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Characteristics of faba bean protein-based high-moisture meat analogues incorporating brewers' spent grain through extrusion

Yue Fan [a], Pratheep K. Annamalai [a,b,c], Bhesh Bhandari [a], Sangeeta Prakash [a,*]

[a] School of Agriculture and Food Sustainability (AGFS), The University of Queensland, St Lucia, QLD 4072, Australia
[b] Centre for Future Materials (CFM), University of Southern Queensland, Toowoomba, QLD 4350, Australia
[c] School of Agriculture and Environmental Sciences (SoAES), University of Southern Queensland, Toowoomba, QLD 4350, Australia

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ABSTRACT

Extrusion is a continuous, versatile process widely regarded as the most cost-effective method for producing high-moisture meat analogue (HMMA). To achieve the desired structural and textural characteristics in meat analogue products, a strategic combination of appropriate ingredients and their optimal ratios is essential. This study investigated the introduction of a fibrous structure in faba bean protein-based HMMA (27 to 39 % protein content) by varying the total solids content (33, 36 and 39 %) and incorporating brewers' spent grain (0, 3 and 6 %) as a structural enhancer. The findings indicate that increasing the solids content promotes fibrillation and improves the overall meat analogue texture. Notably, brewers' spent grain (BSG), an underutilised food by-product, was successfully incorporated into the formulation, promoting fibrous structure formation and enhancing meat texture. However, optimisation is necessary, as while a 3 % BSG inclusion improved extrudate quality, a 6 % inclusion had detrimental effects. This study contributes to the development of sustainable food systems by demonstrating spent grain's potential to strengthen meat analogue structure and texture, while contributing to the positioning of faba bean protein as an untapped, sustainable plant protein source. Further, this study proposes an alternative pathway for repurposing fermented waste (i.e., BSG) by converting it into a functional ingredient that enhances its economic value while reducing environmental impact.

1. Introduction

As a nutrient-dense food, animal meat has been an integral part of the human diet since ancient times. However, the consumption of animal-derived meat is associated with several environmental issues, including climate change, disruption of the phosphorus cycle, damage to the nitrogen cycle, and a decline in biodiversity caused by large herds of livestock (Rubio et al., 2020). Besides, animal meat production has adverse effects extending beyond the environment to human health and animal welfare. While animal-derived proteins are excellent sources of numerous essential nutrients such as protein, iron, and vitamin B12, there is evidence that excessive consumption may contribute to the development of several chronic diseases, including cardiovascular disease, metabolic disease, and cancer (Klurfeld, 2018; Neacsu et al., 2017; Rubio et al., 2020). In addition, the production and consumption of animal-based foods is always accompanied by ethical and religious concerns. The development of cell-cultivated meat can reduce the environmental burden to some extent, but it will remain controversial due to concerns regarding animal origin, cost, and scalability (Kirsch et al., 2023). With the growing concern over animal-derived foods, the necessity of replacing conventional animal-derived foods with plant-derived foods that can provide the majority of human nutritional requirements has become widely recognised.

In numerous studies, it has been demonstrated that plant-based meat alternatives (PBMAs) have a lower ecological footprint than conventional meat (De Boer & Aiking, 2011; Neacsu et al., 2017; Van der Weele et al., 2019; Van Mierlo et al., 2017). Further, PBMAs contain no cholesterol, low saturated fat, and high bioactive compounds and fibre, making them a healthier alternative to conventional meat products. Currently, most of the research and commercial development on plant-based meat rely on soy (Grabowska et al., 2014; Schreuders et al., 2019; Zahari et al., 2020) or pea proteins (Diaz et al., 2022; Osen et al., 2014; Yuliarti et al., 2021) due to their excellent emulsifying and gelling properties, as well as their superior ability to form fibrous structures. Nevertheless, numerous other plant proteins, which could offer nutrient rich meat analogues and are abundantly available, have not been

* Corresponding author.
E-mail address: s.prakash@uq.edu.au (S. Prakash).

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evaluated for their potential as primary protein sources. To develop sustainable plant-based meat analogues with wide range of sensory properties and nutrients, and to reduce overdependence on soy and pea proteins, there is a need to investigate underexplored protein materials.

The faba bean (*Vicia faba* L.) is one of the undervalued sources of protein for plant-based meat production. In recent years, it has emerged as a high-quality plant protein source that provides a sustainable and healthier eating pattern (Martineau-Côté et al., 2022). A small amount of fertilizer is required to cultivate faba beans due to their nitrogen-fixing capacity, specifically their ability to fix atmospheric nitrogen and deposit it in the soil to increase soil fertility (Jensen et al., 2010). Aside from its environmental benefits, faba bean delivers high nutritional value. In contrast to most plant proteins, faba bean protein is a complete protein containing all nine essential amino acids (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine) along with a well-balanced amino acid profile. In addition, it is a source of bioactive peptides. There is evidence that faba bean peptides released after digestion exhibit antioxidant, anti-inflammatory, antidiabetic, antihypertensive, and cholesterol-lowering properties, suggesting the potential of faba bean protein as a functional food ingredient to combat the rising incidence of non-communicable diseases (Martineau-Côté et al., 2022). Although the presence of antinutritional components may limit its effectiveness in some situations, faba bean protein offers significant nutritional benefits, making it a viable alternative to animal proteins in certain circumstances. Besides, faba bean protein has a neutral taste, allowing it to be directly incorporated into food products without masking agents. More importantly, it exhibits favourable emulsifying and gelling properties, enabling the development of an interlaced, fibrous matrix (Nivala et al., 2021; Raikos et al., 2014). Hence in this study, faba bean protein was chosen for its environmental and nutritional benefits, as a promising alternative to conventional soy or pea protein sources.

The lack of sensory appeal of the current meat alternatives is one of the major hindrances to their consumption. Creating a fibrous structure with globular plant proteins is a major challenge for achieving sensory attributes resembling meat. It is necessary for plant proteins to undergo structuring processes (e.g., extrusion, shear cell technology, freeze structuring, spinning processes and 3D printing) to acquire fibrous structures and micropores. Extrusion is the most widely used technique for the fabrication of meat analogues among all the structuring techniques available. As a continuous process, extrusion allows for a high level of productivity, making it ideal for large-scale production. It is a highly efficient technology that integrates several unit operations, including mixing, kneading, heating, shearing, shaping, and forming, into one process. Under the combined effects of pressure, heat and mechanical shear, biopolymer materials are plasticized to produce a molten mass within the extrusion barrel and then forced to exit through a narrow opening (i.e., die) at the discharge end of the barrel to attain the desired shape (Zhang et al., 2022). This process involves a variety of changes, including denaturation and complexation of proteins, gelatinisation and degradation of starch, oxidation of lipids, and degradation of antinutrients and phytochemicals (Zhang et al., 2022). Due to its versatility, food extrusion is used in this study as a cost-effective method to introduce fibrous structures.

A proper combination of formulation and processing conditions is critical to the characteristics of the final product, with both factors significantly influencing attributes such as texture, structure, and overall quality. A formulation that relies solely on plant proteins often fails to achieve the desirable properties for meat analogue products, leaving a coarse mixture with poor consistency and inferior mouthfeel (Huang et al., 2022). Hence, it is imperative that a structure enhancer be incorporated into the construction of meat analogues to ensure acceptable dimensional stability. Brewers' spent grain (BSG), a by-product of the brewing industry, is used as a structure enhancer in this study. BSG contains a high fibre content up to 50 % (Lynch et al., 2016), which can facilitate fibrous structures during extrusion by entrapping water that would otherwise be located between proteinaceous structures (Diaz et al., 2022).

A growing concern regarding the repurposing of industrial wastes and by-products has led to BSG becoming a more popular ingredient in human foods over the past few years. Moreover, BSG, in addition to proteins and dietary fibres, consists of nutrients which possess health-promoting properties. As a rich dietary fibre source, BSG is particularly valuable for its viscous fibres, which facilitate cholesterol and fat excretion, accelerate transit time, increase faecal weight, and even alleviate ulcerative colitis symptoms (Ikram et al., 2017). Further, the phenolic compounds (e.g., hydroxycinnamic acids) present in spent grain have a wide range of health benefits, including antioxidant, anti-inflammatory, anti-atherogenic, and anticarcinogenic effects (Ikram et al., 2017).

The rationale behind utilising BSG in this study is to address the dual challenges of food sustainability (with a plant-based structural enhancer) and waste (industrial by-product) utilisation. The successful integration of BSG provides an alternative approach for repurposing food waste into a functional ingredient, thereby enhancing its economic value and reducing environmental impact. This simple ingredient composition qualifies the meat substitutes developed in this study as clean-label products, which are characterized by a simple ingredient list free of artificial additives, preservatives, and other synthetic ingredients (Singh et al., 2021). This also aligns with global efforts to promote circular economy principles in food systems. Since BSG is incompatible with faba bean protein due to its low solubility, it has the potential to enhance fibrous structure formation during extrusion by promoting phase separation, with faba bean protein serving as the continuous phase and BSG serving as the dispersed phased (McClements & Grossmann, 2021).

With the aim of utilising only plant-based ingredients and industrially adaptable processing, our prior study investigated enzymatic crosslinking to introduce desired texture (Fan et al., 2024). Aside from our previous study, no research has been conducted on using brewers' spent grain (BSG) as an additive in meat analogues. Furthermore, research on the use of faba bean protein to create meat analogues are limited (do Carmo et al., 2021; Ferawati et al., 2021). The utilisation of these ingredients maximizes sustainability benefits and improves the nutritional profile of meat substitutes. Hence, this study investigates the processability of faba bean-based meat analogues through extrusion while varying moisture content (61 %, 64 %, 67 %) and incorporating BSG as a structural enhancer (0 %, 3 %, 6 %).

The effect of moisture content and integration of BSG on the physicochemical properties of the extrudates (structure, texture, and water retention capacity) was examined. Microstructure observation and cutting strength tests were conducted to determine the degree of fibre formation. Aside from the structure, two other key properties of meat products were evaluated, namely the texture and water retention capacity. For a better understanding of their performance in the construction of meat analogues, faba bean protein and spent grain were assessed for solubility, water absorption capacity, and pasting characteristics. Moreover, by demonstrating how BSG can enhance the structural and textural properties of plant-based meat products, this study paves the way for its application as a cost-effective and environmentally friendly additive in food systems. This would open the door for additive-free products, composed entirely of natural ingredients without the inclusion of synthetic or artificial ingredients, and eventually allow meat substitutes to take the place of traditional animal meat.

2. Materials and methods

2.1. Materials

Faba bean protein isolate was purchased from Australia Plant Proteins Pty Ltd., Australia, which contains 88 % protein, 5–6 % fat, 0.5 % carbohydrates, and 2–3 % ash. Brewers' spent grain flour was purchased from Grainstone Pty Ltd., Australia, containing 50 % dietary fibre, 26 % protein, 9 % fat, 14 % carbohydrates and 0.2 % ash. Considering the aim of this work is to produce a meat analogue with fibrous characteristics, chicken breast meat was selected as the reference product since it is considered one of the healthiest meat options due to its low fat and high protein content (Kralik et al., 2018).

2.2. *Water absorption index and water solubility index of raw ingredients*

Water absorption index (WAI) and water solubility index (WSI) at room temperature of raw ingredients were determined. 1 g of faba bean protein powder or BSG powder was suspended in 10 mL of distilled water, gently mixed at room temperature for 30 min, and then centrifuged at 3000*g* for 15 min (Jakobson et al., 2023). Following centrifugation, the supernatant was carefully decanted, and the sediment was weighed to determine the WAI. The supernatant was collected and dried to determine the dissolved solids that were used to calculate the WSI. WAI and WSI were calculated using Eqs. (1 and 2), respectively. All measurements were conducted in triplicates.

$$WAI = W_S/W_I \tag{1}$$

$$WSI\ (\%) = W_{DS}/W_I*100 \tag{2}$$

where W_S is the weight of the sediment; W_I is the weight of the initial powder weight; W_{DS} is the weight of the dissolved solids.

2.3. *High-moisture extrusion processing*

A laboratory *co*-rotating twin-screw extruder (HAAKE PolyLab OS Extruder, Rheomex PTW 16/40 ThermoFisher Scientific, England) was used to process meat analogue samples. Fig. 1 illustrates the extruder setup along with the screw configuration. The extruder was driven by a motor of 7 kW fitted to a gear box with a 1:5.4 gear ratio. The screw diameter was 16 mm and the screw length to diameter ratio was 40:1. For reducing expansion and promoting fibrous texture formation, a rectangular cooling die (265 mm length × 25 mm width × 5 mm height, made in-house) with a temperature of 35 °C was attached to the end of the extruder.

The extrusion conditions were determined based on preliminary results, which identified a maximum barrel temperature of 145 °C and a screw speed of 200 rpm as optimal for achieving the desired structural and textural properties while ensuring a smooth extrusion process. The high extrusion temperature was achieved using electric heaters attached to thermocouples. The temperature profile across the barrel zones (Fig. 1) was set follows: 45 °C in the feed zone, 60 °C in zone 1, 80 °C in zone 2, 100 °C in zone 3, 120 °C in zone 4, 130 °C in zone 5, 145 °C in zones 6 and 7, 120 °C in zone 8, and 80 °C in zone 9.

As shown in Table 1, three different feed moisture contents with varying levels of faba bean protein isolate (FBP) and brewers' spent grain (BSG) (F1-F9) were prepared to assess the effect of composition on the HMMA properties. In the preliminary experiments, a range of moisture contents was examined to ensure a smooth extrusion and a dimensionally stable extrudate without any breakage and ruptures. It was found that a solids content of less than 33 % produced a porous structure and unstable texture, whereas a solids content greater than 39 % resulted in an excessively rigid texture or caused material blockage within the extruder. Therefore, the feasible solids content range was determined to be 33–39 %. The impact of BSG is evaluated at this solids content range (33 %, 36 %, and 39 %) by varying its incorporation levels (0 %, 3 % and 6 %) in HMMA formulation. The solid ingredients were mixed with the required amount of water prior to extrusion and then fed into the extruder using a single-screw feeder.

Table 1
Formulations for the plant-based meat samples.

Sample	FBP (%)	BSG (%)	Water (%)	FBP:BSG *
F1	33	0		N/A
F2	30	3	67	10:1
F3	27	6		4.5:1
F4	36	0		N/A
F5	33	3	64	11:1
F6	30	6		5:1
F7	39	0		N/A
F8	36	3	61	12:1
F9	33	6		5.5:1

* N/A indicates that the ratio of FBP (faba bean protein isolate) to BSG (brewers' spent grain) is not applicable. In F1, F4 and F7, only faba bean protein is present, and no brewers' spent grain is present.

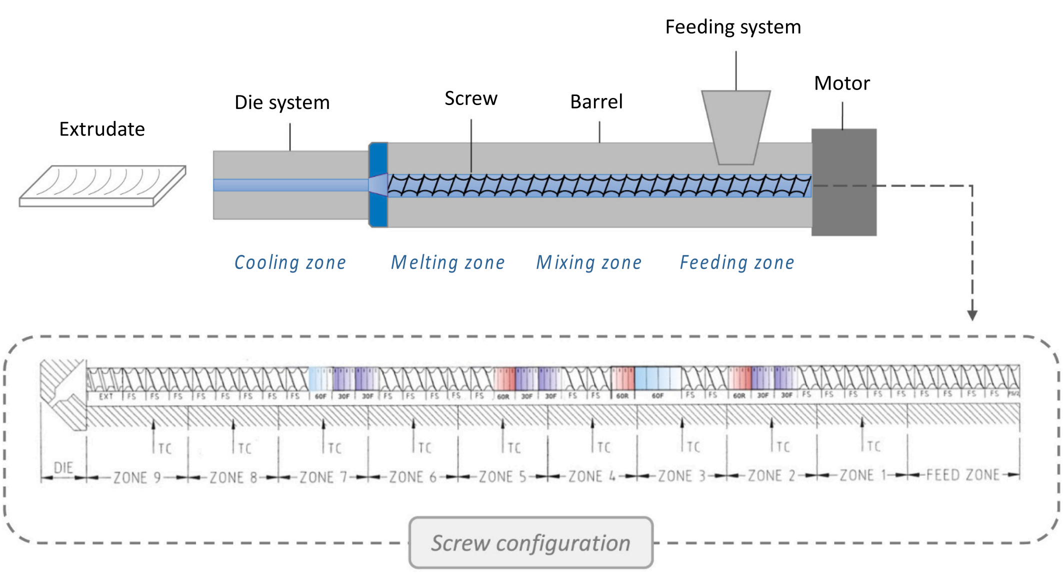


Fig. 1. A schematic diagram of the extruder showing typical elements and screw profile used in this study. The numbers in the screw configuration indicate the angle (in degrees) between the screw elements, while the letters "F" and "R" indicate the forward and reverse orientations of the screw, respectively. "FS" refers to the forward segment that transfers material forward.

2.4. Pasting properties of different FBP:BSG blends

The paste viscosity characteristics of raw material blends with varying ratios of faba bean protein isolate to brewers' spent grain (see Table 1) were investigated by a Rapid Visco Analyser (Model RVA-4, Newport Scientific Pty. Ltd., Warriewood NSW, Australia) according to the AACC Standard Method 76–21.02. In this analysis, 3.5 g powder was dispersed in 25 mL distilled water. It was programmed to run a heating and cooling cycle in which samples were maintained at 50 °C for 1 min, heated to 95 °C in 3.7 min (heating and cooling rate: 12 °C/min), maintained at 95 °C for 2.5 min before cooling to 50 °C in 3.8 min, and finally maintained at 50 °C for 2 min. The curve parameters were evaluated by the RVA software (Thermocline for Windows v2.6, Newport Scientific Pty. Ltd., Warriewood NSW, Australia). Peak viscosity (mPa·s); trough viscosity (mPa·s); final viscosity (mPa·s); pasting temperatures (°C) were recorded. All measurements were conducted in triplicates.

2.5. Scanning electron microscopy (SEM)

A scanning electron microscope (TM4000Plus, Hitachi High-Tech Science Co., Tokyo, Japan) was used to examine the microstructure of the meat analogue samples. The freshly prepared samples were cut into small pieces to fit the specimen stage in the microscope. To ensure a stable structure and conductive surface, specimens were treated prior to observation. First, the specimens were fixed with chemical fixative solution (2.5 % glutaraldehyde in phosphate-buffered saline) to stabilize their structure (Sreejith et al., 2022). The fixed specimens were then rinsed with phosphate-buffered saline and dehydrated by incubating them in a series of ethanol solvents (concentration 30–100 %) (Sreejith et al., 2022). Following dehydration, the specimens were placed in polyporous pots in 100 % ethanol and dried using a critical point dryer (Autosamdri-815, Tousimis Research Co., Rockville, USA) (Sreejith et al., 2022). Dry specimens were mounted on metal stubs with sticky carbon discs, which were coated with a conductive metal (platinum) via a compact coating unit (CCU-010, Safematic GmbH, Zizers, Switzerland). Lastly, the processed specimens were placed in the vacuum chamber of a scanning electron microscope and photographed at an accelerating voltage of 15 kV at a magnification of 800 times.

2.6. Cutting strength measurements

Further to the microstructure observation, a cutting strength test was conducted to quantify the fibrous formation in meat analogues using a texture analyser (TA.XTplus, Stable Micro System Co., Godalming, UK). The test was performed by penetrating a knife blade into the sample to a depth of 6 mm at a speed of 2 mm/s. As shown in Fig. 2, samples were cut longitudinally (lengthwise) and transversely (crosswise) in the direction of the extruder's outflow (Ferawati et al., 2021). If the longitudinal and transverse cuts have similar values, it indicates that the meat analogue sample has a uniform texture and no fibrous layers (Ferawati et al., 2021). The difference value (D value), calculated by subtracting the lengthwise cutting strength (LW) from the crosswise cutting strength (CW), was recorded. All measurements were performed in triplicates.

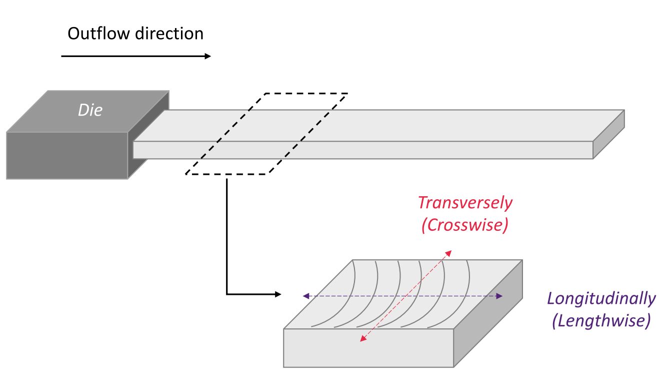


Fig. 2. Cutting directions (crosswise and lengthwise) for cutting strength measurements on high-moisture meat analogue (HMMA).

2.7. Texture

Texture profile analysis (TPA) of the meat analogue samples was performed on a texture analyser (TA.XTplus, Stable Micro System Co., Godalming, UK). A double compression test was conducted using a cylindrical Perspex attachment probe (diameter 10 mm) to evaluate the hardness, cohesiveness, and chewiness of the samples. Samples were cut into cubes with dimensions of 25 × 25 × 5 mm (L x W x H) and equilibrated at room temperature (22–25 °C) for 1 h prior to measurements. The following parameters were used: 1 mm/s pre-test speed, 2 mm/s test and post-test speed, 3 mm target distance, 5 s wait time and 5 g trigger force (Dick et al., 2021). Based on the two-cycle force-time curve obtained, the hardness, cohesiveness and chewiness of the samples were determined using the Eqs. (3–5), respectively. All measurements were performed in triplicates.

$$\text{Hardness} = \text{F}_{max\rightarrow \text{C1}} \tag{3}$$

$$\text{Cohesiveness} = \text{A}_2/\text{A}_1 \tag{4}$$

$$\text{Chewiness} = \text{F}_{max\rightarrow \text{C1}} {}^*\text{A}_2/\text{A}_1{}^*\text{D}_2/\text{D}_1 \tag{5}$$

where $F_{max\rightarrow C1}$ is the maximum force of the first compression; A_1 or A_2 is the area of work during the first or second compression; D_1 or D_2 is the distance of the detected height during the first or second compression.

2.8. Water retention capacity

The water retention capacity of the sample was determined using a centrifugal method. The meat analogue sample (approximately 5 g) was placed in a 50 mL tube with tissue at the bottom (Sakai et al., 2021). In a centrifuge (5702R, Eppendorf Corporate, Hamburg, Germany), the tube was centrifuged at 3000 *g* for 10 min at 35 °C (Sakai et al., 2021). The water retention capacity (WRC) was calculated using Eq. (6). All measurements were conducted in triplicates.

$$WRC\ (\%) = W_2/W_1 \tag{6}$$

where W_2 is the weight after centrifugation and W_1 is the weight before centrifugation.

2.9. Statistical analysis

One-way ANOVA (at a 95 % confidence level) and post-hoc Tukey's test (at a 5 % significance level) were used to determine significant differences, using GraphPad Prism software version 9.5.1.

3. Results and discussion

First, the raw ingredients (FBP and BSG) were analysed for their hydration properties to provide information on the inherent functional properties, which could significantly affect their behaviour during the extrusion process and, eventually, the characteristics of the final product. Subsequently, different FBP/BSG blends were evaluated for their pasting properties. A preliminary extrusion trial was conducted to determine the feasible solids content range for obtaining extrudates with the desired properties. Following the trial, three different levels of solids content with varying ratios of faba bean protein and BSG were assessed for their effects on the structure, texture and water-holding capacity of PBMA. The formulations with ideal fibre formation were identified using scanning electron microscopy (SEM) and cutting strength measurements. Ideal fibre formation is characterized by a well-defined, fibrous structure that provides a meat-like texture. This can be observed through well-aligned fibres in SEM images and a significant difference between lengthwise and crosswise cutting strengths in cutting strength measurements. In addition, the effect of solids content and BSG incorporation on the texture and water retention capacity was assessed.

3.1. Raw ingredients analysis

3.1.1. Water solubility index and water absorption index

Water solubility index (WSI) and water absorption index (WAI) are two of the most important functional properties of ingredients that determine the quality of the final meat analogues. Both are indicators of the hydration properties. The water solubility index reflects the ability of an ingredient to dissolve in water, while the water absorption index indicates its ability to absorb water. Table 2 presents the hydration properties of faba bean protein and brewers' spent grain.

Faba bean protein displayed a significantly higher water solubility index (50.0 %) than the brewers' spent grain (1.3 %). Solubility is a prerequisite for the construction of the food matrix. A high solubility of the protein promotes the formation of a uniform network, allowing meat analogues to attain comparable textural and structural properties as real meat. Unlike faba bean protein, BSG serves as a structural enhancer responsible for strengthening matrix integrity. BSG is a lignocellulosic material composed of 50 % fibre, which is a rich source of cellulose, hemicellulose, and lignin (Ikram et al., 2017). Plant fibres are characterized by a high degree of crystallinity, making them notoriously difficult to dissolve (Mohammed et al., 2023). The incorporation of insoluble components may promote the formation of fibrous structures through phase separation (McClements & Grossmann, 2021).

Conversely, faba bean protein had a lower water absorption index than brewers' spent grain, with values of 3.9 and 5.2, respectively. The two main components of brewers' spent grain, cellulose and hemicellulose, have been demonstrated to be effective at absorbing water, which is attributed to the hollow cavities present within them (Mohammed et al., 2023). In addition, the inherent high crystallinity of cellulose and hemicellulose allows them to retain liquid within the interfibrillar region (where they are located), further enhancing their ability to absorb water (Mohammed et al., 2023). Through its superior capacity for absorbing water, BSG may facilitate fibrous structures during the extrusion process by capturing water that would otherwise be located between proteinaceous structures (Diaz et al., 2022).

Briefly, faba bean protein and brewers' spent grain exhibit different hydration properties, enabling them to perform their respective functions in the construction of plant-based meat. Faba bean protein creates the matrix, forming a protein-rich phase (Dekkers et al., 2018). The low solubility of brewers' spent grain makes it incompatible with faba bean protein, forming a polysaccharide-rich phase (Dekkers et al., 2018). During extrusion, high shear force causes proteins to deform and elongate in the direction of the extruder's outflow, with polysaccharides dispersed throughout the protein matrix (Huang et al., 2022). A combination of two incompatible biopolymers can strengthen phase separation, thereby facilitating fibrillation (Beniwal et al., 2021). Moreover, the superior water absorption capacity of brewers' spent grain enables it to trap water that would otherwise be located between proteins, facilitating fibrillation even more effectively (Diaz et al., 2022).

Table 2
Water solubility index (WSI) and water absorption index (WAI) of raw ingredients.*

	Faba bean protein	Brewers' spent grain
WSI	50.0 % ± 1.7 % [a]	1.3 % ± 0.6 % [b]
WAI	3.9 ± 0.1 [b]	5.2 ± 0.2 [a]

* Different lowercase letters within rows indicate a significant difference ($p < 0.05$).

3.1.2. Pasting properties of FBP/BSG blends

To gain insight into the pasting behaviour, a rapid viscosity analysis was conducted to monitor the changes in viscosity caused by heating and cooling food ingredients in water. This analysis involves the application of shearing, heating, and cooling over time to produce a viscosity curve of the material, which indicates its pasting properties. The pasting behaviour of different FBP/BSG blends is displayed in Fig. 3. To understand the influence of BSG on pasting behaviour, formulations designed at the same moisture content for extrusion (see Table 1) are compared in one plot for clarity. In Fig. 3, a significant reduction in viscosity was observed when a moderate amount of BSG was added (F2, F5 and F8, 3 % BSG in the designed formulation) compared with pure faba bean protein powder (F1, F4 or F7). The viscosity further decreased as BSG incorporation increased (F3, F6 and F9, 6 % BSG in the designed formulation), exhibiting flat curves rather than the fluctuating curves observed in the other blends.

Table 3 provides the curve parameters. Peak viscosity and trough viscosity refer to the maximum and minimum viscosities attained during heating or pasting. Viscosity at the end of the pasting cycle is final viscosity. It was found that the pure FBP sample (F1, F4, F7) had the highest peak, trough, and final viscosities, followed by the blends containing a moderate amount of BSG (F2, F5, F8) and the blends containing a high amount of BSG (F3, F6, F9). The results indicate that BSG addition adversely affected the pasting properties of the samples, and this effect was more pronounced with higher BSG levels. By increasing the proportion of BSG in FBP/BSG blends, the higher fibre content resulted in a decreased sample viscosity. It has previously been demonstrated that BSG has an extremely low solubility compared to FBP (Table 2), which indicates that it is difficult to dissolve in water. Viscosity is well known to be a concentration-dependent property, the higher the concentration of soluble components, the more viscous the system (Kar & Arslan, 1999). As a result, the reduced faba bean protein concentration and the insolubility of BSG will inevitably lead to a reduction in viscosity.

Pasting properties refer to the changes in food ingredients that occur when heat is applied in the presence of water, which significantly impacts the final product. The pasting temperature refers to the point at which the sample increases viscosity. According to Table 3, no pasting temperature was recorded when 6 % BSG was incorporated, suggesting that the blends lost their pasting capabilities after adding 6 % BSG. The pasting profiles (Fig. 3) confirm this statement, showing that the viscosity of the blends with 6 % BSG (F3, F6, F9) remained stable throughout the heating process without exhibiting any peaks. With the addition of 3 % BSG, the pasting characteristics were reduced, although certain pasting properties were retained, as evidenced by the detection of pasting temperature. A similar phenomenon was reported by Nikolić et al. (2022), who investigated the effect of dietary fibre content on the pasting properties of wheat flour. Their findings indicated that refined wheat flour with low fibre content exhibited superior pasting properties compared to whole-grain wheat flour with high fibre content.

To facilitate fibrillation during extrusion while maintaining the desired pasting properties, BSG (structural enhancer) must be applied in an appropriate quantity. Compared to a 6 % BSG incorporation, a 3 % incorporation offers greater potential to enhance the structure of meat analogues without significantly affecting the desired pasting characteristics. Supporting evidence is provided by structure images (*Section 3.2.1*).

3.2. Effect of composition (BSG and moisture content) on HMMA

3.2.1. Structure

Fig. 4 presents the visual appearance and microstructures of cross-sections of the meat analogue samples prepared with varying levels of faba bean protein isolate (FBP) and brewers' spent grain (BSG) with a slice of chicken meat as the reference animal meat structure. Since the

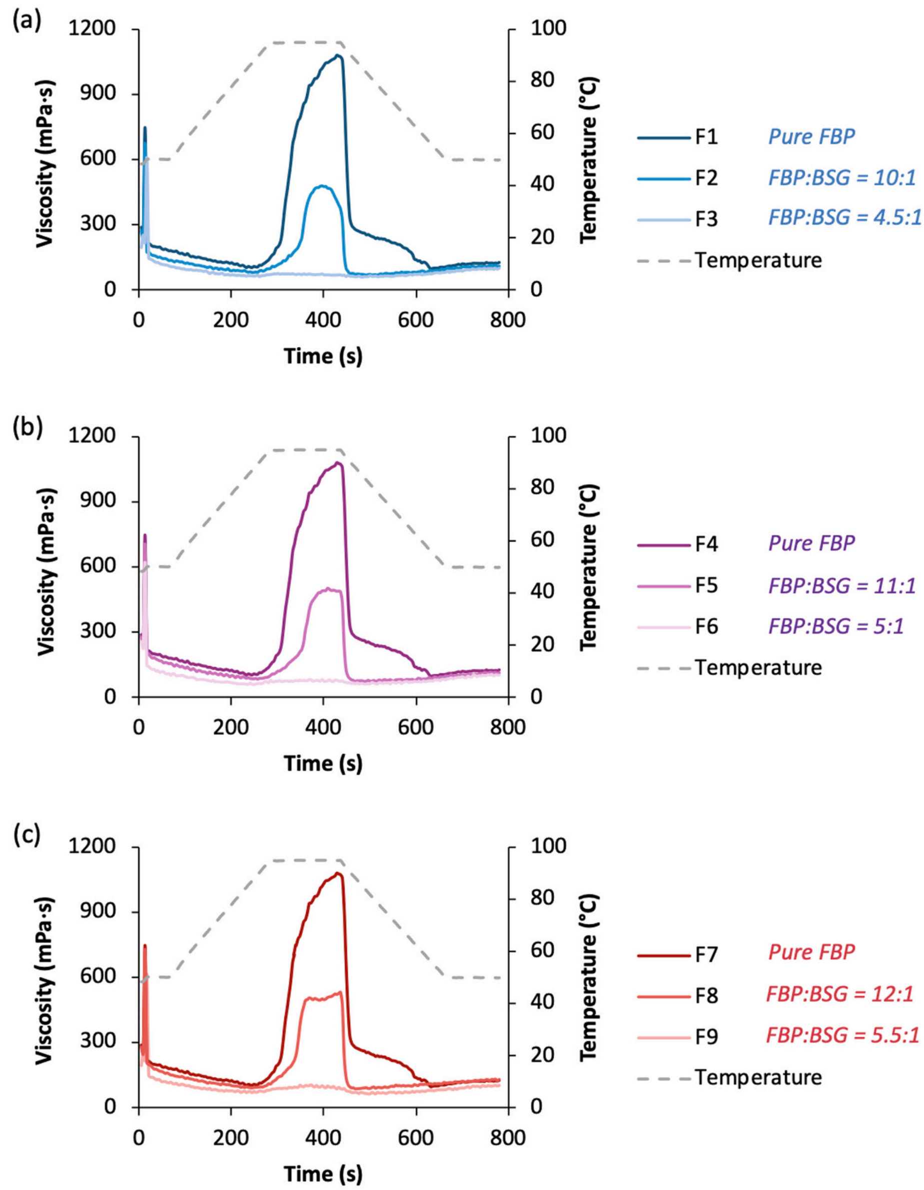


Fig. 3. RVA pasting profile of raw material blends with varying ratios of faba bean protein isolate (FBP) to brewers' spent grain (BSG). Formulations that are designed to achieve the same moisture content (%) for extrusion are presented in one graph for comparison: (a) F1-F3 at 67 %, (b) F4-F6 at 64 %, (c) F7-F9 at 61 %.

focus of this study is to mimic the microstructure and texture properties comparable to animal meat, no colourant was added to the samples for colour comparison. As shown in Fig. 4a, it is evident from the cross-sections that samples containing only faba bean protein isolate (F1, F4, F7) could produce fibrous structures perpendicular to the extrusion direction. This suggests that a fibrous structure can be introduced in the faba bean protein-based meat analogue formulation through extrusion, demonstrating the potential of faba bean protein as the primary protein source for the development of fibrous meat analogues.

When 3 % of BSG was introduced (F2, F5, F8) (in Fig. 4a), more fibrous structures were observed indicating the influence of BSG on the microstructure. This fibrous microstructure formation is slightly comparable to that of chicken meat. However, a further increase in the BSG to 6 % (F3, F6, F9) resulted in more granular structures than fibrous structures on the cross-section surfaces (Fig. 4a). Microstructures in Fig. 4b, support these macrostructural observations, showing that samples with 3 % BSG had a more uniform distribution of fibres than those with the same solids content. Therefore, a moderate BSG loading (3 %) facilitated the formation of finer and straighter fibres, whereas a higher loading (6 %) disrupted fibre formation resulting in granular particles on cross section. The results of the water absorption index (Table 2) indicate that BSG is more effective at absorbing water than

Table 3
Pasting properties of faba bean protein isolate (FBP) and brewers' spent grain (BSG) blends.*

	FBP: BSG	Peak viscosity (mPa·s)	Trough viscosity (mPa·s)	Final viscosity (mPa·s)	Pasting temperature (°C)
F1/ F4/ F7	Pure FBP	1062 ± 23 [a]	95 ± 2 [a]	127 ± 3 [a]	95 ± 0 [a]
F2	10:1	486 ± 12 [bc]	67 ± 2 [bc]	110 ± 6 [b]	95 ± 0 [a]
F3	4.5:1	108 ± 11 [d]	60 ± 2 [c]	102 ± 5 [c]	–
F5	11:1	513 ± 5 [b]	73 ± 2 [bc]	114 ± 1 [b]	95 ± 0 [a]
F6	5:1	111 ± 4 [d]	58 ± 4 [c]	102 ± 6 [c]	–
F8	12:1	443 ± 21 [c]	83 ± 12 [ab]	123 ± 4 [ab]	95 ± 0 [a]
F9	5.5:1	119 ± 1 [d]	64 ± 0 [bc]	106 ± 2 [c]	–

* Different lowercase letters within columns indicate a significant difference ($p < 0.05$).

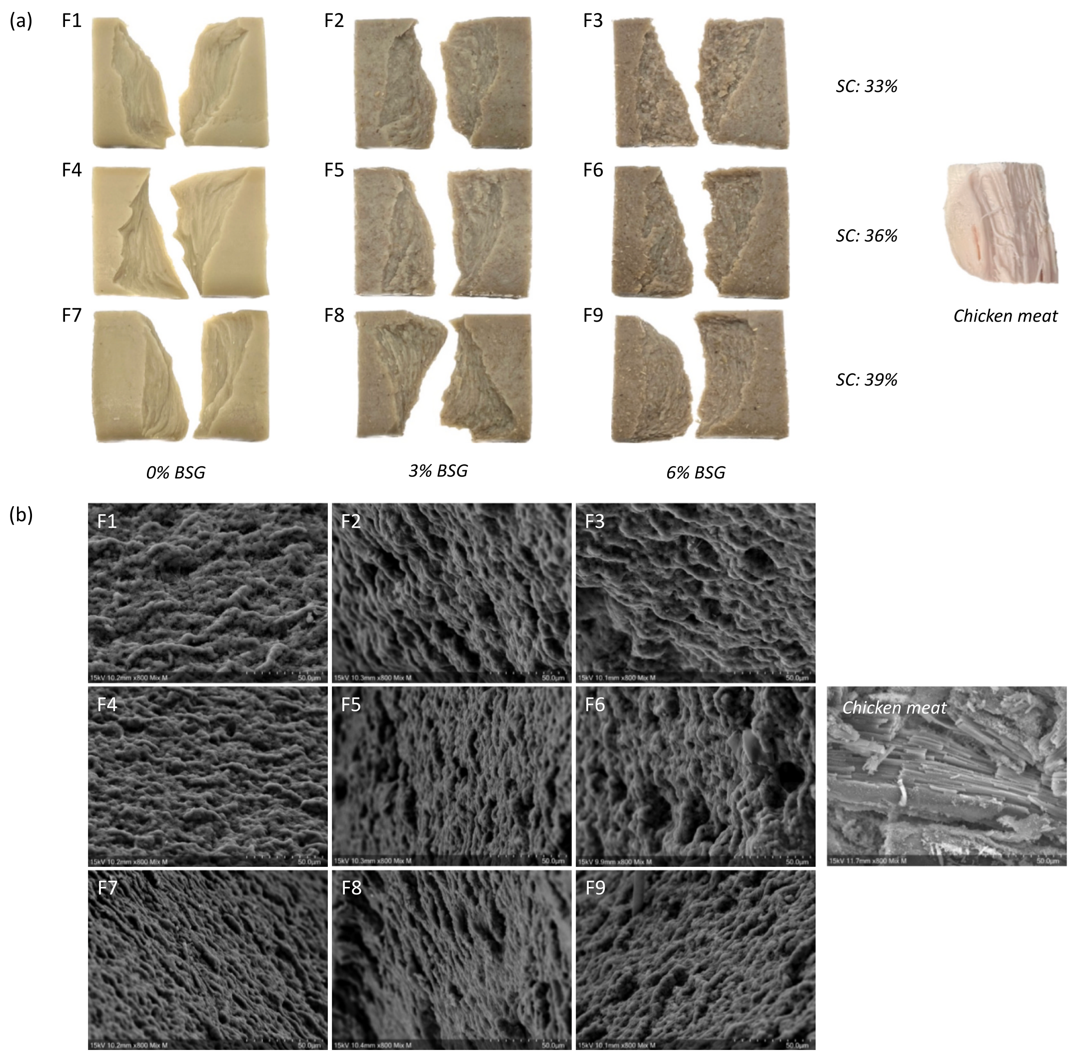


Fig. 4. Comparison of (a) visual observation and (b) cross-sectional scanning electron microscopic images (x 800 magnification) of the high-moisture plant-based meat analogue (HMMA) samples processed under the same extrusion conditions (145 °C, 200 rpm), and a chicken breast meat. Samples with the same brewers' spent grain (BSG) content and varying solids content (SC) are arranged vertically.

FBP. The moderate addition of BSG contributes to the entrapment of free water and the prevention of its accumulation between proteinaceous structures, thereby promoting the formation of fibrous structures during extrusion (Diaz et al., 2022; Zhang et al., 2022). However, excessive fibre addition to high-protein-containing extrudates interferes with protein molecular chain aggregation, disrupting the network formation

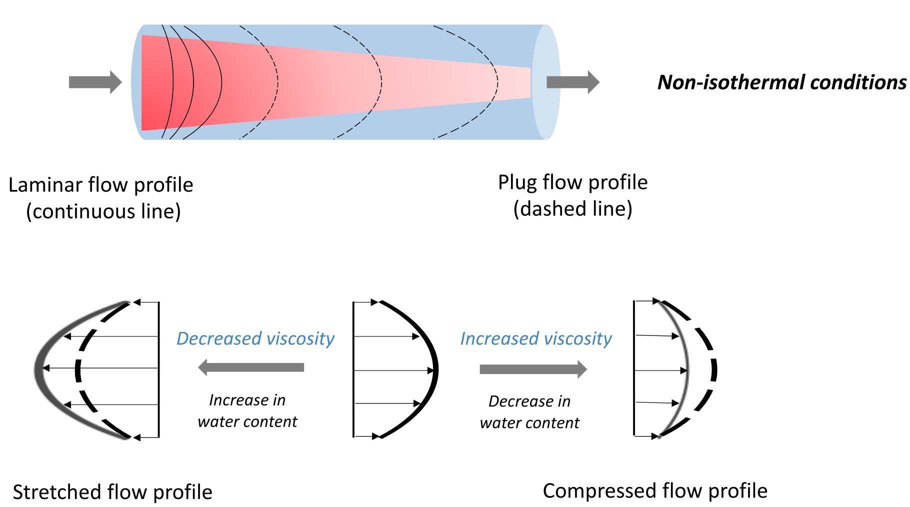


Fig. 5. Flow conditions of melting mass in the long cooling die.

(Diaz et al., 2022; Lynch et al., 2016). The RVA results also provide explanations for this phenomenon. In general, high BSG additions severely impair pasting properties, or in other words, reduce viscosity to a great extent, while moderate additions retain desirable pasting properties with slight viscosity reductions. An increase in viscosity facilitates the formation of fibrous structures. The study conducted by Kaunisto et al. (2024) developed a thermodynamic finite element model of the fibre formation process during extrusion of high-moisture meat analogues, demonstrating that a higher viscosity can facilitate fibrous structure formation by affecting Cahn-Hilliard mobility.

Additionally, higher solids content (SC) facilitated the development of a more apparent fibrous structure, as indicated by the column direction (Fig. 4b). Among the samples containing 3 % BSG, sample F8 closely resembled chicken meat in terms of microstructure (Fig. 4b). This difference could be attributed to variations in viscosity due to differing water content.

Fig. 5 illustrates the flow of molten feed within the cooling die channel. As the melt temperature decreases along the cooling die channel, the plasticized mass solidifies, affecting the flow characteristics of the extrudate. Specifically, under non-isothermal conditions, the flow profile elongates until solidification of the molten mass occurs (Zhang, Zhang, et al., 2023). Subsequently, the solidified material retains a plug flow state after passing through the cooling channel (Zhang, Zhang, et al., 2023). The solidified extrudate is then shaped at the die exit according to the desired product appearance. The flow behaviour of the material during solidification plays a critical role in fibre formation. When feed material contains a higher solids content, its viscosity increases, allowing for greater compression of the melting feed during solidification, resulting in a more defined fibrous structure (Zhang, Zhang, et al., 2023). Additionally, a higher solids content facilitates a higher degree of polymerization, further enhancing the fibrous structure (Chen et al., 2011).

3.2.2. Cutting strength

The influence of extrusion on the fibrous structure formation, was analysed by measuring the 'cutting strength' of the extrudates. Fig. 6 compares the differences in cutting strength measured when samples are cut longitudinally (lengthwise) and transversely (crosswise) in the direction of the extruder's outflow. In general, a greater difference between lengthwise (LW) and crosswise (CW) cutting strengths indicates a more pronounced fibrous structure *(as described in Section 2.6)*. The difference value (D value) is calculated by subtracting the lengthwise cutting strength from the crosswise cutting strength. A positive D value indicates greater crosswise cutting strength while a negative value indicates greater lengthwise cutting strength.

Samples with a D value exceeding 1 N (D value >1) possess a noticeable fibrous structure, namely F2 (1.31 N), F5 (1.98 N), F7 (1.25 N) and F8 (2.33 N). Notably, three of these samples (F2, F5 and F8) incorporated 3 % BSG, with the formulation having the highest solids content (F8) displaying the most prominent fibrous structure. These observations align with the structural findings (*Section 3.2.1*). To create anisotropic fibrous structures, it is necessary to introduce two incompatible biopolymers, reinforcing the phase separation between the plant proteins, with one phase acting as a dispersed phase and one phase acting as a continuous phase. Polysaccharides have been shown to play a crucial role in altering the conformation of plant proteins within the extruder (Beniwal et al., 2021; Kyriakopoulou et al., 2019; Kyriakopoulou et al., 2021; McClements & Grossmann, 2021).

Fig. 7 illustrates the conformational changes of protein which take place during the extrusion process. These changes can be divided into three stages: unfolding, aligning, and cross-linking (Chen et al., 2023). A schematic diagram of equipment for extrusion processing is shown in Fig. 1. Depending on the function of each section, the extruder can be divided into feeding, mixing, melting, and cooling zones. In the mixing and melting zones, biopolymer materials are plasticised to produce a semi-solid mass in the barrel under the combined effects of heat, pressure and mechanical shear, where the spherical structure of proteins is unfolded through the hydrolysis of peptide bonds (Samard et al., 2019).

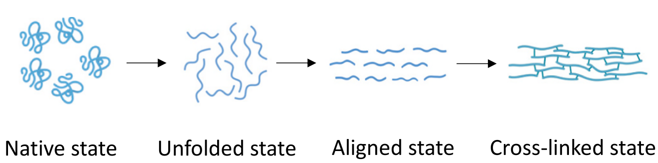
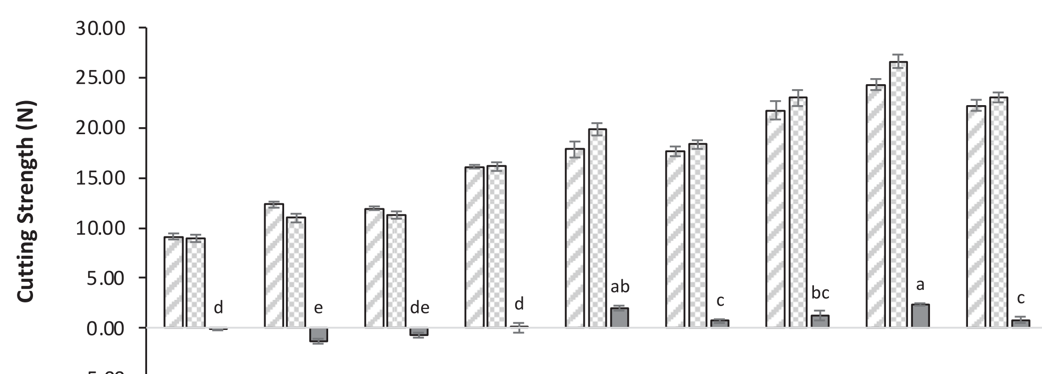


Fig. 7. Schematic illustration of fibrous structure formation under shear forces and heating during the extrusion process.



	F1	F2	F3	F4	F5	F6	F7	F8	F9
Lengthwise (N)	9.11	12.34	11.93	16.10	17.84	17.62	21.73	24.30	22.21
Crosswise (N)	8.99	11.03	11.27	16.14	19.82	18.34	22.98	26.63	23.03
CW-LW	-0.12	-1.31	-0.66	0.04	1.98	0.72	1.25	2.33	0.82
Brewers' spent grain content	*0%*	*3%*	*6%*	*0%*	*3%*	*6%*	*0%*	*3%*	*6%*
Solids content	*33%*			*36%*			*39%*		

Fig. 6. Differences in the cutting strengths measured lengthwise and crosswise of meat analogue samples with varying formulations (F1-F9), where different alphabets (lower case) above the bars indicate a significant difference ($p < 0.05$).

As a result, the denatured proteins lose their tertiary and higher structures, transitioning to a "*molten globule*" state. High shear forces cause these meta-stable proteins to deform easily and elongate along the mass flow direction. Upon reaching the die system (cooling zone), a laminar flow of melt occurs in the longitudinal direction of the die due to the gradual cooling of the extrudate, where protein molecules are realigned to form a fibrous structure (Ryu, 2020; Zhang, Zhao, et al., 2023). During this process, the elongated polysaccharides (as the dispersed phase) are embedded within the protein matrix (as the continuous phase) (McClements & Grossmann, 2021). As cooling progresses, fibrous proteins gradually lose flexibility and rapidly aggregate due to hydrophobic interactions, with hydrophilic polysaccharides inhibiting lateral fibrous protein aggregation, thereby enhancing extrudate and therefore, improving the fibre degree of the extrudate (Huang et al., 2022).

Interestingly, the formulation consisting solely of 39 % faba bean protein (F7) achieved a fibrous level comparable to formulations with BSG (3 %) incorporated. This suggests that faba bean protein holds promise as a protein source for developing meat analogues with fibrous structures. The heat-induced gelation of proteins, critical for meat analogues production, relies on various bonding and interaction patterns, including hydrogen and covalent bonds, along with electrostatic and hydrophobic interactions (Dangi et al., 2022). Gels derived from faba bean protein have been found to display significant interactions in terms of compressive stress and strain at fracture, contributing to superior gelling performance (Dangi et al., 2022). Due to the superior gelling properties of faba bean protein, an interlaced, fibrous structure can be achieved with minimal textural additives, allowing additive-free or more sustainable formulations (Nivala et al., 2021; Raikos et al., 2014).

Moreover, the D values for formulations F1-F3 are negative, while those for the remaining formulations are positive, suggesting varying orientations of fibrous formations within the matrix. The flow behaviour within the cooling die significantly influences fibre formation during solidification. As depicted in Fig. 5, a decrease in viscosity elongates the laminar flow profile, while an increase in melt viscosity compresses it

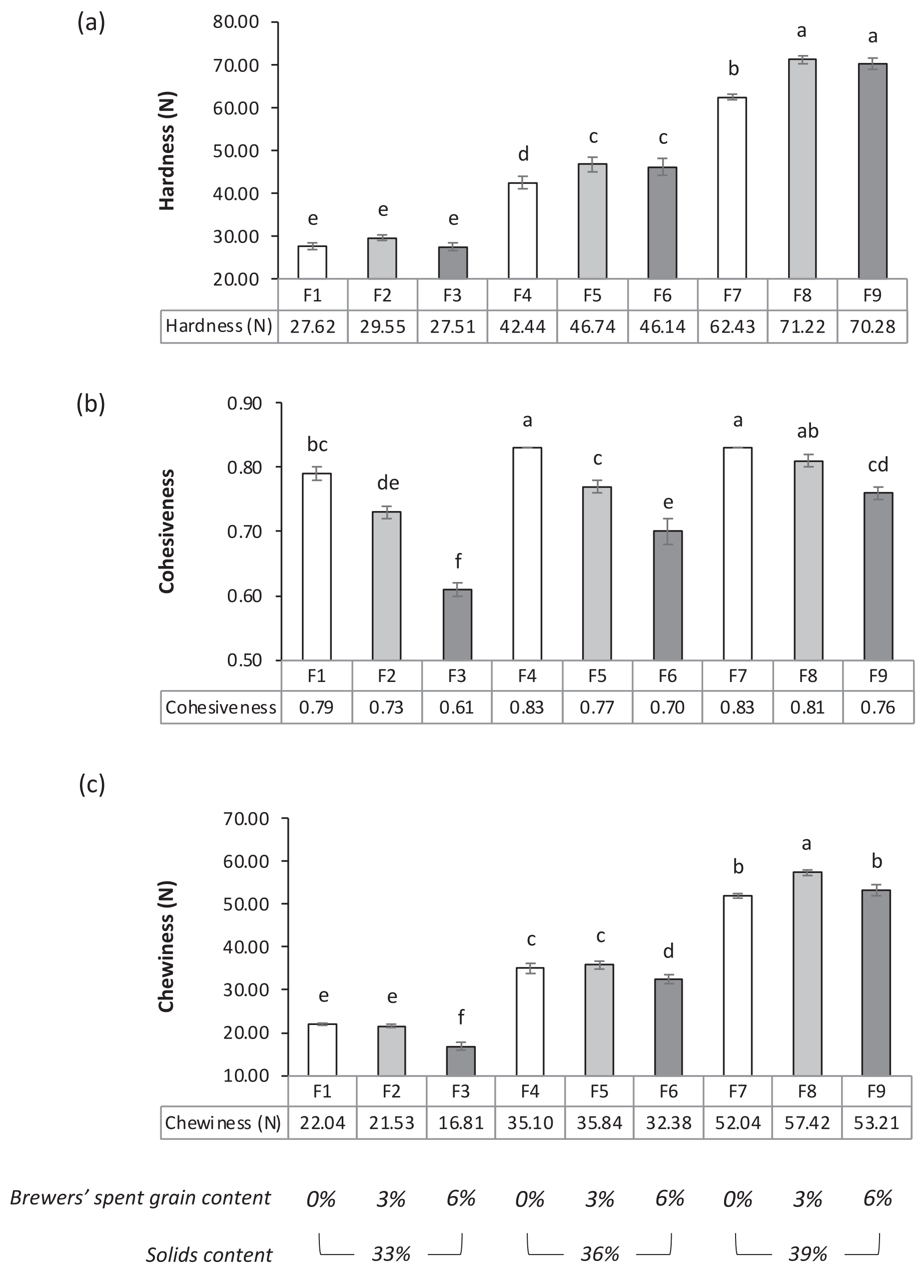


Fig. 8. TPA results (hardness, cohesiveness, and chewiness) of the meat analogue samples with varying formulations (F1-F9). Samples with the same brewers' spent grain content are denoted by same-coloured bars, where different alphabets (lower case) above the bars indicate a significant difference ($p < 0.05$).

due to increased fluid friction (Zhang, Zhang, et al., 2023). The higher water content in F1, F2 and F3 resulted in lower viscosity, causing the laminar flow profile to stretch within the cooling die channel. Consequently, specimens exhibited lower resistance to cutting blade intrusion in the crosswise direction compared to the lengthwise direction (Fig. 2).

It is worth noting that the samples containing 3 % BSG (F2, F5 and F8) tend to have greater cutting strength than equivalent samples with the same solids content. This phenomenon can be explained by the results of the texture analysis relating to hardness (*Section 3.2.3*).

3.2.3. Texture

To determine the effects of BSG content and moisture level on the texture of meat analogues, hardness, cohesiveness, and chewiness were assessed (Fig. 8). Hardness is an indicator of meat products' toughness and maturity, which is determined by their resistance to deformation (Jonkers et al., 2021). Cohesiveness refers to the ability to cohere or bind together under a compressive or tensile stress, which describes the internal bonding and structural integrity (Rosenthal & Thompson, 2021). As for chewiness, it is defined as a sensation of sustained, elastic resistance brought about by laborious mastication, which indicates the effort required to chew them into a swallowable form (Godschalk-Broers et al., 2022). Since all three of these textural properties are critical to the sensory experience of meat products, they are frequently evaluated in meat studies.

The incorporation of 3 % BSG increased the values for hardness and chewiness, whereas the values decreased when the BSG was increased to 6 % (Fig. 8a & 8c). A simple formulation of plant proteins typically fails to achieve the desirable adhesiveness for meat analogue products, leaving a coarse mixture with poor consistency and inferior mouthfeel (Huang et al., 2022). Hence, it is imperative that a binding agent be incorporated into the construction of meat analogues to ensure acceptable dimensional stability. The incorporation of polysaccharides like crude fibre can improve the protein network structures during the extrusion process by combining with the exposed reaction sites of the protein molecular chain (Zhang et al., 2022). The excessive addition of crude fibre, however, adversely affects the protein network by interfering with the aggregation behaviour of protein molecular chains. In addition, Chiang et al. (2019) reported that there is a direct correlation between the fibre degree and the level of texturization, hardness, and chewiness of the extrudates. The presence of abundant fibrous structures is responsible for a harder and chewier texture. In terms of cohesiveness (i.e., the strength of the internal bonds that constitute the body of the product), higher BSG content was associated with lower values (Fig. 8b). A possible explanation is that BSG was unable to substitute for the strong internal bonds formed by the faba bean protein component upon extrusion (Diaz et al., 2022).

Further, a noticeable improvement in all three textural properties was achieved following the increase in solids content from 33 % to 36 % (F1-F4, F2-F5, F3-F6) as well as from 36 % to 39 % (F4-F7, F5-F8, F6-F9). The process parameters play an important role in determining the texture of the end product through their influence on system parameters, such as mechanical energy input and mean residence time. Feed moisture content is one of the most critical process parameters. By serving as a lubricant, water reduces the viscosity of meat-analogue dough, which further reduces the friction generated between the material, the barrel, and the screw during extrusion cooking (Ryu, 2020). In this case, an increase in solids content (or a decrease in water content) increased the residence time within the extruder, increased the thermomechanical energy input applied to the extrudate, and ultimately improved the agglomeration level in the final product (Chen et al., 2010; Chen et al., 2011). Another explanation for this phenomenon is that samples formed by coagulation during thermal processing differ in density. Two chemical reactions were involved in the thermal processing of the protein samples: denaturation, which is a permanent alteration of the protein structure, and coagulation, which is the setting of the protein (Lee et al., 2016). Upon reaching denaturation temperature, chemical bonds (hydrogen and disulphide bonds) are broken, leading to the unravelling of the helical structure of proteins (Cozzone, 2002). Coagulation occurs after denaturation, in which unfolded protein molecules form aggregates as a result of intermolecular interactions. Due to stronger intermolecular interactions, greater protein content leads to denser aggregates or, more precisely, a more extensive network of protein cross-links, which in turn improves the mechanical properties of meat analogues (Diaz et al., 2022).

3.2.4. Water retention capacity

The water retention capacity (WRC) of a food product is a measure of its ability to retain water, which is a critical factor affecting meat quality and yield. From consumers' perspective, meat products with a low water retention capacity are perceived as having inferior palatability due to their lack of juiciness and tenderness (Bowker, 2017). Moreover, it reduces the visual appeal of packages due to excess purging (Bowker, 2017). From manufacturers' perspective, an enhanced water retention capacity can improve protein functionality, increase processing and cooking yields, and enhance the adhesion of marinades to meat (Bowker, 2017). For these reasons, a high-water retention capacity is always desirable in meat products.

Fig. 9 depicts the effect of BSG and solids contents on the water

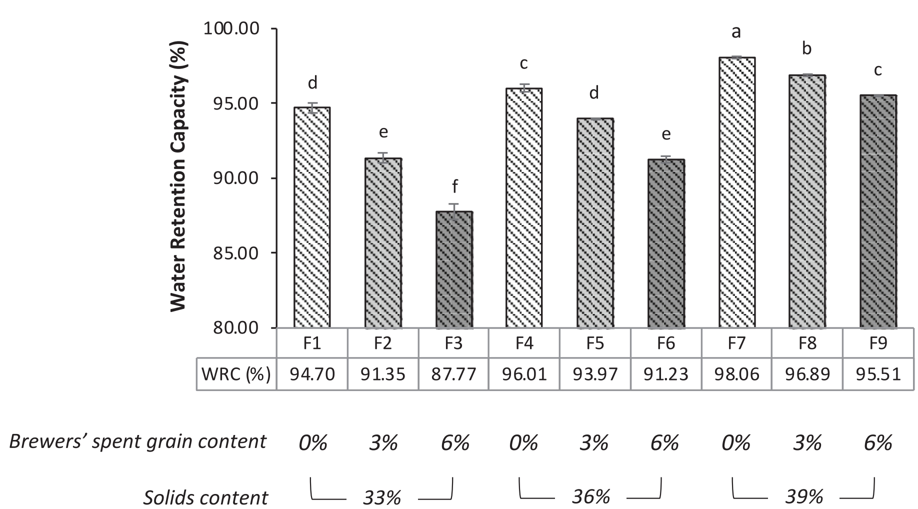


Fig. 9. Water retention capacity of samples with varying formulations (F1-F9). Samples with the same BSG content are denoted by same-coloured bars, where different alphabets (lower case) above the bars indicate a significant difference ($p < 0.05$).

retention capacity of the HMMA samples. As was evident, the inclusion of BSG reduced the samples' ability to retain water, the greater the amount of BSG incorporated, the lower the water retention ability. BSG is a lignocellulosic material that contains approximately 28 % lignin, 28 % non-cellulosic polysaccharide (primarily arabinoxylans), and 17 % cellulose (Mussatto et al., 2006). Compared with protein components, fibre components such as cellulose and lignin are extremely poor at retaining water (Boulos et al., 2000; Muhonen et al., 2022). It is due to the presence of hollow cavities or capillaries within the fibre components, as previously discussed in *Section 3.1.1. Water solubility index and water absorption index*. Additionally, samples with a higher solids content are more capable of retaining water. In the presence of greater solids, protein-protein and protein-water interactions became intense, therefore facilitating the water retention capacities of the meat analogues (Zhang et al., 2017). The study conducted by Zhou et al. (2022) compared various commercial plant-based meat analogues with animal meat products, reporting that the water retention capacity ranged from 88 % to 98 %. Similarly, the formulations developed in this study, with a water retention capacity ranging from 87.77 % to 98.06 %, fall within or closely align with the acceptable range observed in meat products.

Briefly, a moderate inclusion of BSG (3 %) promoted fibre formation by strengthening phase separation. This resulted in improved texture characteristics such as hardness and chewiness, but at the expense of reduced cohesiveness. In the presence of excessive amounts of BSG (6 %), fibre formation was disrupted by interfering with the aggregation behaviour of protein molecular chains, ultimately adversely affecting all three texture properties, namely hardness, cohesiveness, and chewiness. For use as a structure enhancer, BSG must be applied in an appropriate amount to enhance fibrillation during extrusion while maintaining the desired pasting properties. According to RVA results, 3 % BSG retained pasting properties with only slight reductions, whereas 6 % BSG resulted in deteriorated pasting properties. Additionally, the increase in solids content led to a more defined structure and improved texture by increasing compression of the melting feed during solidification and allowing a higher degree of polymerization. In terms of water retention capacity, an increase in the BSG incorporation impaired the capacity, while an increase in the solids content enhanced it. Considering the improved structure, texture, and water retention capacity, extrusion of meat analogues with a higher solids content may be an optimal approach for future studies.

4. Conclusions

This study demonstrated the potential of faba bean protein as a primary protein source for constructing plant-based meat analogues without any synthetic processing aids or rheology modifiers. In doing this, this study has also underscored the beneficial impact of brewers' spent grain (BSG) on the physical attributes of the final extruded product. The incorporation of 3 % BSG promoted dimensionally stable meat analogue samples with fibrous formation and enhanced the microstructural and textural characteristics. However, further increase in BSG loading level inhibited such fibrous formation due to poor protein aggregation which impacted adversely on the internal structure. Moreover, when the BSG content was maintained at a constant level, this study observed an increase in the fibrous structure with higher solids content, upon greater compression of the melting feed during solidification.

Overall, this study has successfully pioneered a clean-label meat analogue formulation with a fibrous structure comparable to chicken breast meat using only a sustainable plant protein (faba bean protein isolate) and upcycled food waste (brewers' spent grain) through extrusion. The findings of this study will have a profound impact on future research by overcoming the key challenge in replicating meat's sensory properties, namely the creation of meat-like fibrous structures from globular plant proteins. This will pave the way for developing plant-based products with properties equivalent to animal meat, offering a sustainable and environmentally responsible alternative to traditional meat products.

CRediT authorship contribution statement

Yue Fan: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Conceptualization. **Pratheep K. Annamalai:** Writing – review & editing, Supervision, Resources, Conceptualization. **Bhesh Bhandari:** Writing – review & editing, Supervision, Conceptualization. **Sangeeta Prakash:** Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization.

Declaration of competing interest

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

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Data availability

Data will be made available on request.

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