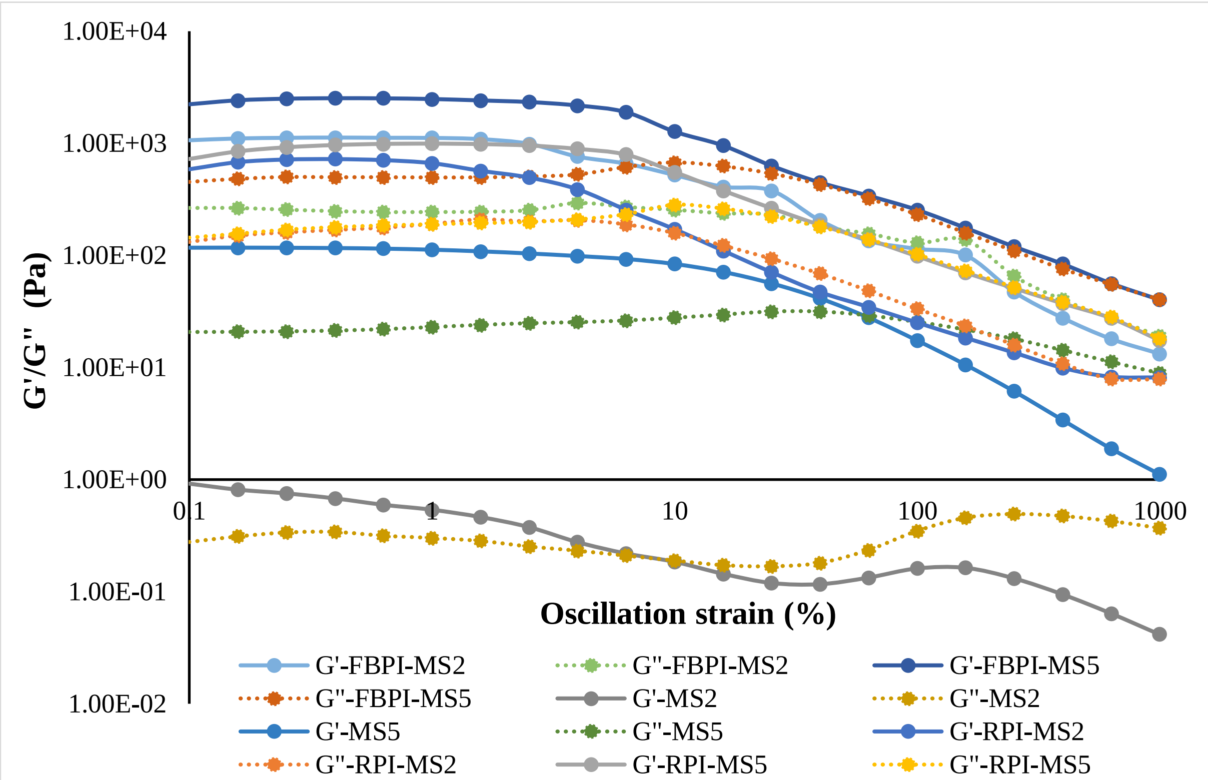


Figure 4.5 Storage modulus (G′) and loss modulus (G″) from amplitude sweep tests of FBPI–MS and RPI–MS composite gels containing 2% and 5% (w/w) MS

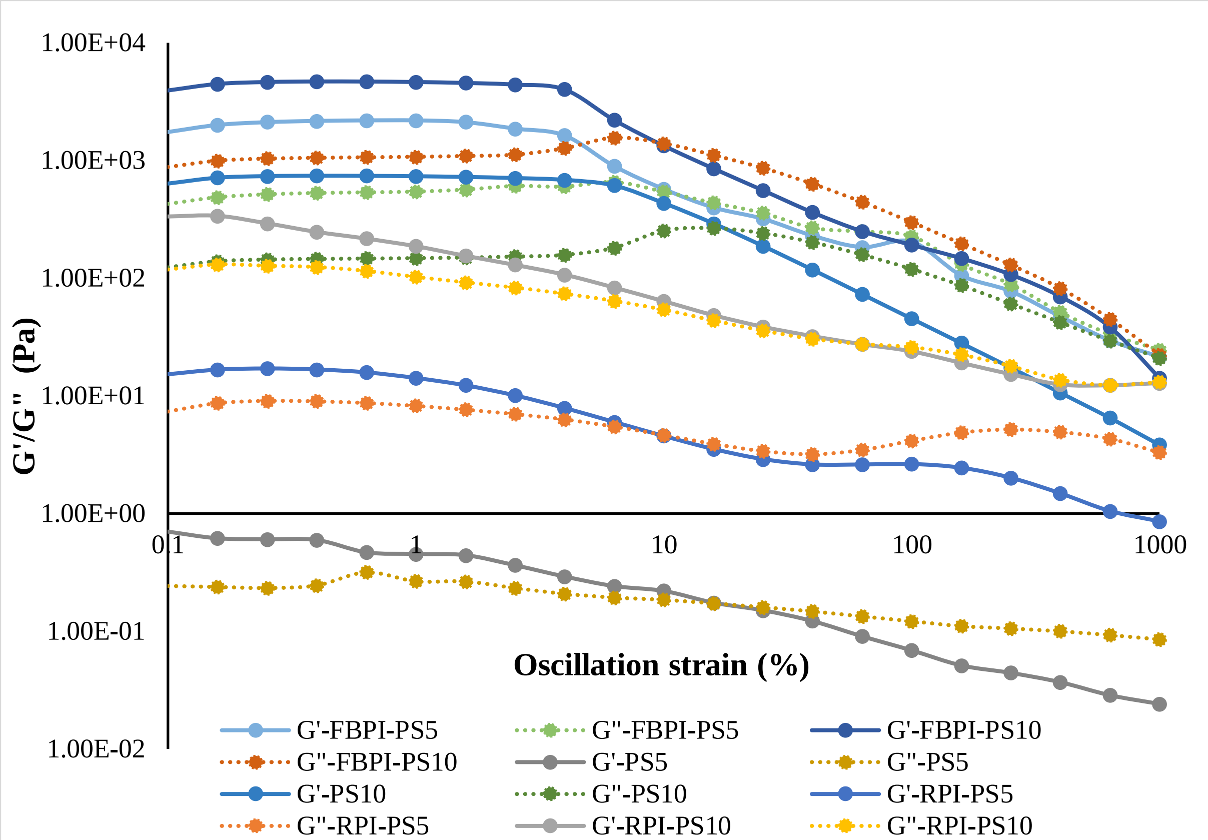


Figure 4.6 Storage modulus (G′) and loss modulus (G″) from amplitude sweep tests of FBPI–PS and RPI–PS composite gels containing 5% and 10% (w/w) PS

As shown in Table 4.4, the crossover point (G′ = G″) varied across the tested samples. FBPI containing 10% (w/w) PS exhibited the lowest crossover strain and formed a distinct statistical group, suggesting reduced elasticity and increased susceptibility to structural collapse under stress (Badjona et al., 2025). This may be attributed to excessive starch content leading to syneresis or phase separation, which compromises gel homogeneity and integrity (Tong et al., 2023). Furthermore, within the FBPI composite systems, increasing the concentration of modified starch did not enhance resistance to strain. On the contrary, higher starch levels appeared to lower the strain threshold at which the gel network failed, likely due to the formation of a denser yet more brittle structure with limited flexibility.

In contrast, FBPI–Me gels displayed improved mechanical resistance with increasing Me concentration, while RPI–Me samples exhibited extremely low crossover strains (<1%), highlighting their fragile structure and liquid-like behaviour. These results confirm that methylcellulose alone is insufficient to stabilize RPI gels under the test conditions.

Notably, RPI composite samples were significantly reinforced by the addition of modified starches. Both MS and PS improved structural resilience, with higher starch concentrations leading to increased crossover strain values. For instance, RPI–MS at 5% (w/w) showed a crossover strain of 36.89%, compared to 22.36% at 2% (w/w) MS, while RPI–PS at 10% (w/w) displayed the highest crossover strain (174.34%), although with a large standard deviation. These results indicated that RPI benefits more significantly than FBPI from starch addition, which is particularly important considering that RPI alone does not form self-supporting gels under the tested conditions. This enhancement in viscoelastic strength may be attributed to the complementary structural role provided by the starch matrix, which compensates for the limited intrinsic gelation ability of RPI. As reported by Gong et al. (2024), the inclusion of rice protein in starch systems leads to the formation of more compact and uniform gel structures, partly due to steric hindrance that restricts intermolecular cross-linking among starch molecules. Moreover, the hydrophilic nature of rice protein plays a role in reducing water migration and enhancing water retention within the gel.

Modified starches, particularly those with high amylopectin content, further contribute by forming continuous, entangled networks that physically embed and stabilize the dispersed protein phase, reinforcing overall structural integrity (Zhang et al., 2025). In protein systems like RPI, which are dominated by globular proteins with low cross-linking potential, the starch matrix acts as a mechanical scaffold, facilitating granule swelling, promoting water immobilization, and enhancing the gel's mechanical resistance (Gong et al., 2024). As Wu et al. (2023) further demonstrated, when rice protein content exceeds 12% (w/w), protein–protein interactions, primarily through disulphide bond formation begin to dominate over starch–protein interactions, contributing to increased gel strength and network density.

On the other hand, FBPI, which contains proteins with higher gelling ability such as vicilin and legumin, can form elastic networks even without starch support, and therefore the relative impact of starch addition is less pronounced in FBPI-based systems (Nilsson et al., 2023).

Finally, the modified starch control samples exhibited low strain resistance overall, except for MS at 5% (w/w) (63.10%) and PS at 5% (w/w) (53.15%), highlighting their limited ability to form stable gels without protein support. Only specific concentrations achieved a level of structural integrity comparable to protein-based systems. Meanwhile, regardless of concentration. Me consistently showed liquid-like behaviour, which can be partially attributed to the complex structural nature of commercial Me. Unlike random copolymers, methylcellulose consists of heterogeneously substituted polymer chains, comprising both heavily and poorly methylated regions (Desbrières et al., 2000) . This heterogeneity induces block-like behaviour along the backbone, which significantly affects gelation. At low polymer concentrations, gel formation is believed to arise from hydrophobic associations among the methylated zones, leading to the formation of a weak percolating network (Chevillard & Axelos, 1997).

Table 4.4 Tan δ at the crossover point for plant-based proteins-hydrocolloids composite gels

Hydrocolloid (type and concentration (w/w))	Composite FBPI sample (tan (δ)	Crossover Strain (%)	Composite RPI sample (tan (δ)	Crossover Strain (%)	Hydrocolloid only sample (tan (δ)	Crossover Strain (%)
0.8% Me	1.08 ± 0.09^{a}	22.48 ± 20.04^{a}	1.33 ± 0.48^{a}	0.46 ± 0.44^{a}	2.53 ± 1.92^{a}	0.10 ± 0.00^{a}
1.6% Me	1.05 ± 0.06^{a}	44.11 ± 21.93^{a}	1.50 ± 0.41^{a}	0.15 ± 0.07^{a}	1.13 ± 0.18^{ab}	1.11 ± 0.67^{a}
2% MS	1.21 ± 0.26^{a}	60.68 ± 28.41^{a}	1.13 ± 0.07^{a}	22.36 ± 27.22^{a}	1.03 0.17^{c}	11.09 ± 6.75^{b}
5% MS	1.11 ± 0.08^{a}	24.98 ± 14.90^{a}	0.96 ± 0.38^{b}	36.89 ± 42.06^{a}	1.10 0.16^{abc}	63.10 ± 0.00^{c}
5% PS	1.20 ± 0.18^{a}	32.19 ± 24.30^{a}	1.05 ± 0.08^{a}	14.01 ± 18.73^{a}	1.01 ± 0.02^{c}	53.15 ± 66.24^{c}
10% PS	1.21 ± 0.09^{a}	12.93 ± 3.38^{b}	1.08 ± 0.12^{a}	174.34 ± 304.75^{a}	1.10 ± 0.09^{bc}	20.49 ± 6.55^{b}

Values within the same column with different superscript letters indicate significant differences ($p < 0.05$).

4.3.2 Water holding capacity (WHC)

WHC is a crucial functional property that reflects the ability of a material to retain water under conditions where free water is limited (Mengozzi et al., 2024). It is influenced by both intrinsic factors, such as protein structure, conformation, amino acid composition, and hydrophobicity, and extrinsic factors, including pH, temperature, and ionic strength (Li et al., 2023). In protein-based gels, WHC is often associated with gel strength, as a well-structured gel matrix can effectively immobilize water within its network, whereas a weak or disrupted gel structure leads to water loss (Mengozzi et al., 2024). In starch-protein matrices, the interactions between starch granules and proteins further influence water retention and distribution within the system (Zhang et al., 2025).

The WHC results for FBPI and RPI composite samples varied significantly depending on the type and concentration of the added hydrocolloid (Table 4.5). Pure FBPI formed a self-supporting gel with a high WHC of 88.71%, confirming its intrinsic gel-forming ability. However, when 0.8% (w/w) Me was added, no gel was formed, and the sample remained in a weak or viscous state. This indicates that at lower concentrations, Me likely interfered with the protein–protein interactions required for gel network formation, possibly by binding available water or occupying critical sites for protein aggregation (Liu et al., 2022). Interestingly, at a higher concentration (1.6% (w/w) Me), a very weak gelation did occur, although the WHC dropped drastically to 45.56%. This suggests that while Me at 1.6%(w/w) still disrupted the protein matrix to some extent, it may have also contributed to network reinforcement by forming its own thermoreversible gel upon heating, a known property of methylcellulose (Peñaranda & Garrido, 2024). The transition from a non-gelling to a gelling state with increased Me concentration may be attributed to the gelation mechanism of Me itself, which forms a three-dimensional fibrillar network when heated. This secondary structure could have compensated for the disrupted FBPI network and helped trap water, albeit less effectively than the original FBPI-only gel (Niemczyk-Soczynska et al., 2019; Niemczyk-Soczynska et al., 2022).

In contrast, the addition of MS at 2% and 5% (w/w) to FBPI resulted in WHC values of 90.35% and 85.64 % respectively, indicating that MS, even at low concentrations, preserved high water retention in these systems. Similar effects were observed by Zhao et al. (2023), in which soy protein isolate (SPI) was combined with either common starch (CS) or waxy maize starch (WS). While SPI–CS complexes formed tighter, more ordered protein–starch networks and yielded stronger gels, the incorporation of WS, significantly improved the WHC and thermal stability of the gels. These results were attributed to the structural properties of WS, particularly its high amylopectin content, which forms continuous, entangled networks that physically embed the protein phase and retain water within the gel matrix.

Conversely, the addition of PS at 5% and 10% (w/w) reduced the WHC, particularly at the higher concentration. This reduction may be attributed to certain physical or chemical modifications such as heat-moisture treatment (HMT) or crosslinking can reduce starch swelling capacity and water absorption by altering molecular structure and hydrogen bonding patterns. These treatments often result in a more compact granular structure with decreased porosity, limiting water uptake and expansion during gelatinization (Rohima et al., 2025).

Regarding RPI composite gels, MS at 2% and 5% (w/w) successfully induced gelation, with WHC values of 66.33% and 93.66% respectively, demonstrating that higher starch content contributed to stronger water retention and gel formation. In contrast, PS at 5% (w/w) did not induce gelation, and although a weak gel was formed at 10% (w/w) PS, the WHC remained relatively low at 63.48%. This outcome can be attributed to the increased starch concentration surpassing a critical threshold required to establish a minimal three-dimensional network. Although cross-linked potato starches exhibit reduced swelling and limited integration into protein matrices due to their restricted granular expansion (Rohima et al., 2025), a higher concentration of starch enhances the solid content, leading to increased granule interactions and structural crowding. These effects promote filler-like behaviour, greater physical entanglement, and the formation of weak junction zones upon heating and cooling (Jiang et al., 2022). As a result, even without significant gelatinization or protein-starch synergy, the elevated solid density at 10% (w/w) PS allowed for the development of a weak yet measurable gel, in contrast to the liquid-like behaviour observed at 5% (w/w).

Table 4.5 Water holding capacity (WHC, %) of FBPI and RPI gels containing Me (0.8 and 1.6 % (w/w)), MS (2 and 5% (w/w)) and PS (5 and 10% (w/w))

Hydrocolloid (type and concentration (w/w)**)**	**FBPI composite gel**	**RPI composite gel**

0.8% Me	No gel	No gel
1.6% Me	45.56 ± 0.07^{a}	No gel
2% MS	90.35 ± 0.18^{f}	66.33 ± 0.41^{a}
5% MS	85.64 ± 0.27^{d}	93.66 ± 0.29^{b}
5% PS	73.68 ± 0.20^{c}	No gel
10% PS	65.62 ± 0.07^{b}	63.48 ± 0.23^{c}
Pure plant protein	88.71 ± 0.17^{e}	No gel

Values within the same column with different superscript letters indicate significant differences ($p < 0.05$).

4.3.3 Texture analysis

The texture analysis, shown in Table 4.6, demonstrated that both the type and concentration of hydrocolloids had a marked impact on the mechanical properties of the gels. Among the FBPI-based composite samples, the gel containing PS at 10% (w/w) exhibited the highest hardness, followed by those with 5% (w/w) PS and 5% (w/w) MS. Although, the 10% (w/w) PS gel was the firmest, it showed slightly lower cohesiveness compared to the 5% MS gel, which achieved a more balanced texture overall. The 5% (w/w) MS formulation combined firmness with good cohesiveness and springiness, suggesting the formation of a well-integrated protein–starch network. This observation aligns with the rheological data, indicating strong resistance to deformation and a broad LVR, WHC. In contrast, the 10% (w/w) PS gel, although structurally dense under SEM, likely formed a more brittle and compact matrix, potentially prone to syneresis and lower flexibility.

FBPI-based composite samples containing Me at 1.6% (w/w) showed noticeably lower values for all textural parameters. The gel was softer, less elastic, and less cohesive, indicating that Me at this concentration disrupted protein network formation. This finding aligns with earlier observations of low WHC and microstructural images showing phase-separated, hollow structures. The FBPI sample with 2% (w/w) MS also resulted in moderate hardness and cohesiveness, suggesting a partially developed network, which was less robust than that of the 5% (w/w) MS sample.

For RPI-based composite gels, the 5% (w/w) MS sample consistently showed superior textural properties. The highest hardness, springiness, and cohesiveness within this protein system. This reflects effective starch–protein interactions and is supported by WHC results and a compact, uniform microstructure observed in SEM analysis. In contrast, the 10% (w/w) PS formulation, although able to form a gel, displayed lower values across all textural parameters. This result indicated a weaker and less cohesive network, which was also evident in its less integrated and more irregular microstructure. The RPI–2% (w/w) MS formulation showed intermediate texture values, indicating limited structuring capacity at the lower starch concentration.

Table 4.6 Textural parameters (hardness, springiness, and cohesiveness) of FBPI and RPI gels containing Me (0.8 and 1.6 % (w/w), MS (2 and 5% (w/w) and PS (5 and 10% (w/w)

Hydrocolloid (type and concentration)	Hardness (N)		Springiness (mm)		Cohesiveness	
	FBPI composite gel	RPI composite gel	FBPI composite gel	RPI composite gel	FBPI composite gel	RPI composite gel
0.8% Me	No gel	No gel	No gel	No gel	No gel	No gel
1.6% Me	150.54 ± 10.93^{c}	No gel	0.18 ± 0.01^{d}	No gel	0.26 ± 0.02^{c}	No gel
2% MS	468.19 ± 83.87^{b}	125.82 ± 37.74^{c}	0.29 ± 0.06^{c}	0.38 ± 0.04^{b}	0.62 ± 0.05^{a}	0.26 ± 0.01^{b}
5% MS	624.34 ± 117.78^{b}	619.32 ± 96.71^{a}	0.83 ± 0.02^{a}	0.57 ± 0.11^{a}	0.63 ± 0.04^{a}	0.44 ± 0.04^{a}
5% PS	888.48 ± 123.60^{d}	No gel	0.39 ± 0.06^{c}	No gel	0.33 ± 0.01^{c}	No gel
10% PS	2025.42 ± 696.32^{a}	270.62 ± 20.05^{b}	0.91 ± 0.06^{a}	0.21 ± 0.06^{c}	0.57 ± 0.05^{a}	0.23 ± 0.04^{b}
Pure Plant protein	166.98 ± 26.99^{c}	No gel	0.72 ± 0.14^{b}	No gel	0.43 ± 0.05^{b}	No gel

Values within the same column with different superscript letters indicate significant differences ($p < 0.05$).

4.3.4 Microstructure analysis

Scanning electron microscopy (SEM) is a widely used technique for examining the surface morphology and microstructural characteristics of food gels. Figures 4.7, 4.8 and 4.9 presents the SEM micrographs of FBPI and RPI composite gels containing modified starches (MS and

PS) and methylcellulose (Me) at 100× (left) and 300× (right) magnifications, along with their corresponding digital photographs of the original wet samples

The Me control samples at both 0.8% and 1.6% (w/w) did not undergo gelation, remaining as viscous liquids. Their freeze-dried SEM micrographs displayed smooth, uniform surfaces without any evidence of a continuous gel network, reinforcing the conclusion that Me alone, at these concentrations, was unable to form a stable gel. Similarly, RPI composite samples containing 0.8% and 1.6% (w/w) Me, did not form gels either. While their SEM images showed porous and loosely structured matrices, this morphology likely resulted from freeze-drying of non-gelled viscous solutions rather than genuine network formation.

These observations are consistent with previous studies reported by Chevillard and Axelos (1997), who reported that low levels of methylcellulose often do not form stable gels in plant-based systems. At low concentrations, the polymer chains may briefly interact through their hydrophobic parts when heated, but without enough entanglement or strong interactions with proteins, the mixture can separate into different phases. This results in cloudy, unstable systems that collapse and release water, rather than forming proper gels.

In contrast, FBPI–Me gels at 1.6% (w/w) formed self-supporting structures with dense, though less ordered, networks and moderate aggregation. SEM images suggesting that FBPI acted as the continuous gel phase, while Me remained partially segregated. This observation aligns with Johansson et al. (2024), who reported that FBPI forms fine-stranded, cohesive gel networks at pH 7, supporting its role as a continuous phase under neutral conditions. Moreover, the structural arrangement is consistent with the intermolecular exclusion effect described by Li et al. (2022), wherein Me at concentrations above 1% tends to separate from globular proteins such as caseins at the molecular level, which promotes protein–protein interactions and the formation of a gel network.

The control samples containing only MS, remained as viscous liquids and did not form self-supporting gels under the tested conditions. The SEM images of these samples revealed loose and porous microstructures, reflecting the effects of dehydration on concentrated MS solutions. These observations highlight the need for additional structuring agents, such as proteins, to facilitate network formation. This aligns with previous studies, where protein–starch combinations formed more stable gels through interpenetrating networks with starch during gelatinization, thereby promoting gel development (Gong et al., 2024).

Composite FBPI–MS gels at 2% and 5% (w/w) formed well-integrated networks with interconnected pores. The FBPI–MS at 5% (w/w) gel exhibited a more porous yet cohesive microstructure, featuring fibrous characteristics and well-defined pore network, typical of a strong gel matrix. These observations are align with (Dobson et al., 2022) who investigated the formation of cold-set dough using MS chemically modified via cross-linking and pea protein isolate. In their study, starch–protein gels were prepared with total solids at 47% (w/w), where protein constituted 30–70% of the dry fraction, and starch made up the remainder. Their microscopy analysis showed that increasing the starch concentration improved network formation by acting as a filler that reinforced protein–protein interactions and enhanced the mechanical properties of the matrix. This particle-filled network model supports the observations in the present study, where higher concentrations of MS appeared to enhance the integration of FBPI into a cohesive gel matrix. The structural similarity suggests that filler-like behaviour of MS, combined with protein interactions, plays a crucial role in developing gel integrity.

Similarly, RPI–MS composite gels at both concentrations displayed network formation, though showed a more compact, less porous, and uniform structure compared to FBPI-MS gels. Notably, the RPI–MS at 5% (w/w) gel displayed an interconnected structure with uniformly distributed pores, suggesting enhanced protein–starch interactions at higher starch content.

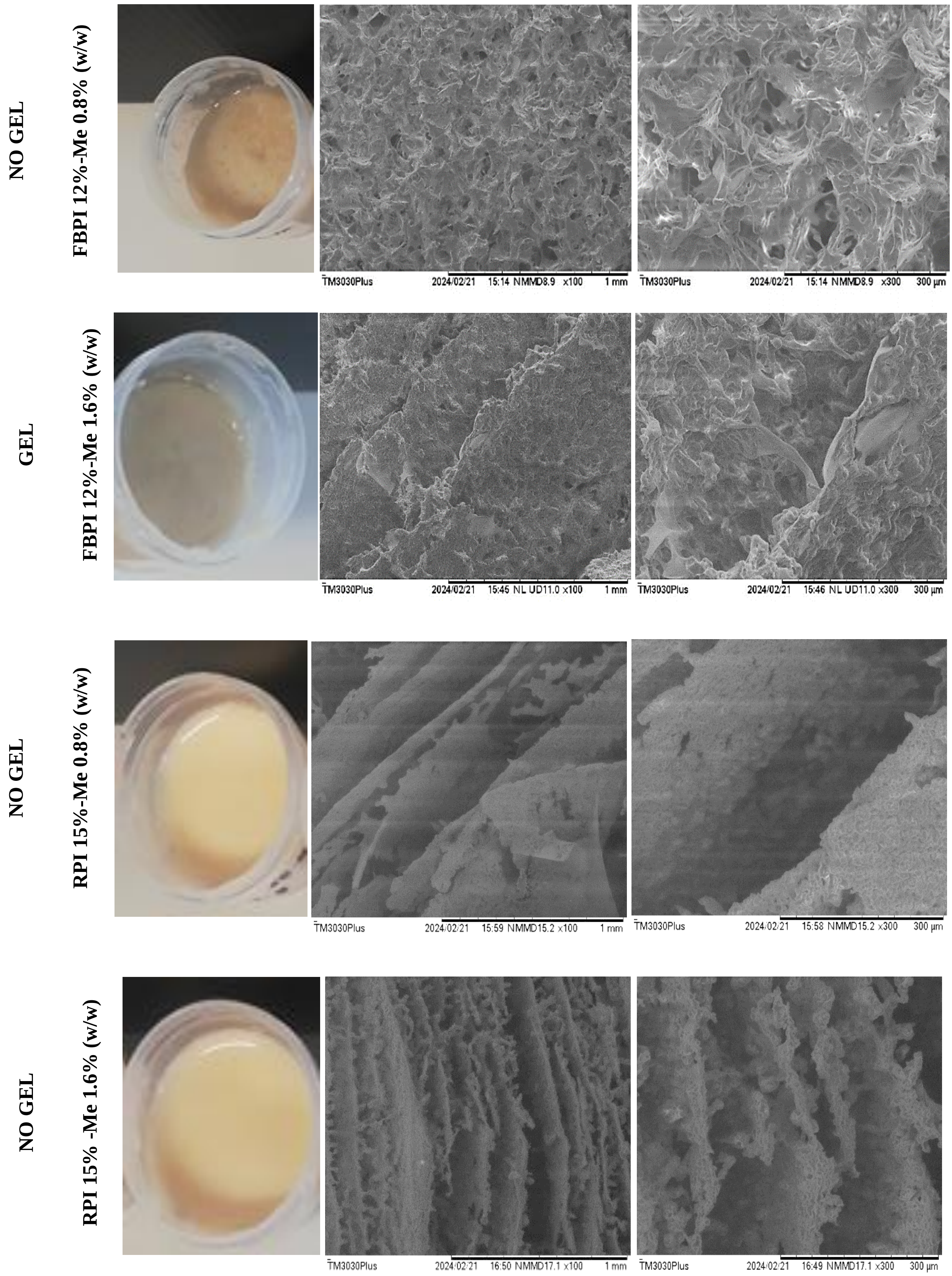
This structural improvement was consistent with its viscoelastic behaviour observed at different strains, as shown in Figure 4.7, and aligns with findings by Wu et al. (2023) who observed that incorporating rice protein (RP) into retrograded rice starch (RS) gels reduced pore size and increased microstructural compactness. They attributed this to the protein’s ability to inhibit molecular rearrangement during retrogradation and enhance water-holding capacity, resulting in a denser, more cohesive matrix. In this case, a similar mechanism may apply, as the higher starch content combined with the cross-linked nature of MS likely enhanced hydrogen bonding and steric effects, thereby stabilizing the gel structure.

Similar to MS control samples, the PS control sample at 5% (w/w) did not undergo gelation, remaining as viscous liquid and its freeze-dried SEM micrographs displayed a similar loosely structured matrices, typical of a non-gelled viscous solution.

Conversely, PS control sample at 10% (w/w) control exhibited a denser, and continuous network with elongated fibrous structures visible under higher magnifications. This structural improvement is likely a result of increased starch concentration, which elevates the solid content and facilitates granule–granule interactions, promoting the formation of weak gel-like networks despite incomplete gelatinization (Gong et al., 2024; Singh et al., 2007). However, the compact architecture and reduced porosity may also be indicative of phase separation or limited water retention, which could predispose the gel to syneresis upon storage. This phenomenon has been associated with amylose crystallization and retrogradation in high-concentration potato starch gels, as reported by Tong et al. (2023) and Singh et al. (2007).

Incorporating PS into FBPI samples resulted in the formation of stable, self-supporting gels as shown in Figure 4.9. FBPI–PS at 5% (w/w) gel showed a continuous, porous network with interconnected cavities, while the 10% (w/w) gel formed a denser and firmer matrix with thickened walls and smaller pores, reinforcing the theory that increasing starch concentration enhances starch-protein interactions, leading to improved network integrity and gel firmness structure (Dobson et al., 2022; Gong et al., 2024)

In contrast, PS incorporation in RPI samples produced contrasting gelation outcomes. The RPI–PS at 5% (w/w) sample failed to form a self-supporting gel, as seen in its glossy, fluid appearance (Figure 4.9). SEM images confirmed a disordered, dispersed microstructure with undefined pores and poor connectivity (Scott & Awika, 2023). However, the RPI–PS at 10% (w/w) sample achieved gelation and displayed a more continuous, fibrous network with visible cavities, indicating enhanced starch-protein entanglement and greater water immobilization. The structural shift observed between 5% and 10% (w/w) PS in RPI-based samples indicated a concentration-dependent reinforcement effect, wherein increased starch content improves network formation not only through physical entanglement but also by enhancing hydrogen bonding among starch and protein molecules (Obadi & Xu, 2021). The abundance of hydroxyl groups in amylose and amylopectin facilitates extensive hydrogen bonding, which is critical in forming junction zones - aggregates that, especially in the presence of hydrophilic, globular proteins like those in RPI, support network cohesion through retrogradation-driven mechanisms (Scott & Awika, 2023). Additionally, PS is a cross-linked potato starch, produced through phosphorylation, which introduces phosphate cross-links between amylopectin chains (Incorporated, 2024). These covalent bridges reinforce the structural stability of the starch network by limiting granule swelling and increasing its mechanical strength, further supporting the formation of a cohesive gel matrix under thermal processing conditions (Punia et al., 2024).


NO GEL
FBPI 12%-Me 0.8% (w/w)
TM3030Plus 2024/02/21 15:14 NMMD8.9 x100 1 mm
TM3030Plus 2024/02/21 15:14 NMMD8.9 x300 300 μm
GEL
FBPI 12%-Me 1.6% (w/w)
TM3030Plus 2024/02/21 15:45 NL UD11.0 x100 1 mm
TM3030Plus 2024/02/21 15:46 NL UD11.0 x300 300 μm
NO GEL
RPI 15%-Me 0.8% (w/w)
TM3030Plus 2024/02/21 15:59 NMMD15.2 x100 1 mm
TM3030Plus 2024/02/21 15:58 NMMD15.2 x300 300 μm
NO GEL
RPI 15% -Me 1.6% (w/w)
TM3030Plus 2024/02/21 16:50 NMMD17.1 x100 1 mm
TM3030Plus 2024/02/21 16:49 NMMD17.1 x300 300 μm

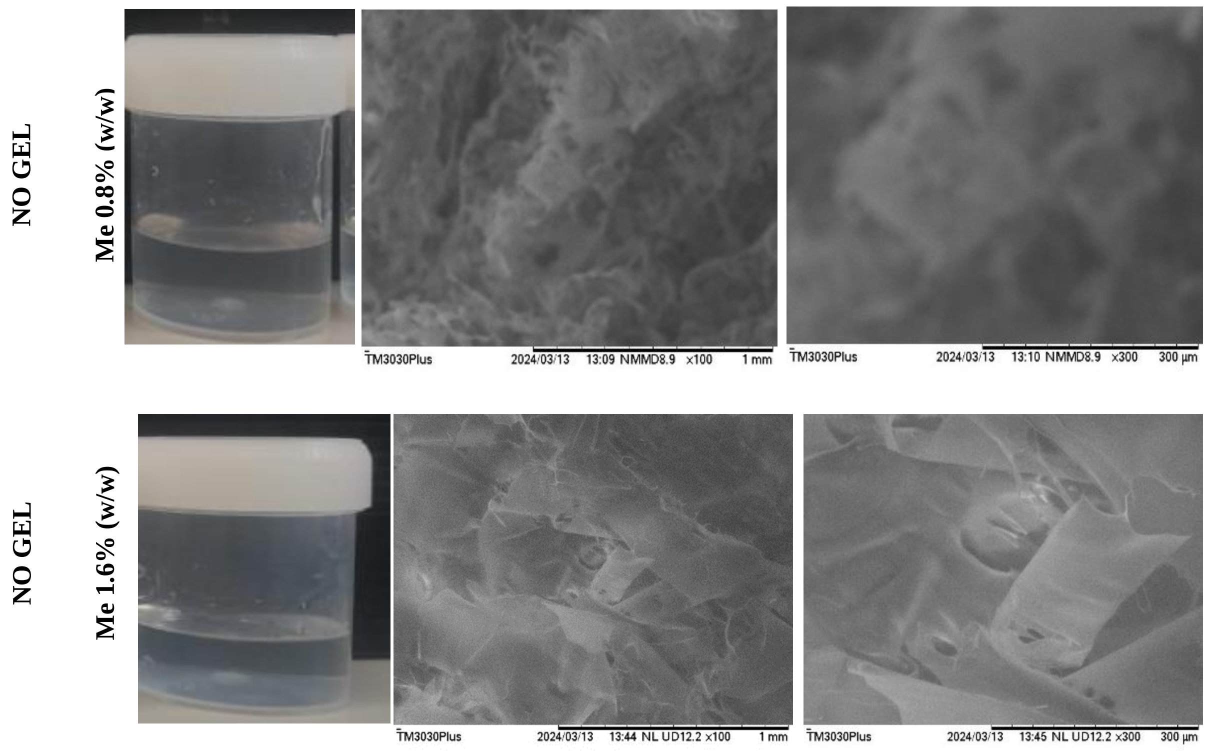
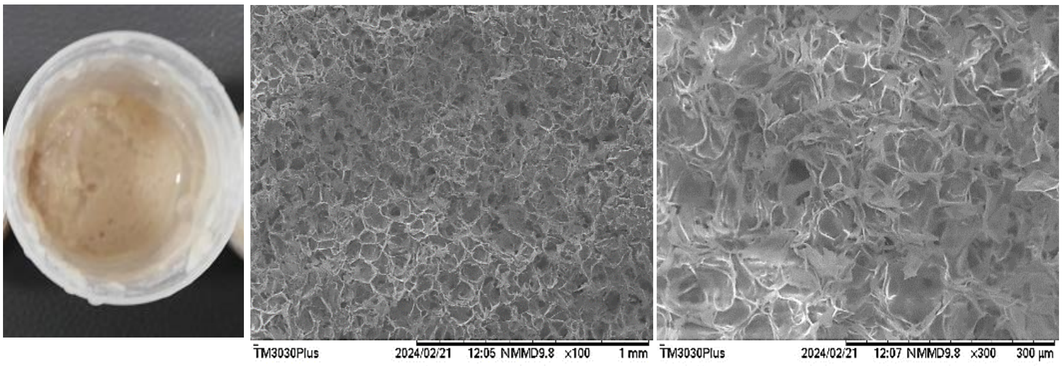


Figure 4.7 Visual appearance and microstructural (SEM) analysis (100× with a 1-mm scale bar, and 300× with a 300-μm scale bar) of FBPI and RPI samples containing Me (0.8 and 1.6 % (w/w), including pure Me.

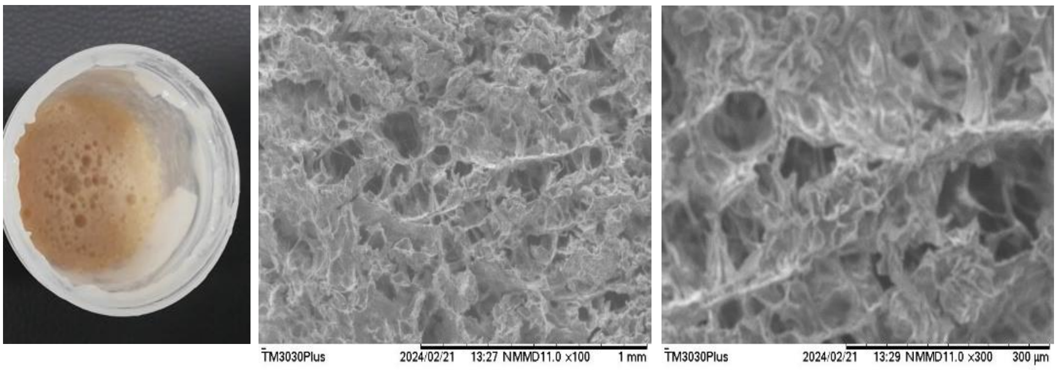
GEL

FBPI 12% MS 2% (w/w)



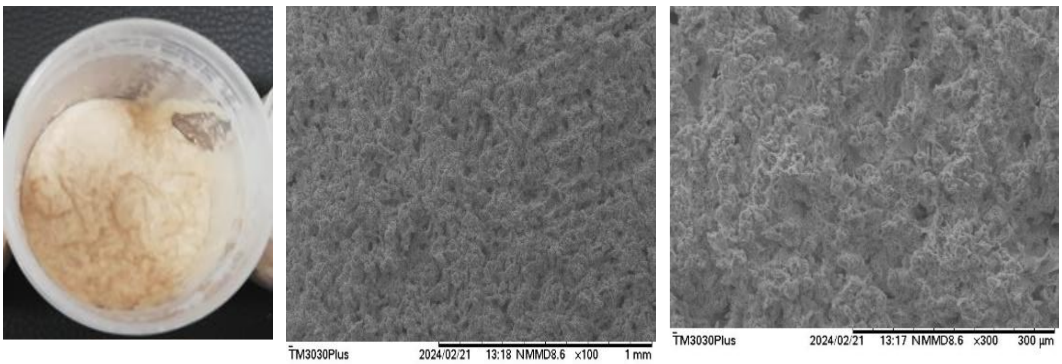
GEL

FBPI 12% -MS 5% (w/w)



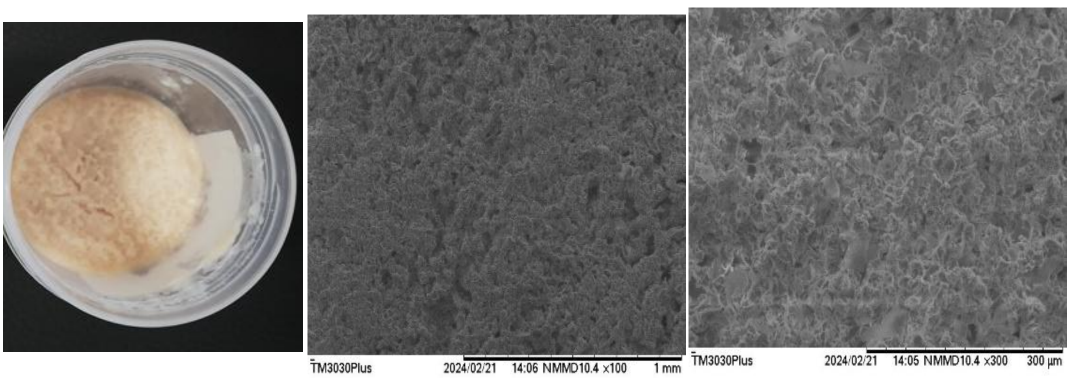
GEL

RPI 15% -MS 2% (w/w)



GEL

RPI 15% -MS 5% (w/w)



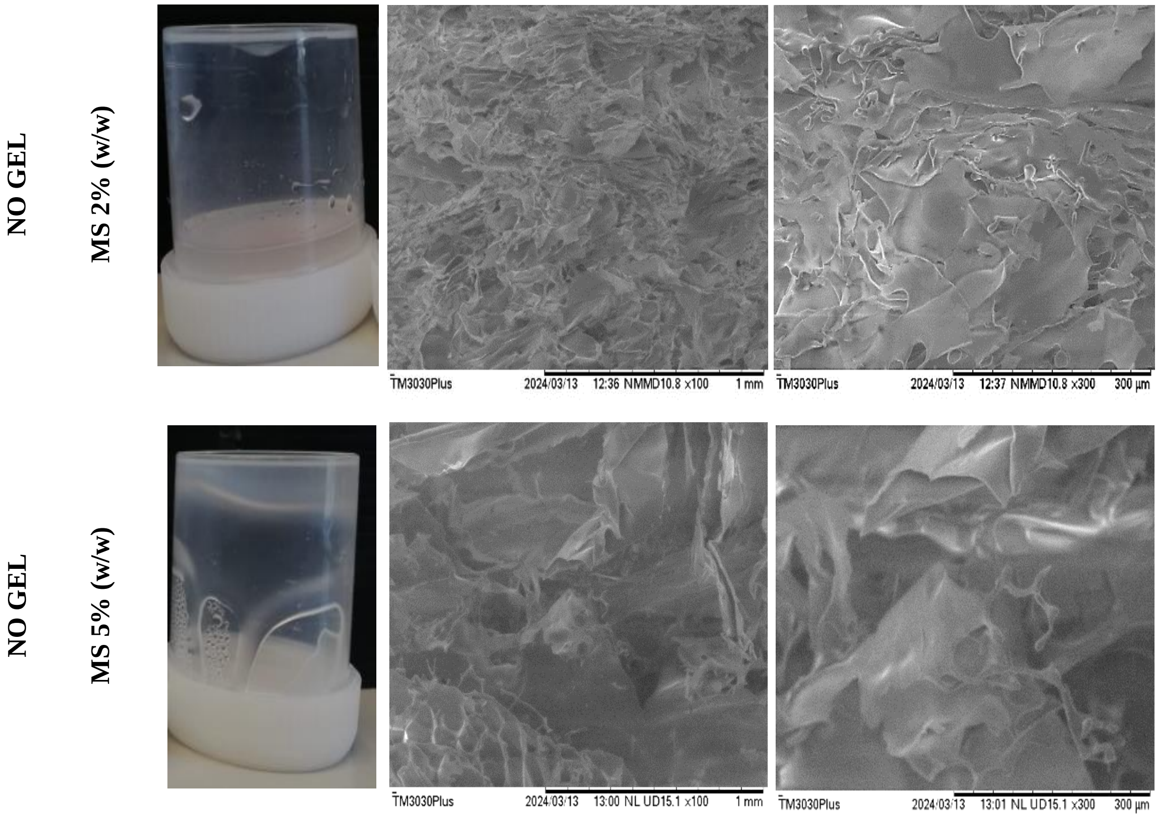
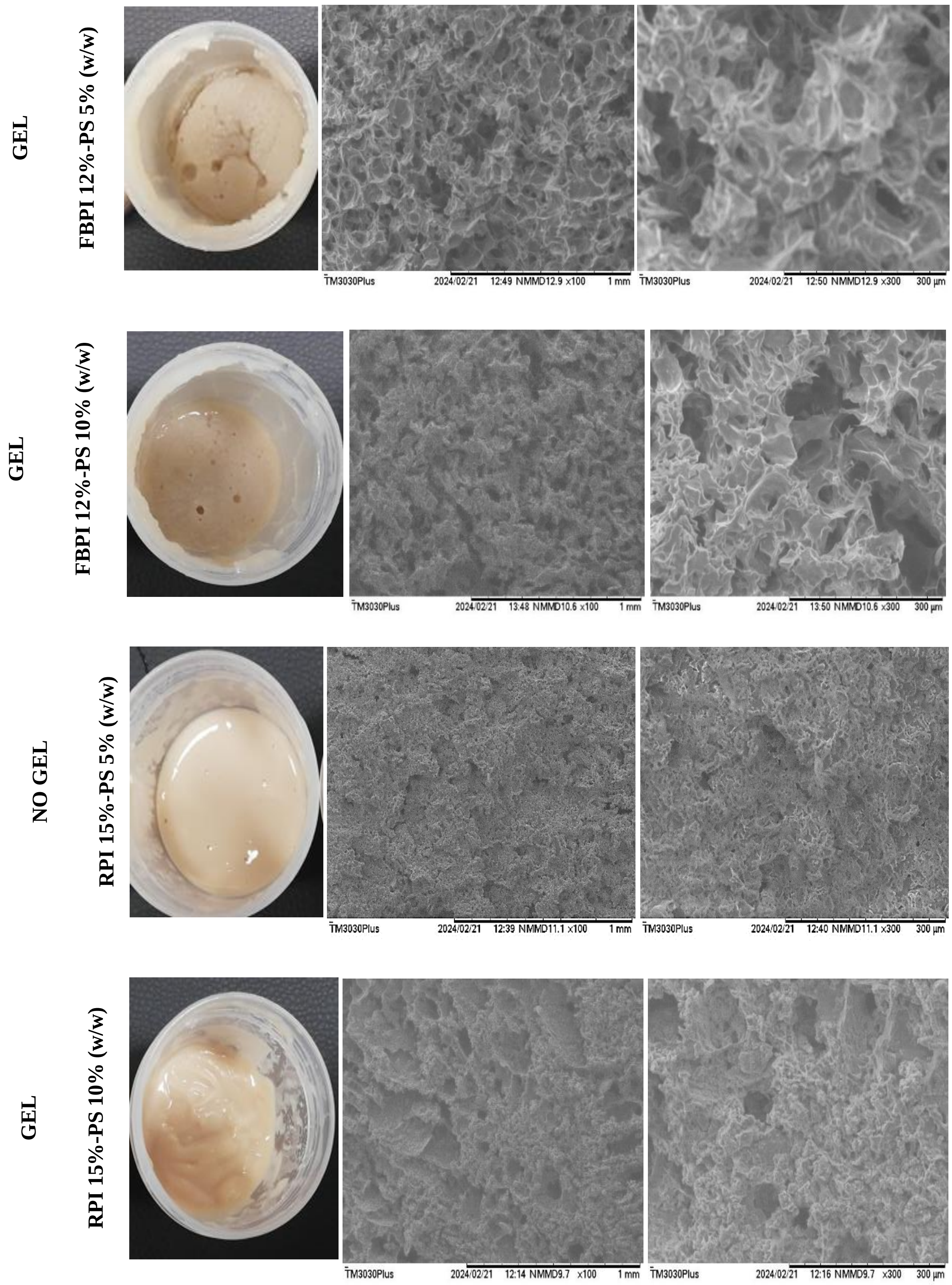


Figure 4.8 Visual appearance and microstructural (SEM) analysis (100× with a 1-mm scale bar, and 300× with a 300-µm scale bar) of the FBPI and RPI samples containing MS (2 and 5% (w/w), including pure MS.


GEL
FBPI 12%-PS 5% (w/w)
TM3030Plus 2024/02/21 12:49 NMMD12.9 x100 1 mm
TM3030Plus 2024/02/21 12:50 NMMD12.9 x300 300 µm
GEL
FBPI 12%-PS 10% (w/w)
TM3030Plus 2024/02/21 13:48 NMMD10.6 x100 1 mm
TM3030Plus 2024/02/21 13:50 NMMD10.6 x300 300 µm
NO GEL
RPI 15%-PS 5% (w/w)
TM3030Plus 2024/02/21 12:39 NMMD11.1 x100 1 mm
TM3030Plus 2024/02/21 12:40 NMMD11.1 x300 300 µm
GEL
RPI 15%-PS 10% (w/w)
TM3030Plus 2024/02/21 12:14 NMMD9.7 x100 1 mm
TM3030Plus 2024/02/21 12:16 NMMD9.7 x300 300 µm

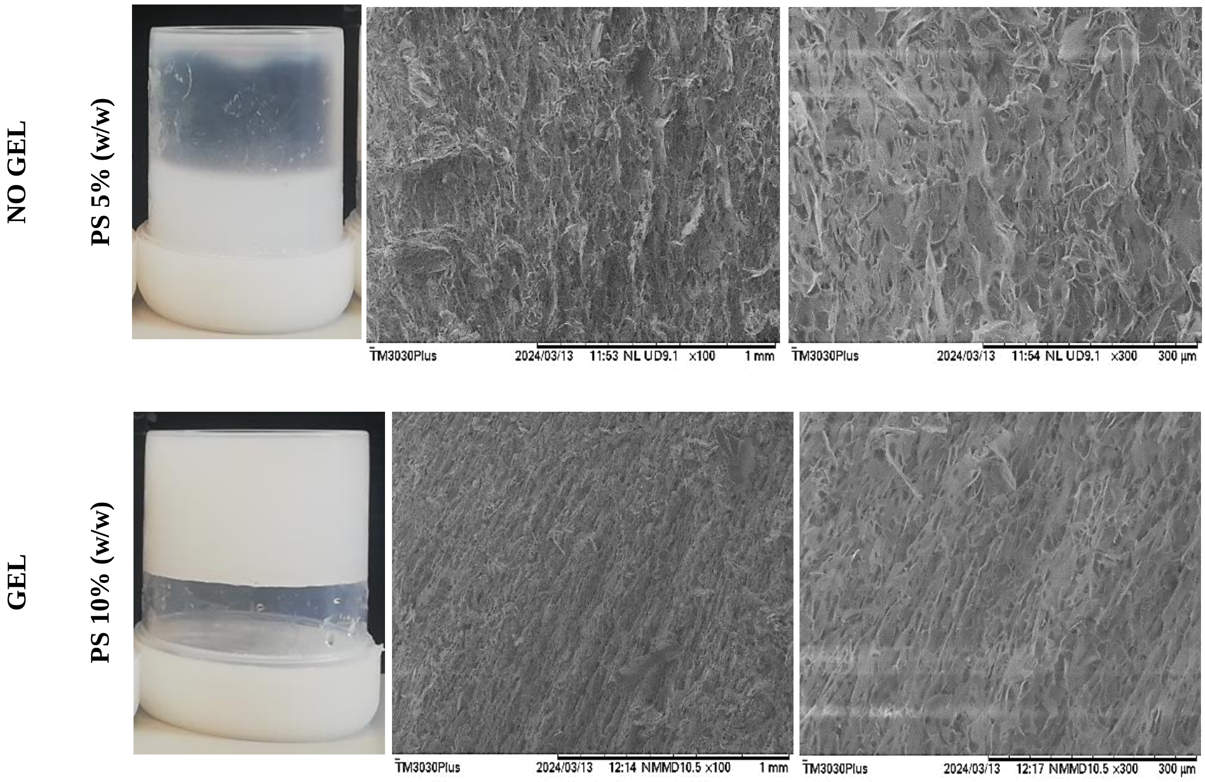


Figure 4.9 Visual appearance and microstructural (SEM) analysis (100× with a 1-mm scale bar, and 300× with a 300-μm scale bar) of FBPI and RPI samples containing PS (5 and 10% (w/w), including pure PS.

4.4 Conclusions

The heat-induced gelation properties of FBPI at 12% (w/w) and RPI at 15% (w/w) were investigated in combination with Me, MS and, PS at varying concentrations. FBPI-based systems consistently formed self-supporting gels, except FBPI 12%-Me 0.8%, while RPI alone did not exhibit gelation under the tested conditions. However, the incorporation of functional ingredients, particularly 5% MS and 10% PS (w/w), significantly improved the gelation and structural properties of RPI based samples.

Rheological analysis revealed that increasing Me concentration enhanced gel strength in FBPI systems, while starches contributed more effectively to network formation in RPI systems. WHC was highest in FBPI alone and in RPI gels with 5% MS (w/w), whereas the addition of Me reduced WHC in FBPI, possibly due to interference with the protein matrix.

SEM imaging further supported these trends, with well-defined network structures in FBPI–MS and RPI–MS gels, along with concentration-dependent improvements in PS-containing formulations.

These results demonstrate the stronger intrinsic gelation potential of FBPI compared to RPI and highlight the critical role of starches, especially MS, in improving the structural performance of proteins with weaker gelling capacity.

Chapter 5. Development of plant based and vegetarian sausages using plant proteins, modified starches, methylcellulose, and egg white powder

5.1 Introduction

The adoption of plant-based foods as substitutes for meat has steadily increased in recent years, driven by growing consumer awareness of environmental sustainability, animal welfare, and health considerations. Nevertheless, the development of meat analogues still faces significant challenges, particularly consumers' reluctance to shift from conventional meat products, which are highly valued for their nutritional profile, sensory attributes, and convenience (Kyriakopoulou et al., 2021). Consequently, considerable research efforts have focused on designing sustainable alternatives that closely mimic the appearance, texture, aroma, and taste of meat.

A wide range of plant-based meat analogues is now available, including strips, chunks, patties, burgers, chicken-like blocks, ground beef-like products, nuggets, steaks, and sausages (Ishaq et al., 2022). The development of these products has been supported by various technologies, including extrusion, fermentation, shearing, spinning and mixing (Kyriakopoulou et al., 2021).

In addition to technological advances, the selection of ingredients such as textured and non-textured proteins, flavourings, fats or oils, binders and colourants plays a key role (Ishaq et al., 2022). However, many of these components are highly processed, which is undesirable due to the growing demand for more natural ingredients and clean label (Kyriakopoulou et al., 2021). Therefore, it is critical to understand the functionality of refined and natural ingredients and how they interact to create products that are more appealing to consumers.

Recent research has emphasized the strategic use of soluble binders, including modified starches, hydrocolloids, and egg-derived ingredients, in combination with legume proteins to improve protein content, tenderness, textural integrity, juiciness, and emulsion stability in meat-free products (Ferawati et al., 2021; Ishaq et al., 2022; Kyriakopoulou et al., 2021).The inclusion of these ingredients, also plays a critical role in stabilizing emulsions and enhancing the gelling properties of plant proteins, which are typically weaker than those found in traditional meat products (Kyriakopoulou et al., 2021; Mohammadi, 2012)

Building upon the findings from Chapter 3, which demonstrated that the addition of egg white powder (EWP) significantly enhanced the gelation behaviour, water-holding capacity (WHC), and textural attributes in FBPI and RPI gels when incorporated at a ratio corresponding to approximately 40 - 50% of the total protein content, the study in this chapter focused on applying these insights to develop a sausage formulation. Specifically, sausage prototypes were developed to investigate the incorporation of EW powder at 8.8%, roughly 43.2% by weight of total protein, to reinforce gel networks, particularly in low-hydrocolloid formulations.

In this chapter, FBPI and RPI were used at 12% and 15% (w/w), respectively. These levels were selected to maintain consistency with the concentrations used in Chapters 3 and 4, ensuring comparability across all sections of the study.

In addition, the impact of hydrocolloid concentration was systematically investigated using both maximum and minimum levels of modified waxy maize starch (MS), modified potato starch (PS), and methylcellulose (Me), as previously defined in Chapter 4.

Accordingly, two formulations were prepared for each plant protein isolate (FBPI at 12% (w/w) and RPI at 15% (w/w)): one with MS (5% (w/w)), PS (10% (w/w)), and Me (1.6% (w/w)) alone, and another combining reduced levels of MS (2% (w/w)), PS (5% (w/w)), and Me (0.8% (w/w)) with 8.8% (w/w) EWP. This design enabled a comprehensive evaluation of the individual and combined effects of hydrocolloid concentration and EWP addition on colour, sensory, and textural properties. This approach aligns with recent advances in the formulation strategies for meat-free analogues, where combinations of plant proteins, soluble binders, and functional additives are optimized to improve product quality, particularly texture and appearance (Kyriakopoulou et al., 2021).

Furthermore, this chapter investigated critical physicochemical and technological parameters, including water activity, pH, cooking yield, colour, and proximate composition (protein, fat, carbohydrate, moisture, and fibre content), which are essential for assessing the nutritional quality, functionality, and consumer acceptability of meat-free sausage alternatives (Ishaq et al., 2022)

Ultimately, this experimental approach aims to develop high-quality meat free analogues with improved texture (hardness, chewiness, springiness and cohesiveness) alongside improved water retention, and nutritional attributes, aligned with current trends toward clean labels and enhanced consumer satisfaction.

5.2 Material and Methods

5.2.1 Materials

The development of plant based and vegetarian sausages formulations involved the following ingredients: unflavoured powders of faba bean protein isolate (**FBPI;** 85% protein, 5.4% fat and 3.8% carbohydrate) and rice protein isolate (**RPI;** 80% protein, 5.3% fat, and 6.3% carbohydrate) both purchased from NZPROTEIN® (Auckland, New Zealand); egg white powder (**EWP;** 85% protein, 0.2% fat, and <2.0% carbohydrate) was purchased from Keto Store NZ Ltd (New Zealand).

The minor ingredients used in this study included commercial methylcellulose (**Me;** Methocel™ A4C; pH of a 2% aqueous solution: 5.0–8.0) and two commercial modified food starches; modified waxy maize starch (**MS;** FIRM-TEX®; pH of a 20% w/w slurry: 4.5–7.0) and modified potato starch (**PS;** PenBind® 850; pH 5.5–8.0). All of these were kindly provided by Hawkins Watts, New Zealand. Technical specification sheets for these ingredients are included in Appendices A1 and A2.

Additional ingredients included, table salt, black pepper, turmeric, cumin powder, paprika powder, chili flakes, balsamic vinegar, psyllium husk, Macro nutritional yeast flakes (Woolworths, New Zealand), whole yellow mustard seeds (Cerebos Greggs Ltd, New Zealand), fried shallot flakes (Oriental Merchant NZ Ltd), miso paste (Tomoechan, Japan), and deodorised coconut oil (Blue Coconut Distribution NZ Ltd). These were all purchased from a local supermarket (Woolworths, Auckland).

A commercial vegan sausage (Italian Herbs; Tonzu, Swanson, Auckland, New Zealand) was also obtained from Woolworths and used as a reference for physicochemical and textural comparisons. Cellulose casing (Viscofan Veggie Casing 24 Home 30.5m) was purchased from D. M. Dunningham Ltd. (Penrose, Auckland, New Zealand).

5.2.2 Preparation of sausages

Sausages were prepared using four different formulations. Two were based on FBPI: a fully plant-based version and a vegetarian version incorporating EWP. The same approach was applied to RPI, resulting in a corresponding pair of formulations. The preparation process for a 300 g sausage batch, adapted from the method described by Keerthana Priya et al. (2022).The formulations for sausages are shown in Table 5.1.

In brief, a methylcellulose dispersion was prepared by dissolving the powder into hot deionized water (approximately half the total required volume) at 90°C for 15 minutes at 150 rpm using a food blender (Thermomix TM5, Vorwerk, Germany). Then, the volume was completed by adding cold water (5°C) and thoroughly mixed for another 15 minutes or until a smooth dispersion was obtained. The dispersion was stored in the fridge at 4°C overnight. (These conditions were optimized during preliminary trials and followed the protocol described by Hawkins Watts).

Next, all dried ingredients including protein powder, starches, and spices were mixed for 3 min in stand mixer (KitchenAid Classic Tilt-Head Stand Mixer, KSM 275W, Whirlpool Corporation, USA) at low speed, followed by the addition of wet ingredients and grated frozen coconut oil. Wet ingredients and grated frozen coconut oil were then added, the mixture was mixed thoroughly at high speed for another 3 minutes or until a homogeneous mixture was achieved.

It is important to note that the addition of 8.8% (w/w) EWP was selected to maintain the plant protein-to-egg white ratios that previously demonstrated the most stable and elastic gel networks gelation behaviour in Chapter 3. In those experiments, the optimal composites samples (FBPI/EW and RPI/EW) incorporated EWP at approximately 40% of the total protein content while keeping the overall protein level constant. This same proportion was applied in the sausage formulations to directly translate the functional behaviour identified in Chapter 3 into the final product matrix.

The mixture was then wrapped or added into the cellulose casing by using a manually operated cold extrusion sausage machine (Stainless steel, manual sausage machine, Polsinelli Enologia, Italy) with an approximate length of 12 cm. The edges were twisted and tied. Following the method described by Keerthana Priya et al. (2022), the sausages were stored at 4°C overnight to ensure shape stability. Subsequently, the sausages were steamed for 30 minutes at 95°C. All freshly prepared sausage samples were allowed to cool to room temperature prior to texture, WHC, and structural analyses. Only the samples for proximate analysis were stored at –18 °C prior to testing.

Table 5.1 Composition (% w/w) of FBPI- and RPI- based sausage formulations with and without EWP

Ingredients	FBPI-H (%w/w)	FBPI-H-E (%w/w)	RPI-H (%w/w)	RPI-H-E (%w/w)
Faba bean protein isolate (FBPI)	12.00	12.00	-	-
Rice protein isolate (RPI)	-	-	15.00	15.00
Methylcellulose (Me)	1.60	0.80	1.60	0.80
Water	43.35	43.35	43.35	43.35
Modified potato starch (PS)	10.00	5.00	10.00	5.00
Modified waxy maize starch (MS)	5.00	2.00	5.00	2.00
Balsamic vinegar	2.40	2.40	2.40	2.40
Egg white powder (EWP)	-	8.80	-	8.80
Deodorised coconut oil	4.47	4.47	4.47	4.47
Psyllium	2.23	2.23	1.57	1.57
Black pepper	0.74	0.74	0.74	0.74
Mustard seeds	2.23	2.23	1.67	1.67
Cumin powder	1.49	1.49	0.69	0.69
Paprika powder	1.55	1.55	1.00	1.00
Chili flakes	0.74	0.74	0.33	0.33
Sal	1.49	1.49	1.49	1.49
Miso paste	3.98	3.98	3.98	3.98
Shallot flakes	4.47	4.47	4.47	4.47
Nutritional yeast	1.49	1.49	1.49	1.49
Turmeric	0.74	0.74	0.74	0.74

Sample codes for the sausages were presented in Table 5.1. FBPI-H and RPI-H referred the plant-based sausage formulations prepared with either FBPI or RPI, respectively, combined with a high level of hydrocolloids. These formulations contained 10% (w/w) PS, 5% (w/w) MS, and 1.6% (w/w) Me.

In contrast, FBPI-H-E and RPI-H-E represented the vegetarian versions of these formulations, in which the level of hydrocolloids was reduced to5% (w/w) PS, 2% (w/w) MS, and 0.8% (w/w) Me, and 8.8% (w/w) EWP was added.

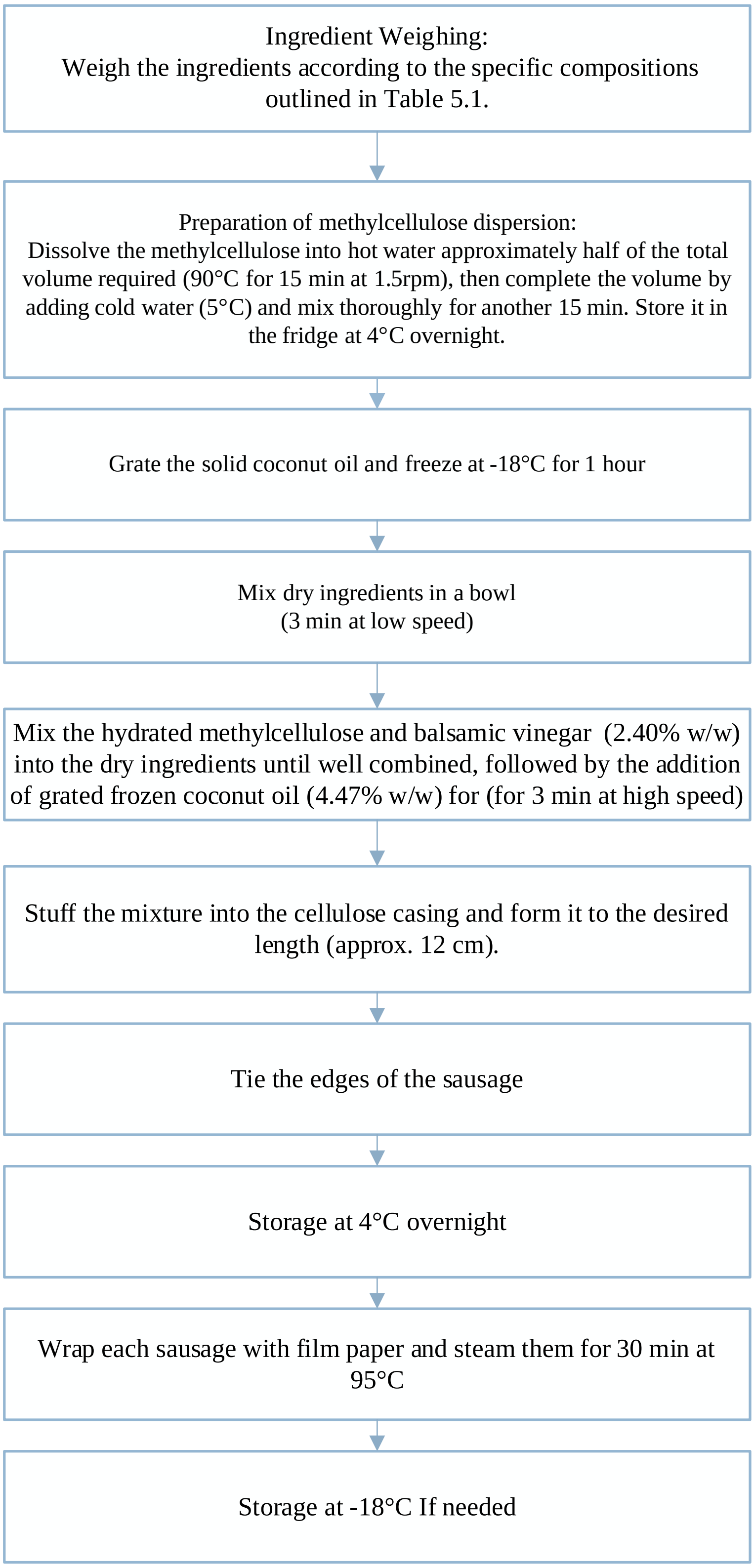


Figure 5.1 Flow diagram of producing a batch of 300 g of plant-based (FBPI-H and RPI-H) and vegetarian sausages (FBPI-H-E and RPI-H-E) using FBPI and RPI.

5.2.3 Analyses of sausages

After cooking at 95°C for 30 minutes by steaming, the sausages were analysed to determine colour, pH, water activity and cooking yield, texture and proximate composition. The commercial sausage was also subjected to steaming and then analysed.

5.2.3.1 Colour determination

The colour of the steamed sausages was analysed using a colorimeter (Minolta Chroma Meter CR-300, Minolta Co. Ltd, Japan). After calibrating the instrument with a CR-300 white calibration plate, sausages were cut into equal slices (5 mm thick) and placed inside a disposable measuring cup. Colour measurement was carried out by placing the measurement cup on the measuring head of the CR-300 colorimeter. Result were expressed numerically in CIE L*, a*, and b* values, which represent the lightness (L) from 0 (black) to 100 (white) range, the redness (+a) and greenness (-a), and the yellowness (+b) and blueness (-b) (Corrêa et al., 2023).

5.2.3.2 Water activity

Water activity of the steamed sausages was measured using a water activity meter (Aqualab 4TEV, METER Group, Inc, USA). After warming up the equipment for 1 hour, samples were sliced evenly and placed halfway into disposable measuring cups. The water activity was then measured by placing the cup in the sample drawer, closing it, and turning the knob from load to read.

5.2.3.3 Cooking yield

Following the procedure described by Keerthana Priya et al. (2022), cooking yield was calculated using the following formula. Raw sausages samples were weighed before steaming at 95°C for 30 minutes and weighed again afterward.

$$Cooking\ yield\ \% = \frac{Cooked\ sausage\ mass\ (g)}{Raw\ sausage\ mass\ (g)}\ x\ 100$$

5.2.3.4 pH determination

According to the method described by Keerthana Priya et al. (2022), 10 g of each steamed sausage sample was homogenized in 90 mL deionised water using a magnetic stirrer for 2 hours at 300 rpm. The pH of each sample was measured at room temperature using a pH meter (Accumet AB150, Fisher Scientific), calibrated with standard pH buffer solutions at pH 4.0 and 7.0 prior to measurement.

5.2.3.5 Proximate composition

After removing the casing, the steamed sausage samples were frozen, labelled, and ground for proximate analysis. Protein content was measured using the Kjeldahl method, where a nitrogen-to-protein conversion factor of 6.25 was applied. Moisture content was analysed using AOAC 925.10 and 930.15 methods. The samples were dried at 105 °C overnight. Ash content was determined using AOAC 942.05 method with a furnace at 550°C. Fat content was analyzed using the Mojonnier method (AOAC 922.06) and Total dietary fibre (TDF) was measured using the Megazyme method (AOAC 991.43) at the Nutrition Laboratory, Massey University, Palmerston North, New Zealand. In this study, dietary fibre content was analysed only once. The second estimation was calculated stoichiometrically from the ingredient composition.

Carbohydrate content was determined by difference using the following formula:

Carbohydrates (%w/w) = 100− (Moisture + Ash + Protein + Fat + TDF)

5.2.3.6 Texture profile analysis (TPA)

The texture of the steamed sausages was evaluated using a Texture Analyser (TA-XT plus, Stable Micro Systems Ltd., UK) following the method described by Keerthana Priya et al. (2022) with slightly modifications. The steamed sausages were sliced into pieces (25mm diameter x 20mm height).

A 35 mm diameter aluminium cylinder probe (P/35) was used to compress the samples twice for a depth of 1 mm. The test settings were as follows: pre-test speed = 2 mm/s, test speed = 2 mm/s, post-test speed = 2 mm/s, target distance = 10 mm, trigger force = 5.0 g, and data acquisition rate = 100 points per second.

Texture parameters (hardness, cohesiveness, chewiness, gumminess, springiness and resilience) were obtained from the force-time curve using Exponent Software (Version 6,1,15,0, Stable Micro Systems Ltd., UK).

5.2.4 Statistical analysis

All samples for colour measurement, water activity, cooking yield, and pH determination were prepared and measured in at least duplicate, and results are presented as mean ± standard deviation (SD). Samples for proximate analysis were prepared in duplicate; however, measurements were not replicated. Statistical analysis was performed using SPSS Statistics 26.0 (IBM, USA). One-way analysis of variance (ANOVA), followed by Tukey's HSD test, was conducted to identify significant differences among groups at a significance level of $p < 0.05$.

5.3 Results and Discussion

5.3.1 Visual appearance and colour determination

Photographs of the cooked sausage samples were taken after preparation, as shown in Figure 5.2. The visual characteristics of sausages formulated with FBPI and RPI, combined with varying proportions of modified starches, methylcellulose, and EW powder, were evaluated.

Overall, all sausage samples exhibited an appealing colour, making them both highly palatable and visually appetizing. The commercial vegan sausage used as a reference displayed a notably lighter appearance with a slight pink hue. Differences in protein source and formulation were reflected in the visual appearance, the vegetarian sausages (FBPI-H-E and RPI-H-E) exhibited a softer, less dry texture when cut, compared to their plant-based counterparts (FBPI-H and RPI-H). When comparing protein sources, sausages made with RPI tended to be drier and grainier, while those made with FBPI had a smoother texture.

Colour is one of the first attributes evaluated by consumers when selecting food products. Therefore, the differences in lightness (L*), redness (a*), and yellowness (b*) observed among the developed sausages highlight the impact of formulation choices, particularly protein source and EWP addition, on the visual quality and potential consumer acceptance of plant-based and vegetarian sausages. The colour attributes of the sausages, including lightness (L*), redness (a*), and yellowness (b*), are presented in Table 5.2.

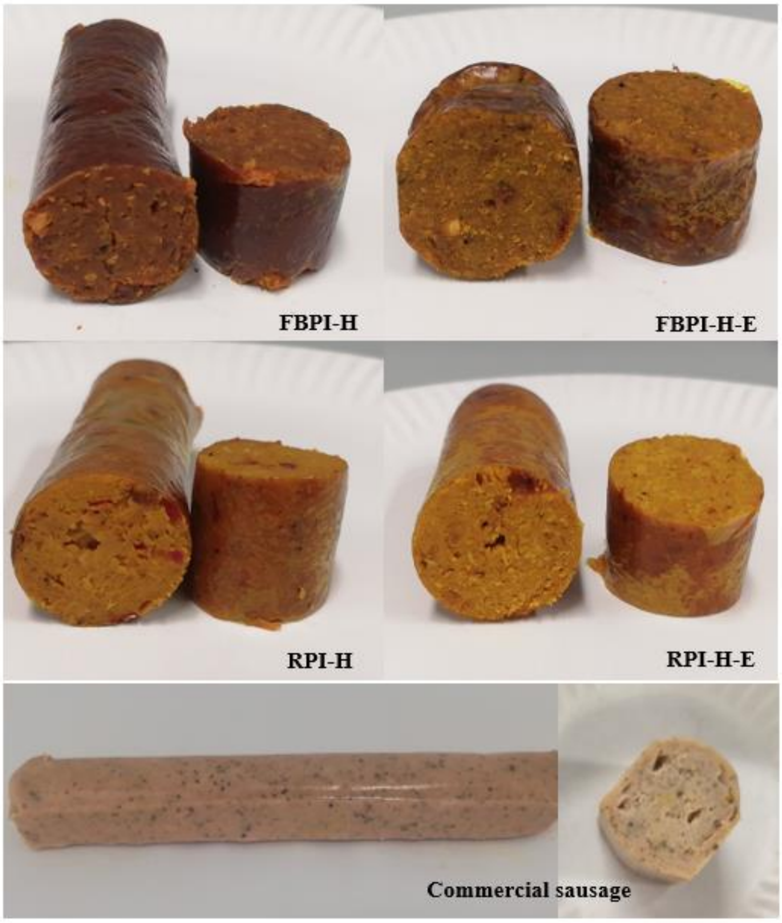


Figure 5.2 Pictures of cooked plant-based sausages (FBPI-H and RPI-H), vegetarian sausages containing EWP (FBPI-H-E and RPI-H-E) and a commercial sausage

Overall, sausages formulated with FBPI exhibited a darker appearance, partly attributed to the naturally darker hue of faba bean protein isolate. The addition of EWP, which has a pale-yellow colour, slightly increased the lightness of both FBPI- and RPI-based sausages; however, the effect was more noticeable in FBPI formulations due to the inherently lighter colour of rice protein isolate in the RPI samples. These visual observations were consistent with the measured L* values. FBPI-H samples showed the lowest lightness value (42.80), indicating the darkest appearance among the samples. The addition of EW powder in FBPI-H-E increased lightness slightly (44.54). RPI-based sausages exhibited significantly higher lightness values compared to FBPI formulations, with RPI-H at 53.09 and RPI-H-E at 53.76, confirming their lighter visual appearance. Commercial sausages exhibited the highest lightness (63.59), significantly lighter than all developed formulations.

In terms of redness (a*), RPI-based sausages showed the highest values, indicating greater red intensity compared to FBPI-based sausages. The addition of EW powder notably decreased redness in both protein systems, particularly in FBPI-H-E (4.95) compared to FBPI-H (7.60). The commercial sausage displayed the lowest redness value (3.43), confirming its paler and less saturated appearance.

The yellowness values (b*) followed a similar trend. RPI-based sausages displayed significantly higher yellowness compared to FBPI formulations. Furthermore, the addition of EW powder increased yellowness in both protein systems, enhancing the yellow hue of the sausages. This observation is consistent with known effects of Maillard reactions during heating, where amino groups in egg white proteins react with reducing sugars yellowness (Katekhong & Charoenrein, 2017). For instance, research on egg white during high-temperature processing found that the yellowness index increases over time as heat treatment progresses, confirming the impact of Maillard chemistry on colour development (Tangduangdee et al., 2023).

Table 5.2 Colour parameters (lightness, redness, and yellowness) of cooked plant based-based and vegetarian sausages. Colour measurements are expressed as CIE L*, a*, b* values

Sausage sample	Lightness (L*)	Redness (a*)	Yellowness (b*)
FBPI-H	42.80 ± 0.26^{c}	7.60 ± 0.28^{b}	11.50 ± 0.09^{d}
FBPI-H-E	44.54 ± 0.06^{d}	4.95 ± 0.13^{c}	15.75 ± 0.28^{c}
RPI-H	53.09 ± 0.24^{b}	8.66 ± 0.13^{a}	29.73 ± 0.31^{b}
RPI-H-E	53.76 ± 0.21^{b}	7.56 ± 0.20^{b}	32.32 ± 0.51^{a}
Commercial	63.59 ± 0.16^{a}	3.43 ± 0.09^{d}	12.17 ± 0.40^{d}

Values within the same column with different superscript letters indicate significant differences ($p < 0.05$).

Lastly, the commercial sausage appeared visually as pale yellow with weak colour saturation. This impression was consistent with its L* (63.59), a* (3.43), and b* (12.17) values, indicating a bright and slightly yellowish appearance with minimal redness compared to the developed sausage formulations.

5.3.2 Water activity

Water activity (Aw) measurements for the steamed sausage samples ranged from 0.917 to 0.971 (Table 5.3). The commercial sausage exhibited the highest water activity (0.971), indicating a greater amount of available water, which could potentially impact its shelf life and increase the risk of microbial growth. In contrast, the partial replacement of modified starches and methylcellulose with EWP in both FBPI- and RPI-based formulations led to a reduction in water activity compared to their counterparts without EWP. This reduction is beneficial for enhancing microbiological safety by limiting the amount of free water available for microbial proliferation. As reported by Amagliani et al. (2016) water activity reflects the portion of water in food that is available for biochemical reactions, thereby affecting the microbial, chemical, and physical stability of a product. Microorganisms such as moulds, yeasts, and bacteria are capable of growing in foods with water activities above 0.6, which is undesirable for certain types of products. However, a water activity range between 0.6 and 0.9 is considered desirable for intermediate moisture foods such as sausages (Kim, 2022).

Table 5.3 Physicochemical properties of steamed plant-based and vegetarian sausages, including pH, cooking yield, and water activity

Sausage sample	Measurable Parameters		
	pH	**Cooking yield (%)**	**Water activity (Aw)**
FBPI-H	4.97 ± 0.02^{a}	99.53 ± 0.01^{a}	0.945 ± 0.01^{a}
FBPI-H-E	5.50 ± 0.01^{a}	99.83 ± 0.08^{a}	0.927 ± 0.00^{b}
RPI-H	4.68 ± 0.38^{a}	99.67 ± 0.03^{a}	0.935 ± 0.03^{c}
RPI-H-E	4.96 ± 0.03^{a}	99.89 ± 0.03^{a}	0.917 ± 0.01^{d}
Commercial	5.15 ± 0.01^{a}	98.17 ± 0.18^{b}	0.971 ± 0.02^{e}

Values within the same column with different superscript letters indicate significant differences ($p < 0.05$).

5.3.3 Cooking yield

All sausage formulations exhibited high cooking yields, ranging from 98.17% to 99.89%. As shown in Table 5.3, the commercial sausage showed a slightly lower cooking yield compared to all FBPI and RPI custom formulations. These differences in yield between all FBPI and RPI samples were not statistically significant. In general, the cooking yield can be expected to increase with higher levels of hydrocolloids and may also be influenced by the type of starch used. However, this trend was not strictly observed in the present study, as cooking yield was slightly higher in sausages prepared with EWP.

The results of high yield indicate that both the protein matrix formed by FBPI and RPI, and the incorporation of EWP, contributed to reducing moisture and fat losses during cooking likely due to the cross-linking properties of EW proteins. Similar observations were reported by Xie et al. (2020), who found that pasta formulations containing EWP showed reduced cooking loss, with starch becoming entrapped within the protein matrix, thereby improving shape retention during cooking.

In addition to the contribution of egg white, the intrinsic binding capacities of the plant proteins themselves also played a role in improving cooking yield. Kim and Chin (2024) investigated and emphasized that FBPI acts as an effective binder during cooking due to its high content of hydrophilic amino acids, particularly glutamic acid and aspartic acid. These amino acids contain carboxyl groups in their side chains, which ionize under physiological pH, increasing the net negative charge and enhancing electrostatic repulsion between protein molecules. This promotes greater protein unfolding and exposes polar residues that can interact with water molecules through hydrogen bonding, thus improving water retention. Similarly, Miedzianka et al. (2023) noted that RPI possesses a high water-binding capacity attributed to the same amino acids, further supporting its effectiveness in retaining weight during thermal processing. Both FBPI and RPI thus play critical roles in minimizing moisture loss, a desirable attribute for improving cooking yields in meat-free products.

Complementing the protein network, the type and structure of starch used also influenced water retention and cooking yield. In particular, cross-linked potato starch (CLPS), demonstrated superior water-holding capacity, likely due to its ability to form robust and resilient gel structures following chemical modification (Wu et al., 2018). As observed by Heo et al. (2017), CLPS granules remained swollen and intact after heating, which helped to retain water within the matrix during thermal treatment. Moreover, as noted, Punia et al. (2024) the functional properties of starches, such as swelling power, solubility, water absorption capacity, and oil absorption capacity are significantly affected by cross-linking. For instance, cross-linked maize starches show reduced swelling power and solubility, which is attributed to the presence of phosphate groups that form covalent cross-bridges between starch molecules. This structural reinforcement enhances granule integrity and intergranular cohesion during heating.

However, the effectiveness of cross-linked starches also depends on their botanical origin. In the case of cross-linked waxy maize starch (CLWMS), which is composed almost entirely of amylopectin, the absence of amylose limits the extent of intermolecular cross-linking (Punia et al., 2024; Zhu et al., 2013). As a result, CLWMS forms weaker gel networks that are more susceptible to granule rupture and water loss during cooking, potentially diminishing its contribution to cooking yield in certain formulations.

5.3.4 pH measurement

The pH values of the sausage samples ranged from 4.68 to 5.50 (Table 5.3). While some numerical differences were observed among the formulations, statistical analysis indicated that these differences were not statistically significant.

The variation in pH across samples may reflect the combined effects of balsamic vinegar (2.40% w/w), which tends to lower pH due to its acidity (pH -3.40), and EWP (8.80% w/w), which may raise pH slightly due to its alkaline character (pH -7.45). These opposing influences were evaluated in the context of the inherent pH of the protein isolates, 7.09 for FBPI and 6.49 for RPI.

This trend aligns with findings by Carhuancho-Colca et al. (2024),who observed that vegetarian sausage formulations can vary in pH depending on protein type and inclusion level. Additionally, pH differences can affect the solubility of proteins and influence flavour, texture, and microbial stability functional in food systems. For instance, rice proteins are more soluble at pH levels above 5 or under strongly acidic conditions, whereas solubility is minimized around pH 5, the isoelectric point of glutelin, the major protein fraction in RPI (Romero et al., 2012). Such reduced solubility is considered beneficial for applications like meat analogues, where a firmer, less soluble protein network is desirable (Amagliani et al., 2017).

From a microbiological perspective, all the developed sausages had a mildly acidic pH (above 4.68) and high-water activity (Aw> 0.9), which are conditions that can support the growth of spoilage microorganisms (Dasiewicz et al., 2024). These characteristics are quite similar to those found in traditional meat products, which are also known to be perishable. However, the food safety concerns for plant-based sausages are somewhat different. Since these products rely on raw plant-derived ingredients, the risk of contamination is closely linked to how those ingredients are sourced and handled. As noted by Kabisch et al. (2024), plant proteins can carry pathogenic microbes from the farming environment, which highlights the importance of ingredient quality and good manufacturing practices in ensuring safety.

5.3.5 Proximate Analysis

Plant-based sausages were developed using either FBPI or rice RPI, in combination with 1.6% (w/w) Me, 5% (w/w) MS, and 10% (w/w) PS. A vegetarian counterpart was also formulated by partially replacing these hydrocolloids with 8.80% (w/w) EWP, while reducing Me to 0.8% (w/w), MS to 2% (w/w), and PS to 5% (w/w). All sausage samples were analysed for their proximate composition; including moisture, ash, crude protein, fat, total dietary fibre (TDF), and carbohydrates on a wet matter basis (Table 5.4). For comparative purposes, the nutritional information obtained from the packaging of the commercial vegan sausage was also included.

The addition of grated coconut oil in the sausage formulation as primary fat source was 4.47% (w/w). It should also be noted, as described in Section 5.2.1, additional fat content in the formulation was contributed by the FPBI and RPI ingredients, which contained 5.4% and 5.3% (w/w) fat, respectively. Based on the ingredient formulation, the initial fat content was theoretically estimated to be approximately 5.12% (w/w) in FBPI-based formulations and 5.27% (w/w) in RPI-based formulations. However, the fat content determined analytically using the Mojonnier method was notably higher, with values ranging from 8.75% to 10.85% (w/w). RPI-based sausages showed slightly lower fat levels (9.50% (w/w) in RPI-H and 8.75% (w/w) in RPI-H-E) compared to those prepared with FBPI (10.50% (w/w) in FBPI-H and 10.85% (w/w) in FBPI-H-E). These discrepancies between theoretical and measured fat content can be attributed to the nature of the analytical method, which quantifies total extractable fat, including bound and entrapped lipids that may not be fully accounted for in the formulation-based estimate (Hyvönen, 1996). Moreover, minor contributions from ingredients such as miso paste and fried onion flakes may have further elevated the total fat recovered. Furthermore, the slightly lower values observed in RPI-based sausages may reflect differences in fat retention capacity. FBPI may form a more cohesive gel matrix that limits fat migration and allows greater entrapment of lipids within the protein network during cooking (Kim & Chin, 2024), while RPI's structure may be less effective in this regard.

Moisture content varied depending on the protein source, with RPI-based sausages showing higher moisture levels than those made with FBPI. Interestingly, in both protein systems, the incorporation of egg white powder (EWP) resulted in a slight decrease in moisture content. While EWP is generally associated with improved water-binding due to its ability to form dense gel networks (Razi et al., 2023), the observed reduction in moisture may be attributed to a more compact protein matrix that holds water more tightly, thereby reducing the proportion of free, measurable moisture. This denser gel formation may lead to lower extractable moisture values, despite enhanced water retention at the structural level. (Guo et al., 2020).

Ash content was higher in FBPI-based sausages than in their RPI-based counterparts. The addition of EWP resulted in a modest increase in ash content across both protein sources, possibly due to the natural mineral content of the EW (Mudau et al., 2021).

A notable increase in crude protein was achieved with the incorporation of EWP. In FBPI-based sausages, protein content rose from 14.75% to 21.55% (w/w), while in RPI-based sausages, it increased from 15.45% to 22.30% (w/w). Given that FBPI, RPI, and EWP contain 85%, 80%, and 85% (w/w) protein, respectively, the estimated protein contents based on formulation were 10.5% (w/w) for FBPI-H, 17.68% (w/w) for FBPI-H-E, 12% (w/w) for RPI-H, and 19.48% (w/w) for RPI-H-E. The higher values obtained through the Kjeldahl method can be attributed to its measurement of total nitrogen, which includes not only protein-derived nitrogen but also non-protein nitrogen (NPN) compounds. Ingredients such as miso paste, nutritional yeast, onion flakes, and various spices and seasonings (i.e., black pepper, mustard seeds, cumin, chili flakes, turmeric), contain naturally occurring NPN compounds such as amino acids, nucleotides, and small peptides (García et al., 2015; Giri et al., 2011; Izcara et al., 2022; Yang et al., 2011). Although not considered major protein sources in the formulation, these ingredients contribute measurable nitrogen content, leading to an overestimation of protein in the final product. Moreover, the application of a universal nitrogen-to-protein conversion factor (N × 6.25) further contributes to this discrepancy, as it does not distinguish between different nitrogen sources, and may result in protein values that exceed those estimated by formulation alone (Mariotti et al., 2008).

In comparison with a commercial vegan sausage (14.4% (w/w) protein), the plant-based formulations developed in this study achieved slightly higher protein levels, indicating a promising alignment with current commercial options in the market.

Total dietary fibre (TDF) remained relatively stable among all samples. Differences observed between FBPI and RPI formulations were mainly due to the psyllium content, which was 2.23% (w/w) in FBPI formulations and 1.57% (w/w) in RPI sausages. Psyllium was the primary fibre source in the formulations and the partial substitution of hydrocolloids with egg white did not notably affect fibre levels.

The lower carbohydrate content observed in the egg white-containing formulations can be attributed to their reduced starch levels. Specifically, FBPI-H-E and RPI-H-E contained only 5% (w/w) PS and 2% (w/w) MS, while the formulations without EWP (FBPI-H and RPI-H) included 10% (w/w) PS and 5% (w/w) MS. Consequently, the carbohydrate content decreased from 18.14% (w/w) and 18.07% (w/w) in FBPI-H and RPI-H to 12.72% (w/w) and 12.10% (w/w) in FBPI-H-E and RPI-H-E, respectively. Compared to a commercial vegan sausage containing 11.9% carbohydrates, the egg white-containing formulations showed slightly higher values, suggesting that their carbohydrate levels fall within the typical range observed in market-available plant-based sausages.

Table 5.4 Proximate composition of plant-based and vegetarian sausages samples

Parameter (g/100g)	FBPI-H	FBPI-H-E	RPI-H	RPI-H-E	Commercial sausage
Moisture	44.3 ± 0.00	42.55 ± 0.10	47.20 ± 0.00	47.05 ± 0.10	N.A.
Ash	4.00 ± 0.00	4.25 ± 0.10	3.10 ± 0.00	3.40 ± 0.00	N.A.
Crude protein	14.75 ± 0.21	21.55 ± 0.07	15.45 ± 0.21	22.3 ± 0.99	14.4
Fat	10.50 ± 0.14	10.85 ± 0.07	9.50 ± 0.14	8.75 ± 0.07	10.1
TDF	8.30 ± 0.29*	8.10 ± 0.40*	6.70 ± 0.18*	6.40 ± 0.00	1.2
Carbohydrate	18.14 ± 0.22	12.72 ± 0.26	18.07 ± 0.25	12.10 ± 1.13	11.9

TDF values marked with an asterisk (*) represent the average of two methods: (1) direct measurement using a standard analytical procedure (Appendix B), and (2) stoichiometric estimation based on the dietary fibre content of each ingredient in the formulation. For the RPI-H-E sample, the TDF value was estimated solely using the stoichiometric method, as analytical data were not available.

5.3.6 Texture profile analysis

The texture properties of the cooked sausage formulations, specifically hardness, chewiness, springiness, and cohesiveness, are presented in Table 5.5. The addition of EWP notably enhanced hardness and chewiness across the formulations. For instance, the FBPI-H-E sample exhibited a hardness of 5418 N, and chewiness of 2449 N.mm, while RPI-H-E showed even higher values, with hardness reaching 6386 N and chewiness 2296 N.mm.

This considerable increase in texture parameters is consistent with findings by Zhao et al. (2022) in a study on preparation and characterization of pea protein isolate-egg white protein composite gels, who reported that egg white proteins, particularly ovalbumin, form strong gel networks through water-binding and gelling interactions. When combined with plant-based proteins, these networks contribute to a cohesive matrix that significantly improves hardness and chewiness. In their study, EWP addition resulted in greater hardness compared to formulations using xanthan gum and gluten, suggesting that egg white can serve as an effective textural enhancer and a substitute for hydrocolloids in meat-free products.

Overall, RPI-based formulations tended to exhibit higher hardness compared to FBPI-based sausages. This observation aligns with the findings of Lee, Choi, et al. (2022), who developed meat analogues using textured rice proteins produced from blends of RPI and soy protein isolate (SPI) at various ratios (25:75 to 100:0, (w/w)), along with corn starch and wheat gluten. Their study reported that increasing the proportion of RPI reduced matrix porosity, resulting in denser, less aerated structures with increased hardness.

With regards cohesiveness tended to decrease with the addition of EWP and was generally lower in RPI-based formulations compared to their FBPI-based counterparts. This trend is supported by Khatkar et al. (2021), who suggested that the low gelling capacity of RPI may hinder protein cross-linking, weakening the internal structure and reducing the ability of the sausage to recover their shape after compression.

The commercial sausage, used as a benchmark, exhibited significantly lower hardness (1195 N) and chewiness (905 N.mm) than the experimental formulations. These observations are in line with Zhao et al. (2022), who reported similarly low texture values in commercial vegetarian sausages made from soy protein concentrate, wheat gluten, and various starches and texturizers (i.e., methylcellulose, hydrolyzed wheat protein, guar gum). The softer structure of the commercial sample may be partly attributed to the inclusion of rice flour, which, as noted by Oppong et al. (2022), reduces hardness in processed products like fish nuggets by softening the protein gel matrix and disrupting the continuous protein network. This effect is closely linked to the low amylose content of rice flour. As explained by Mariotti et al. (2018). The low amylose level weakens starch granules during and after heating, as amylose molecules leach out more readily. While this leaching can temporarily increase viscosity, excessive swelling often leads to granule rupture. As a result, the starch fails to form a strong, cohesive matrix, reducing its ability to retain water and contributing to a softer, less structured final product.

Table 5.5 Textural parameters (hardness, chewiness, springiness, and cohesiveness) of sausage samples

Sausage sample	Hardness (N)	Chewiness (N.mm)	Springiness (mm)	Cohesiveness
Commercial	1195 ± 43[a]	905 ± 11[a]	0.93 ± 0.03[a]	0.82 ± 0.01[a]
FBPI-H	2798.85 ± 216[b]	1876 ± 1275[b]	0.91 ± 0.00[a]	0.74 ± 0.01[b]
RPI-H	3820.32 ± 73[c]	2300. ± 61[c]	0.84 ± 0.00[b]	0.72 ± 0.03[b]
FBPI-H-E	5418.23 ± 332[d]	2449 ± 380[c]	0.75 ± 0.05[c]	0.60 ± 0.02[c]
RPI-H-E	6386.59 ± 134[e]	2296. ± 358[c]	0.70 ± 0.03[c]	0.51 ± 0.07[d]

Values within the same column with different superscript letters indicate significant differences ($p < 0.05$).

Interestingly, the commercial sausage showed the highest values for springiness and cohesiveness, which is expected given its relatively lower hardness and chewiness. This reflects a more elastic and deformable structure compared to the denser, firmer experimental sausages.

5.4 Conclusions

This study demonstrated the potential of FBPI and RPI, combined with modified starches, methylcellulose, and EWP, to create plant-based and vegetarian sausages with favourable textural, nutritional, and physicochemical characteristics. The addition of EWP played a pivotal role in enhancing cooking yield, protein content, and textural parameters such as hardness and chewiness, establishing its value as a functional ingredient in low-hydrocolloid systems.

Textural analysis revealed that sausages formulated with FBPI exhibited a softer and less dense texture compared to those prepared with RPI. RPI-based formulations displayed significantly higher hardness and chewiness, indicating a firmer structure that required more effort to chew. However, they also showed lower springiness and cohesiveness, reflecting reduced elasticity and weaker structural integrity. In contrast, the commercial sausage formulated with whole soybeans, tapioca flour, rice flour, psyllium husks and spices and herbs presented a more balanced texture profile, with the highest values for springiness and cohesiveness, resulting in a product that was softer, more elastic, and easier to chew compared to the experimental samples.

The incorporation of egg white powder (EWP) not only enhanced the protein content but also contributed to a reduction in carbohydrate levels due to the partial replacement of modified starches, highlighting its value as a complementary ingredient in meat analogues. The inclusion of 8.8% (w/w) EWP in the sausage formulations was guided by the optimal ratios identified in Chapter 3, where EWP comprising approximately 40–50% (w/w) of the total protein content significantly enhanced gelation, water-holding capacity (WHC), and texture. Although this proportion was initially determined based on ingredient weight, subsequent calculations confirmed that EWP represented approximately 39.6% (w/w) of the total protein in the final formulation. This finding validated the initial formulation strategy and demonstrated that the selected EWP level effectively reinforced the structural and functional properties of low-hydrocolloid systems, thereby supporting the objectives outlined for this chapter.

Differences in fibre content were primarily due to the lower level of psyllium incorporated in the RPI-based sausages, while variability in fat content, on the other hand, can be explained by differences in fat retention capacity and the nature of the analytical method used.

Across all formulations, methylcellulose and modified starches contributed similarly by enhancing water-binding capacity and supporting gel structure formation. This, in turn, improved water activity (Aw > 0.90) and cooking yields (>99%), which are important for maintaining juiciness and achieving a texture closely resembling fresh meat. However, the high-water activity observed could also increase susceptibility to microbial spoilage, underscoring the need for appropriate storage and handling conditions.

Colour analysis demonstrated that FBPI formulations produced sausages with darker, less yellow appearances, while RPI formulations resulted in lighter, more yellow products. The addition of EWP notably increased lightness and yellowness, particularly in RPI-based sausages. Water activity measurements showed that all FBPI and RPI formulations had lower

Aw values compared to the commercial sausage, with RPI formulations exhibiting slightly lower Aw than FBPI samples.

Lastly, pH values ranged from 4.68 to 5.50; the inclusion of EWP increased the pH across formulations, while RPI-based sausages maintained overall lower pH levels due to the inherent acidity of the RPI.

Chapter 6. Conclusions and Future Recommendations

6.1 Conclusions

This study explored the gelation behaviour and functional properties of faba bean protein isolate (FBPI) and rice protein isolate (RPI) through a stepwise progressive approach; first, by combining them with egg white powder (EWP) (Chapter 3); then with methylcellulose and modified starches (Chapter 4); and ultimately, by applying these systems in the development of plant-based and vegetarian sausages (Chapter 5). The results across all chapters demonstrated that, when paired with functional ingredients such as EWP, methylcellulose, and modified starches, FBPI and RPI have strong potential as base ingredients for developing meat alternatives with desirable textural and nutritional qualities.

The addition of EWP significantly enhanced the gelation of both FBPI and RPI. Particularly, blends containing 40–60% EWP (relative to total protein) exhibited improved storage modulus (G′) and loss modulus (G″), indicating stronger viscoelastic properties. EWP addition notably increased hardness, cohesiveness, and springiness in both protein systems, with FBPI-based gels showing better elasticity and structural recovery than RPI-based counterparts.

Methylcellulose and modified starches (waxy maize and potato starch) were effective in supporting gel formation, especially in FBPI systems. RPI alone failed to form strong gels; however, its structural performance was greatly improved by the inclusion of modified starches, particularly at higher concentrations (MS at 5% and PS at 10%). Methylcellulose reduced gel strength and water-holding capacity (WHC) in FBPI gels likely due to competition with protein–water interactions.

Sausages formulated with FBPI were softer and smoother, whereas RPI-based samples were firmer and more granular. The inclusion of EWP (8.8% w/w), accounting for ~40% of the total protein, significantly enhanced protein content, hardness, and chewiness, while reducing carbohydrate content. Despite these improvements, springiness and cohesiveness remained lower than in commercial references, indicating room for optimization.

6.2 Future recommendations

To further improve the quality, functionality, and commercial viability of the of the sausage prototypes developed in this study, encompassing both plant-based and egg-containing formulations, several future research directions are recommended.

First, conducting consumer sensory evaluation trials will be essential to assess acceptability, with a focus on texture, flavour, and appearance. Second, the versatility and functional stability of the formulations should be evaluated under various cooking methods, such as baking and frying, to simulate real-world culinary applications. Third, it is important to investigate the microbiological stability, oxidative resistance, and moisture retention of the sausages during storage to ensure long-term product safety and quality. Additionally, standardizing the pH of all formulations prior to gel preparation could help isolate the effects of the protein and additive systems, eliminating pH as a confounding variable in gelation behaviour. Lastly, rheological testing, including time–temperature and frequency sweeps under different environmental conditions (e.g., salt levels and pH), would provide deeper insights into the viscoelastic properties of starch–protein composite gels, aiding in formulation refinement.

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Appendices

Appendix A1: Technical specification of modified waxy maize starch (FIRM-TEX®)

technical specification

Ingredion

FIRM-TEX Starch

FIRM-TEX is a modified food starch derived from waxy maize. It is used as a binder and texturiser in cooked meat products and other applications where a low gelatinisation temperature is required.

Chemical and Physical Properties

	Min.	Max.
Moisture %		14
pH (20% w/w slurry)	4.5	7.0
Viscosity MVU		
End	340	540

Microbiological Limits

	Max.
Total Plate Count/g	10,000
Yeast/g	200
Mold/g	200
E. coli	Negative
Salmonella	Negative

Meets NFPA specification for thermophilic bacteria.

While the information below is typical of the product, it should not be considered a specification.

Physical Appearance

	Typical
Colour	White to Off-white
Form	Fine Powder

Nutritional Data/100g

	Typical
Calories, Kcal[1]	360
Calories from Fat	0
Total Fat, g	<0.1*
Saturated Fat, g	0
Trans Fat, g	0
Cholesterol, mg	0
Sodium, mg	148
Total Carbohydrate, g	89.9
Dietary Fiber, g	0
Total Sugars, g	<0.1*
Added Sugars, g	0
Other Carbohydrate, g	89.9
Protein, g	0.2
Vitamin D, mcg	0*
Calcium, mg	3
Iron, mg	<0.2*
Potassium, mg	<10*
Ash, g	<0.2

* Not present in level of quantification

Certification

Kosher
Halal

Packaging and Storage

FIRM-TEX is packaged in multi ply kraft paper bags. FIRM-TEX should be stored in a clean, dry area at ambient temperature and away from heavily aromatic material.

Shelf Life

The best before date for FIRM-TEX is 24 months from the date of manufacture.

Regulatory Data

Source	Waxy Maize
Labelling	Food Starch- Modified
E#	1442
INS#	1442

Features and Benefits

FIRM-TEX has excellent water binding capacity making it ideally suited for applications in the meat industry. It can be used to partially replace skim milk powder and to reduce moisture loss in vacuum packaged meats. Using FIRM-TEX at 2-4% can significantly improve consistency, extended shelf life, and decrease "drip" in a wide variety of smokehouse cooked meats.

This starch also imparts good viscosity and a smooth short texture in high pH soups and sauces. Its low gelatinisation temperature allows it to function in dry mix products added to "hot water".

Effective Date: April 21, 2021 06863102

The information described above is offered solely for your consideration, investigation, and independent verification. It is up to you to decide whether and how to use this information. Ingredion Incorporated and the Ingredion group of companies make no warranty about the accuracy or completeness of the information contained above or the suitability of any of their products for your specific intended use. Furthermore, all express or implied warranties of noninfringement, merchantability, or fitness for a particular purpose are hereby disclaimed. Ingredion Incorporated and the Ingredion group of companies assume no responsibility for any liability or damages arising out of or relating to any of the foregoing.

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Appendix A2: Technical specification of modified potato starch (PenBind® 850)

technical specification

Ingredion

PenBind® 850 05690400

PenBind® 850 is a modified potato food starch with favorable gelling and texturizing properties. It has low viscosity with short texture and forms gel that will melt when heated. It is extremely white in color and bland in taste.

Chemical and Physical Properties

	Min.	Max.
Moisture %	-	18.0
pH	5.5	8.0
Viscosity (SIM-M711)		
Viscosity Peak, MVU	700	1200

Physical Appearance

	Typical
Color	White to Off White
Form	Fine Powder

Screen Test

	Typical
% on U.S.S 100	<2.0

Microbiological Limits

Initial testing is done on a single composite sample against a limit of m. If result is above m, the three class sampling and acceptance below is used.

	n	c	m	M
Total Plate Count/g	5	3	10,000	100,000
Yeast/g	5	3	200	1,000
Mold/g	5	3	200	1,000
Coliform	5	3	100	1,000

Where n = # of samples tested; c = maximum allowable number of results between m and M; m = upper target limit; M = maximum acceptable value.

E. coli	Negative
Salmonella	Negative

Nutritional Data/100 g

	Typical
Calories	339
Calories from fat	0
Total Fat, g	<0.1*
Cholesterol, mg	0
Sodium, mg	133
Total Carbohydrate, g	84.7
Dietary Fiber, g	0
Total Sugars**, g	<0.1*
Added Sugars, g	0
Other Carbohydrate, g	84.7
Protein, g	0.1
Vitamin D, mcg	0
Calcium mg	7
Iron, mg	<0.4*
Potassium, mg	<20*
Ash, g	0.3

* Not present at level of quantification.
** "Total Sugars" in this product may contribute to "Added Sugars" for nutrition labeling purposes in the final consumer product.

Certification

Kosher pareve
Halal

Packaging and Storage

PenBind® 850 can be packaged in multi ply Kraft paper bags and totes. PenBind® 850 should be stored in a clean, dry area at ambient temperature and away from heavily aromatic material.

Shelf Life

The best before date for PenBind® 850 is 24 months from the date of manufacture.

Regulatory Data

Source	Potato

United States

Meets FCC (Food Chemical Codex) requirements.

Labeling	Food Starch-Modified

Canada

CFDA Regulation	B.16.100, Table XIII
Labeling	Modified Potato Starch

Features and Benefits

- Clear Dispersion with Short Texture
- Bland Flavor
- Texturizing
- Forms Reversible Gel
- Low Viscosity

Effective Date: September 8, 2022 Next Review Date: September 8, 2025

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Westchester, Illinois 60154
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708.551.2600

1600 – 90 Burnhamthorpe Rd., West
Mississauga, Ontario L5B 0H9
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905.281.7950

www.ingredion.us

Appendix B: Proximate analysis results of plant-based and vegetarian sausages

Sausage A corresponds to FBPI-H, Sausage B to RPI-H, Sausage C to FBPI-H-E, and Sausage D to RPI-H-E.

1/2

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TO:	**Sung Je Lee**	**AT:**	**SF&AT**
SUBJECT:	**Final Report**	**DATE:**	**20/05/24** ON NP963121222FLORI
TRIAL:	**TN24-239**	*SAMPLES RECEIVED:*	**25/03/24**

Number of pages in this report: 2 Client Reference:

Testing initiated: *15/04/24* Testing completed: *20/05/24*

TN24-239 Vegan Sausages Results are on an as received basis

NutLab ID	Sample Name	Moisture %	Ash %	Crude protein %	Fat %	TDF %
TN24-239-01	Vegan Sausages A	44.3	4.0	14.9	10.4	8.1
		44.3	4.0	14.6	10.6	
TN24-239-02	Vegan Sausages B	47.2	3.1	15.6	9.4	6.8
		47.2	3.1	15.3	9.6	
TN24-239-03	Vegan Sausages C	42.6	4.3	21.6	10.8	7.8
		42.5	4.2	21.5	10.9	
TN24-239-04	Vegan Sausages D	47.1	3.4	23.0	8.8	
		47.0	3.4	21.6	8.7	

Methodology

Moisture : (Feed, forages), AOAC 925.10, 930.15

Ash : Furnace 550°C AOAC 942.05 (Feed, meat)

* *Crude protein : Kjeldahl, N-P = 6.25*

Fat : (Mojonnier) Acid, (Flour, Baked, extruded products) AOAC 922.06

Total Dietary fibre : Megazyme, AOAC 991.43

*Tests marked with an asterix are currently outside the scope of the Nutrition Laboratory's accreditation

Wibha Desai
IANZ Key Technical Person

Karl Dale
IANZ Key Technical Person