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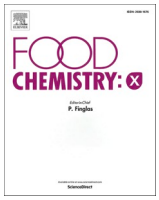

Potential of hydrolyzed wheat protein in soy-based meat analogues: Rheological, textural and functional properties

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ABSTRACT

Hydrolyzed proteins, which are considered to possess significant bioactive properties such as antioxidant and high digestibility, have garnered increasing interest as food ingredients. This study investigates the feasibility of using hydrolyzed wheat gluten (HWG) and soy protein concentrate (SPC) in various ratios to create meat analogues using high-moisture extrusion technology. Results indicate that meat analogues with 40% HWG addition to SPC have a better texture and greater similarities in terms of hardness, chewiness, and toughness to chicken meat than meat analogues with 40% wheat gluten (WG) addition to SPC. Additionally, the meat analogues with HWG showed high antioxidant capacity, protein digestibility, and amino acid composition, indicating potential health benefits. These findings indicate that HWG could serve as a texture modifier to improve both the texture and nutritional content of meat analogues.

Introduction

The consumption of meat has increased alongside human consumption levels. However, studies indicate that a diet high in red meat is associated with various health issues such as obesity, type 2 diabetes, cardiovascular disease, stroke, and colorectal cancer. This is primarily due to the high fat content present in meat, which is considered a risk factor for health (Willett, et al., 2019). As a result, people are now focusing on healthier diets and exploring alternatives to meat that are nutritionally beneficial. Plant-based meat analogues that resemble animal meat in appearance, taste, and flavor are gaining popularity among consumers (Zhang, et al., 2021). In comparison to meat burgers, meat analogues contain significantly less energy, saturated fat, sodium, and no cholesterol, rendering them a healthier option for individuals (Sun, et al., 2021). The food industry currently uses high moisture extrusion as the primary method of producing meat analogues. This involves mixing protein materials and water in a barrel containing screws and subjecting the mixture to high temperature, pressure, and shear. The texturized meat analogues are then extruded through a cooling die mounted at the end of the extruder (Cheftel, et al., 1992).

Meat analogues have advantages over animal meat, but there are also some drawbacks. Recent research, as according by Zhou, et al., (2021), has indicated that meat analogues are digested slower in the small intestine compared to beef. It is noteworthy that animal meat possesses an amino acid composition akin to that of the human body. These findings underscore the significance of developing meat analogues that align with human nutritional requirements. Hydrolysis can be used to modify plant proteins, producing low molecular weight fragments that improve their biofunctionality, solubility, and bioavailability. Hydrolyzed protein has excellent nutritional properties, good amino acid balance, and high digestibility (Etemadian, et al., 2021). Moreover, it is well-established that these meat analogues can exert a positive influence on human health, aiding in the maintenance of well-being and disease prevention. These benefits encompass immunomodulatory, antimicrobial, antioxidant, antithrombotic, hypocholesterolemic, and antihypertensive actions, as elucidated by Montesano et al. (2020). Wheat gluten, which is derived from protein in wheat flour responsible for the dough formation, possesses favorable bioactive properties when hydrolyzed. Hydrolyzed wheat gluten (HWG) generally has good functional properties, including high solubility, water retention capacity, foaming, and emulsification properties (Tang, et al., 2023). HWG has been also found to possess good bioactive properties that can improve human health when used as a food additive, according to Karami, et al., (2019). WG serves as an exceptional raw material for manufacturing meat analogues, but it remains uncertain whether its hydrolysates possess the same remarkable capabilities. Furthermore,

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previous studies, such as the work by Tuck et al. (2014), have suggested that HWG can serve as a texture modifier. This suggests that HWG could enhance the texture of meat analogues, potentially making them more appealing to a broader consumer base, including the elderly. Taking these factors into account, we chose to incorporate HWG as one of the key raw materials for producing meat analogues in this study.

Soy protein is a popular choice for meat analogues due to its balanced amino acid composition and good gelatinization ability (Zhang, et al., 2021). Soy protein is available as soy protein concentrate (SPC, 65 %-80 % protein) and soy protein isolate (SPI, 85 % protein or higher) based on its protein content. According to Cheftel, et al. (1992), meat analogues made from SPC formulations are easier to extrude and texture than those made from SPI under similar conditions. While there have been studies on high moisture extrusion with SPC as the main ingredient, the focus has mostly been on texture and structure (Chiang, et al., 2019; Pietsch, et al., 2019). However, there is still a gap in research on high moisture extrusion with hydrolyzed protein and SPC, as well as the nutritional and functional properties of the extrudate.

The aim of this study was to assess the feasibility of using HWG and SPC as raw materials for meat analogues, an area that has not been extensively explored. The rheological properties of the two materials were analyzed at different ratios, followed by processing the two materials using high moisture extrusion to evaluate differences in texture, functional properties, and protein digestibility. Additionally, meat analogues containing 40 % HWG addition to SPC and chicken breast were included for comparison. The results of this study could provide valuable insights into the benefits of HWG as a texture modifier and nutritional enhancer in the development of meat analogues.

Materials and methods

Materials

Soybean protein concentrate (SPC) was purchased from Hao Xiang (Guangzhou, China), wheat gluten (WG) was purchased from Wanbang (Henan, China). SPC contains approximately 75.3 % protein, 8.5 % moisture, 4.4 % ash, 14.9 % carbohydrate, and 2.4 % fat. WG contains around 81.3 % protein, 3.7 % moisture, 2.1 % ash, 12.2 % carbohydrate, and 1.1 % fat. Neutrase (8×10^5 U/g) from Bacillus amyloliquefaciens was kindly provided by Novozymes (Bagsvaerd, Denmark). Fresh chicken breasts were purchased from a local supermarket in Harbin, China, and stored at 4 °C before use. 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) were both purchased from Solarbio (Beijing, China). Throughout the study, deionized (DI) water was used.

Preparation of HWG

WG was initially dissolved in DI water at a mass concentration of 15 %. The pH of the resulting suspension was then adjusted to 7.1, and the temperature was carefully maintained at 48 °C. Subsequently, neutral protease was introduced to achieve a final enzyme-substrate ratio of 3000 U/g. Following an incubation period of 5 h at the specified temperature and pH, the system was subjected to heating at 95 °C for 15 min to deactivate the enzyme. Finally, the resulting hydrolysate was freeze-dried to yield a powdered sample.

Determination of the degree of hydrolysis

The degree of hydrolysis (DH) of the HWG was determined using the pH-stat method. The DH of a protein is defined as the ratio of cleaved peptide bonds (h) to the total number of bonds per unit weight (h_{tot}). The DH of the HWG was calculated according to Eq. (1):

$$DH(\%) = \frac{hx100}{h_{tot}} = \frac{BxN_bx100}{\alpha xM_pxh_{tot}} \quad (1)$$

where, B represents the amount of base consumed; N_b is the normality of the base; α is the average dissociation constant of α-NH2 groups; M_p is the amount of protein (g); and h_{tot} denotes the total peptide bonds (meqv/g protein). Using the above method, the DH of the HWG prepared and calculated was found to be 11.21 %.

Rheological properties of protein blends

To start, SPC was mixed with HWG and WG in different proportions (0 % HWG, 20 % HWG, 40 % HWG, 60 % HWG, 40 % WG). Next, this resulting 5 g of protein blend was addition to 20 mL of DI water and stirred at 700 r/min for 2 h, then left to stand for 12 h at 4 °C. Rheology was then measured using a Haake rheometer (Mars 40, Thermo Fisher Scientific Inc., Germany) with a plate geometry sensor (diameter 35.00 mm; gap 1.00 mm). Viscosity was measured at a constant temperature of 25 °C and at a shear rate ranging from 1 to 100 s^{-1}. Allow the sample to stand on the plate for 5 min before performing the viscosity shear test, and the flow behavior was described by a power-law model (Eq. (2).

$$\tau = K\gamma^n \quad (2)$$

where, τ, K, γ, n was the shear stress (Pa), consistency index ($Pa{\cdot}s^n$), shear rate (s^{-1}), and flow behavior index, respectively. Oscillation tests were conducted over a range of angular frequencies from 0.1 to 100 rad/s at a constant temperature of 25 °C. In temperature scanning tests, samples were carried out at a constant frequency of 1 Hz, first gradually rising from 25 °C to 150 °C at a rate of 5 °C/min and remaining constant at 150 °C for 2 min, followed by cooling to 25 °C with a rate of 5 °C/min. Storage modulus (G′) and loss modulus (G″) were recorded automatically during the measurements.

SDS-PAGE of SPC, WG, and HWG

The SDS-PAGE method used in the present study was based on the method of Dai et al. (2022). SPC, WG, and HWG were mixed with reducing sample buffer (containing 14.4 mmol/L β-mercaptoethanol) and adjusted to a protein concentration of 2 mg/mL. The above mixture was kept in boiling water for 5 min, followed by centrifugation at 25 °C and 10,000g for 10 min. The supernatant obtained was carefully loaded onto polyacrylamide gels (12 % running gel and 5 % stacking gel) and electrophoresed under a constant current of 25 mA. and images were obtained using a gel imager (Gel Doc EZ, Bio-Rad, USA).

High moisture extrusion

SPC was mixed with HWG and WG in various proportions (0 %, 10 %, 20 %, 30 %, 40 %, 50 %, 60 % HWG, and 40 % WG) using a mixer (SYH-5, Changzhou Yi Rui Dry, China). The resulting mixtures were extruded with high moisture content in a twin screw extruder (Process 11, Thermo Fisher Scientific Inc., Germany) with a screw length/diameter ratio of 44:1 at a feed rate of 7.0 g/min and a moisture content of approximately 60 %. The extruder had seven barrels with temperatures of 40, 60, 80, 100, 120, 150, and 150 °C, respectively. The extrusion process was cooled using a die at a constant temperature of 20 °C.

Preparation of chicken meat sample

The chicken breasts were placed in sealed bags and heated to an internal temperature of 75–80 °C in a hot water bath. Subsequently cooling at room temperature for 30 min, the surface was carefully blotted dry and cut into fixed pieces for subsequent analysis.

Color measurement

The meat analogues and chicken meat samples were sliced into 10 × 10 × 1 mm (L × W × H) squares, with three copies of each sample

prepared from different locations. Color characterization was performed using a colorimeter (ZE6000, Nippon Denshoku Inc., Japan) with a C/2, D65/2 light source. Prior to testing, the colorimeter was calibrated using white cardboard, and the samples were then placed sequentially at the test points. L* from 0 to 100 means that the color is getting brighter (black to white).

Texture analysis

The texture of samples was measured in accordance with our previous study of Zhang, et al. (2022). A texture analyzer (TA. XT Plus, Stable Micro Systems, UK) was used to measure the hardness, chewiness and toughness data of the samples. Then samples were cut into 15 × 15 × 5 mm blocks and held on a platform with a clamp. The texture analyzer was used with the TA-46 MORS probe and compression test mode. The probe is pierced into the sample at a speed of 1.0 mm/s and reaches 3 mm where the probe returns, the trigger force of the process is 10 g. Each test was divided into two shears, with the first shear vertical to the extrusion direction and the second shear parallel to it. The fiber degree was calculated as the ratio of hardness in the vertical direction (F_v) and in the parallel direction (F_p). The texture of each sub-sample was tested 10 times and the results were averaged.

Scanning electron microscopy (SEM)

The microstructure of the samples was investigated using a scanning electron microscope (S-3400 N, Hitachi, Japan). Meat analogues and cooked chicken meat samples were cut into thin slices and washed twice with a sodium phosphate buffer (PBS, 0.01 M, pH 6.8) and absolute ethanol before being soaked in a 5 % glutaraldehyde solution for 24 h. Subsequently, the sample was rinsed with *tert*-butanol then dried. The dehydrated samples were coated with gold particles, and images were taken at a magnification of × 1500.

Determination of DPPH radical-scavenging activity

DPPH radical-scavenging activity was evaluated according to Karami, et al. (2019). To prepare the sample dispersions (1.0 wt%), the freeze-dried extrudate powder samples were added to DI water and continuously stirred for 2 h, respectively. The dispersions were then incubated overnight at 4 °C to ensure complete dissolution of the samples. An equal volume of DPPH stock solution (0.2 mM of 95 % ethanol) was mixed with the dispersions. The above mixtures were incubated for 30 min in light-free conditions, followed by absorbance at 517 nm obtained with a UV spectrophotometer (UV-2600, Shimadzu, Japan). The blank was ethanol instead of DPPH solution, and control was ethanol instead of sample solution. The DPPH radical-scavenging activity was calculated by Eq. (3).

$$DPPHradicalscavengingactivity(\%) = \left[1 - \frac{\left(A_{sample} - A_{blank}\right)}{A_{control}}\right] \times 100 \quad (3)$$

$ABTS^{\bullet+}$ radical-scavenging activity

$ABTS^{\bullet+}$ radical-scavenging activity was determined according to the method of Karami, et al. (2019). An equal volume of 7.4 mM ABTS and 2.6 mM potassium persulphate was mixed and incubated in the dark for 12 h to produce $ABTS^{\bullet+}$. The absorbance of the $ABTS^{\bullet+}$ solution was regulated to 0.7 ± 0.02 (734 nm) with PBS buffer (0.01 M, pH 7.4). The sample dispersions were mixed with the $ABTS^{\bullet+}$ solution at a ratio of 1:8 (v/v). The absorbance (734 nm) of the mixtures was measured after incubation in the dark for about 6 min. The blank was prepared using PBS buffer. The $ABTS^{\bullet+}$ radical-scavenging activity was calculated by Eq. (4).

$$ABTSradicalscavengingactivity(\%) = \left(1 - \frac{A_{sample}}{A_{blank}}\right) \times 100 \quad (4)$$

In vitro protein digestibility

The in vitro protein digestibility (IPVD) was measured using the technical approach reported by Bhat, et al. (2019). To determine the IPVD, the Kjeldahl method was used to determine the protein content of samples and chicken samples. Samples equivalent to 437.50 mg protein were then weighed, cut into 2 × 2 × 2 mm squares and added to 17 mL of 0.1 M HCl (pH 1.9 ± 0.1) containing pepsin (enzyme: substrate ratio = 1:100 w/w) to simulate the gastric digestion phase. After continuous stirring for 1 h at 37 °C, sodium phosphate buffer solution (pH 8.0, 0.1 M) and pancreatic enzyme were added to simulate the intestinal digestion phase. The trypsin was inactivated by adjusting pH 2.0 after 2 h of digestion at 37 °C. Supernatant was collected after centrifugation (4000 × g, 15 min), and the bicinchoninic acid (BCA) method was used to measure the protein concentration. A blank was prepared by digesting a mixture without the addition of samples. The IVPD (%) was obtained using Eq. (5).

$$Proteindigestibility(\%) = \frac{P_{sample} - P_{blank}}{P} \times 100 \quad (5)$$

where, P_{sample} and P_{blank} were the protein content of the digested sample and blank, respectively. P referred to the protein content of the undigested sample.

Amino acids analysis

The lyophilized powder sample was put into the test tube with 15 mL of 6 M HCl solution. After the addition of four drops of phenol, the tubes were set in a refrigerant for 3–5 min and filled with nitrogen. The tubes were then sealed and kept in a hydrolysis oven for 22 h at 110 ± 1 °C. After cooling, the hydrolysis solution was filtered and 50 mL of DI water was made up, and then dried under reduced pressure (40–50 °C). Next, 2 mL of sodium citrate buffer was taken to dissolve the dried sample. The samples were then determined by an automated amino acid analyzer (LA8080, Hitachi, Tokyo, Japan).

Statistical analysis

All experiments were performed at least three times, and the results were expressed as the mean ± standard deviation. One-way analysis of variance (ANOVA) and Duncan's test were performed on the experimental results using SPSS 25 software (SPSS Inc., Chicago, IL, USA) at a significance level of $p < 0.05$.

Results and discussion

Rheological behavior analysis

Gel rheology of protein blends under shear

The protein blends inside the extruder are subject to two major controllable factors, one being the shear forces generated by the constant rotation of the screw with the engaging element and the other being the temperature of the barrel inside the extruder. The combined effect of these factors results in the protein material becoming a meat analogue with a meat fiber-like structure. To gain more insight into the changes that occur in the protein blend inside the extruder, we have used rheology to analyze it. Rheology involves applying dynamic oscillatory shear or heat to the material to obtain changes in its viscoelasticity and network structure. As shown in Fig. 1**A & B**, we examined the rheological behavior of protein mixtures under non-thermally induced shear at room temperature in this study. A decreasing trend in the viscosity of the blends with increasing shear rate was observed,

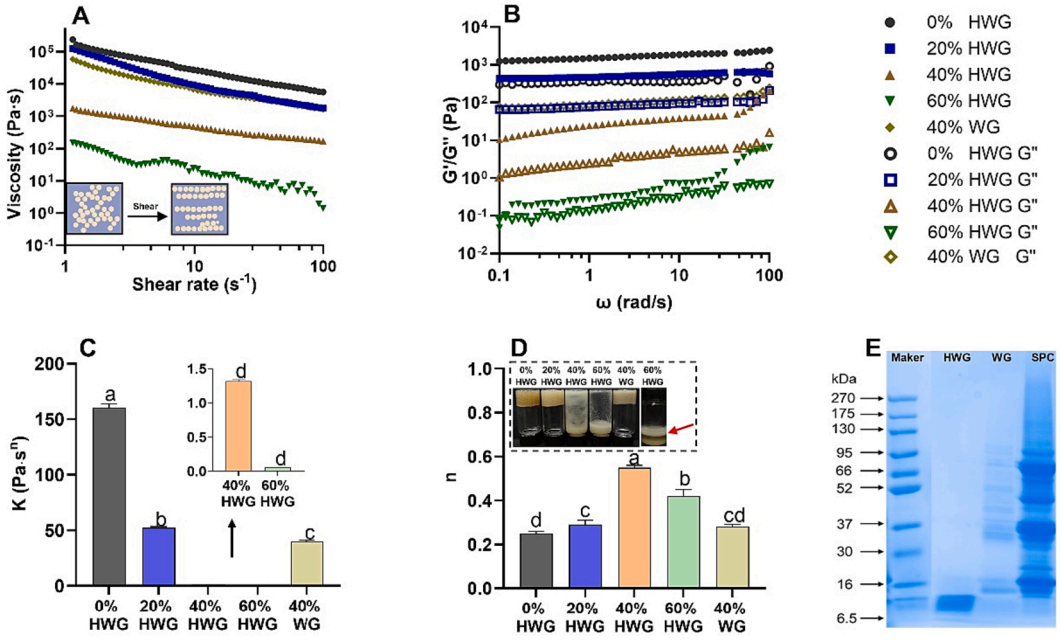

Fig. 1. A represents shear stress-apparent viscosity relationship; B represents variation of storage modulus (G′) and loss modulus (G″); C and D represent the consistency coefficient K and the flow behavior index n, respectively. The dotted line box in D highlights inverted vials containing different protein mixtures; E represents electropherograms of the protein raw material.

indicating shear-thinning behavior. For globular soy proteins, this may be due to the shear-induced formation of shear flow within a range of shear rates that drives the alignment of globular proteins (Fig. 1**C**) (Vermant, 2001). The viscosity of the mixtures tended to decrease with the gradual increase of the HWG proportion. In the present study, the HWG band showed its molecular weights mostly between 6.5 kDa and 16 kDa, and a very small fraction at approximately 37 kDa and 95 kDa (Fig. 1**E**). Generally, WG consists mainly of gliadin and glutenin, with molecular weights of 25–100 kDa and 500–10000 kDa, respectively. This indicates that most of them are hydrolyzed into small molecular peptides in HWG (Zhang, et al., 2020). In particular, gliadin is responsible for the viscosity of WG. Therefore, the decreasing viscosity of SPC-HWG blends is caused by the small molecular weight of HWG. Similar results were reported in the research of Lamsal, et al. (2007) that showed low viscosity of proteins owing to the reduction of their molecular weight by protein hydrolysis. Additionally, the viscosity of the SPC-HWG blend with 40 % HWG added is lower than that of the SPC-WG blend with the same amount of WG added, providing further evidence.

Expressed by the consistency coefficient (k) and the flow behavior index (n), the power law model is commonly used in fluid foods to represent protein blends, as shown in Fig. 1**C & D** ($R^2 > 0.9$). The n value is dimensionless, and closer to 1 indicates a closer approximation to Newtonian fluid behavior and a weaker shear thinning behavior. The *n*-value of the blends increased from 0.25 to 0.55 as the proportion of HWG increased from 0 % to 40 %, indicating an enhanced flow behavior. However, at 60 % HWG, the n value decreased, showing a poorer fluid behavior that could be related to the stratification phenomenon in its protein blend (Fig. 1**D**). As expected, the K value of the gels increased with increasing HWG concentration, as the consistency coefficient was related to the viscosity (Lamsal, et al., 2007). The viscosity, n values of the protein blends were consistent with the phenomena observed in the inverted vials containing the protein blends, with the 0 % HWG, 20 % HWG, and 40 % WG blends all sticking to the bottom of the inverted vials, exhibiting strong viscosity and poor flowability.

As the material undergoes deformation, its elasticity and viscosity can be expressed in terms of the storage modulus (G') and loss modulus (G''), respectively. Both G' and G'' exhibited an increasing trend with increasing angular frequency, as shown Fig. 1**B**, indicating that all modules exhibit frequency dependence over the entire range of frequencies (1–100 rad/s). This demonstrates the critical role of non-covalent physical cross-linking in maintaining protein gel networks (Anvari & Joyner, 2017). The reason behind this may be that during brief oscillations at high frequencies, the protein molecular chains become entangled into a temporary cross-linked junction region, leading to the increased viscoelasticity (Chen, et al., 2021). In addition, all protein mixtures have a higher G' than G'', indicating that the protein blends prefer to form solids with better elasticity. Moreover, both G' and G'' showed a decreasing trend with increasing proportion of HWG added, suggesting that the addition of HWG reduces the strength of the network structure of the protein blend gels (Zhang, et al., 2023). This could be attributed to the high solubility of HWG molecules, which may increase the mobility of soy proteins and result in a decrease in both G' and G''.

Gel rheology of protein blends under temperature

Low-frequency (1 Hz) temperature scans enable non-destructive characterization of thermally induced gelation and the linear viscoelastic properties of different protein blend samples. Fig. 2 shows the gelation curve of protein blends for heating followed by cooling, with a maximum temperature of 150 °C to simulate the extruder's temperature conditions. As the temperature increases, the G' and G'' of protein blends rise, possibly because thermally induced denaturation of the soy protein molecules exposed the hydrophobic and sulfhydryl groups. This causes interactions between protein molecules, mainly hydrophobic, and disulfide bonding leading to protein aggregation and gel network formation (Zhao, et al., 2020). With further temperature increases, more protein molecules incorporate into the gel network structure, rearranging the network and increasing the viscoelasticity of protein blends (Renkema & van Vliet, 2002). Viscoelasticity still increases during the temperature decrease stage, but with less variation. This is due to the further increase in van der Waals forces and hydrogen bonding in protein blends, resulting in gel stiffening (Xia, et al., 2021).

As the proportion of HWG increased in the protein blends, G' and G'' decreased over heating and cooling. According to the finding of Tuck, et al. (2014), HWG acts as a plasticizer for protein molecules in gel

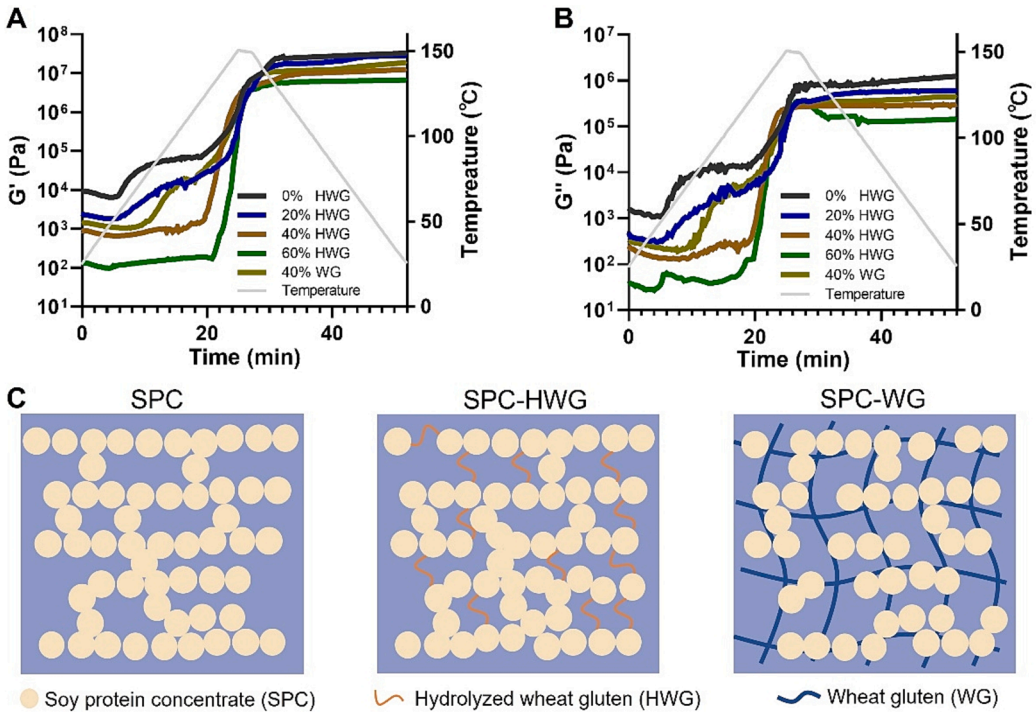

Fig. 2. A and B represent storage modulus (G′) and loss modulus (G″) under temperature variation respectively; C represents the three binding types of SPC, SPC-HWG, and SPC-WG gels.

formation, allowing a rigid gel structure to become more flexible. This is because HWG molecules form "bridges" between soy protein molecules, reducing the local friction coefficient between protein molecules and lowering viscoelasticity in macroscopic gel properties. Ji, et al. (2023) also found that soy protein hydrolysate acted as a plasticizer in soy protein-WG blends, reducing G' of protein blend gels. In contrast, the SPC-WG blend creates a strong gluten network due to its high disulfide bond level linked by cysteine residues. This network combines with spherical soy protein to form a distinct sphere-fiber structure, giving SPC-WG gels high elasticity (Qiu, et al., 2023). Fig. 2**C** shows the three different molecular network structures of SPC, SPC-HWG, and SPC-WG schematically.

Characterization of high moisture meat analogues

Texture analysis

Fig. 3 shows that different protein blends yield meat analogues with varying textures during high moisture extrusion. The texture analyzer measures the hardness, chewiness, and toughness values to provide a clear description of the meat analogue's physical properties. In addition, the fiber degree, which describes the meat analogue's fiber structure, is commonly used to quantify the degree of fibrousness. In previous studies (Dou, et al., 2022), the fiber degree was evaluated as the ratio of parallel hardness to vertical hardness (Fig. 3**A**), with values above 1 indicating a favorable fibrous structure. As shown in Fig. 3**B**, the fiber degree tended to increase with higher HWG ratios. This may be due to the HWG peptide chain's exposed cysteine residues, which bind to soy protein to create a strong fibrous network structure (Tuck, et al., 2014). However, excessive HWG addition led to decreased fibrillation, which suggests that HWG addition is optimal up to a certain point. Additionally, the hardness, chewiness, and toughness of meat analogues all decreased as HWG addition increased, which is consistent with the temperature scanning rheology measurements. As previously mentioned in section 3.1.2, HWG acted as a plasticizer and short chain peptides linked to soy protein molecules, creating a more flexible gel network structure that reduced the meat analogues hardness, chewiness, and toughness (Fig. 3**C1 & *C2*, D1 & D2, E1 & E2**). Notably, meat analogues with the same amount of WG addition (40 %) as HWG addition had a higher fiber degree, suggesting that WG has a greater ability of forming the fiber network structure due to higher abundance of gliadin and glutenin. However, the meat analogues with 40 % HWG addition had lower hardness, chewiness, and toughness and were more similar to chicken values (Grabowska, et al., 2014). This suggests that the addition of HWG creates a softer and easier to chew meat analogue while maintaining a similar fiber level to chicken, making it an ideal option for elderly people with weaker teeth and jaw muscles (Munialo, et al., 2020).

Visual and microscopical analysis

Visual images and microstructures of chicken meat and meat analogues with varying proportions of HWG (0 % to 50 %) added in SPC, as well as meat analogues with 40 % WG added in SPC, are displayed in Fig. 4. To observe the microstructure of the meat analogue, thin slices were cut and then freeze-dried, while visual images were taken by cutting in the vertical extrusion direction and tearing to show the fibrous structure (Fig. 4**A & B**). When only SPC was used as the raw material, it was difficult to form a fiber structure after extrusion, and the microscopic image showed a clear cross-linked polymeric structure with small pores (Fig. 4**A1 & B1**). This is probably because of globular proteins in soy protein when subjected to high-temperature extrusion, causing the thermal unfolding of these proteins and leading to the formation of heat solidification gels (Renard, et al., 2006). As the proportion of HWG increased, the fibrous structure of the meat analogues became more abundant, with the richest filamentous fibrous structure observed at the HWG addition of 40 % (Fig. 4**A5 & B5**), consistent with the change of fibrous degree in Fig. 3**B**. However, when HWG was added in reaching half the amount, the filamentous fibers gradually twisted and cross-linked, leading to a disordered fiber structure that could no longer provide a macroscopic meat-like fiber characteristic while reasserting a gel state. Meat analogues made by adding 40 % WG to SPC also had a rich fibrous structure (Fig. 4**A7 & B7**), consistent with previous research by Chiang, et al. (2019) and Grabowska, et al. (2014). The microscopic images of chicken meat showed a dense and compact fiber structure

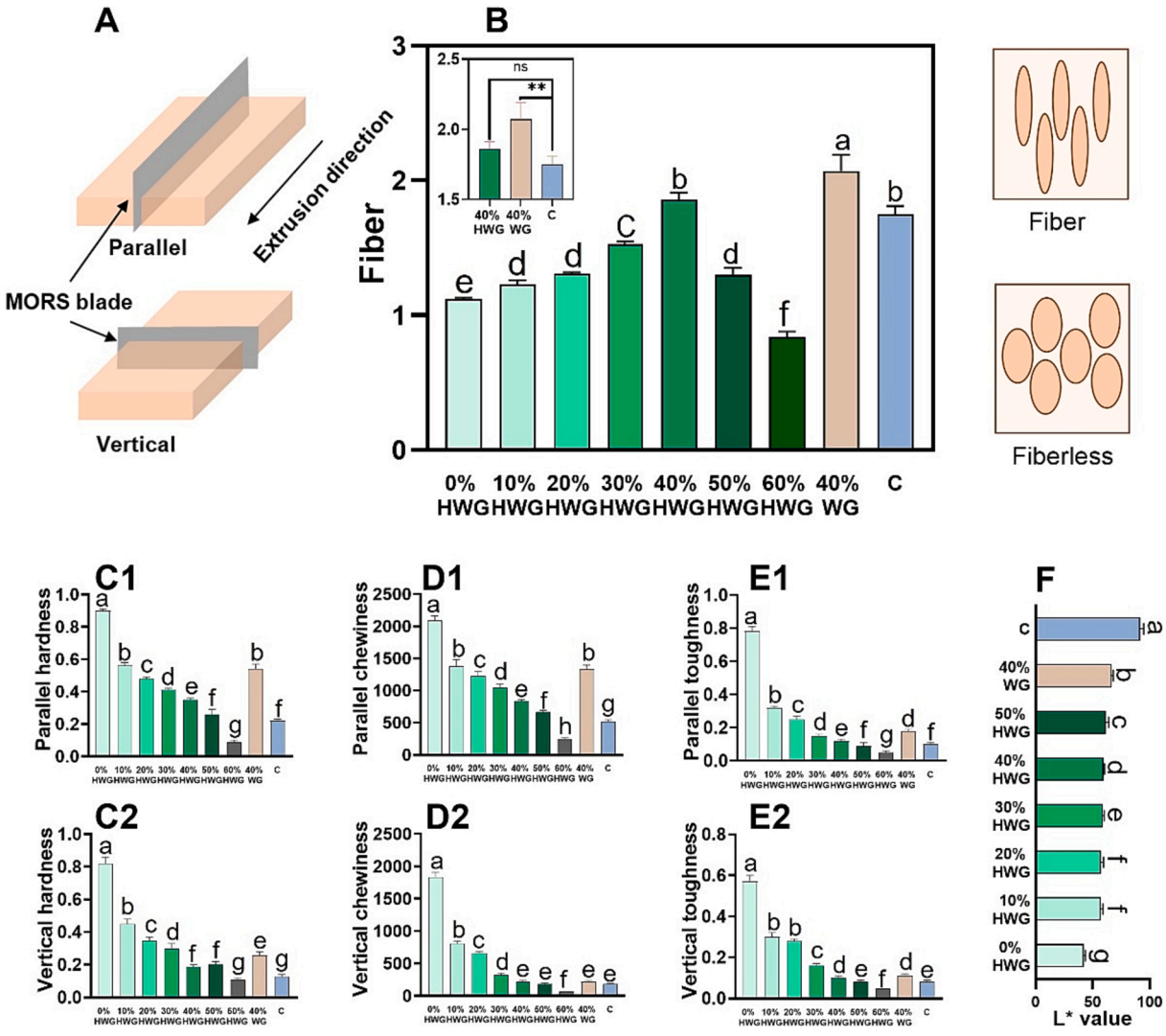

Fig. 3. A represents the different cutting types of the probes for the determination of the composition of extrudate; B represents the variation in fiber degree; C1 & 2, D1 & 2, and E1 & 2 are the hardness, chewiness, and toughness (parallel and vertical) respectively; F represents the L* of the extrudates, where C is chicken.

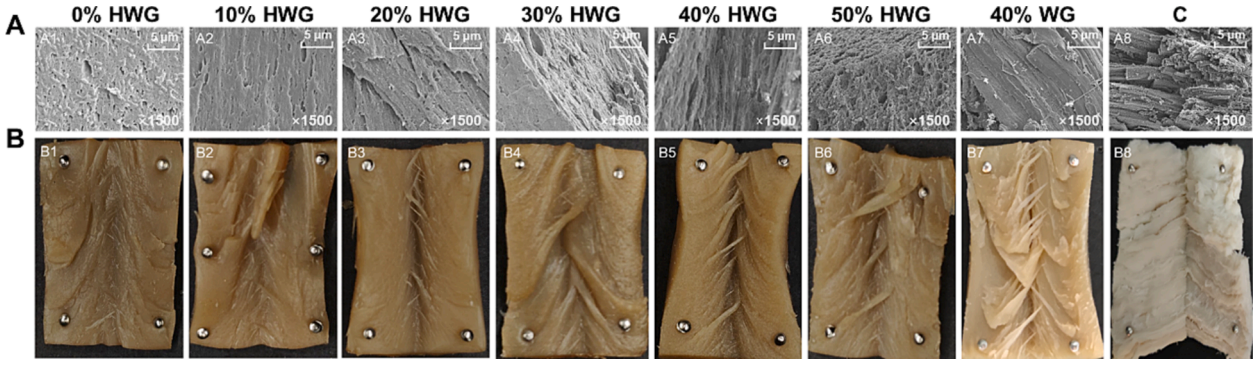

Fig. 4. A represents a SEM image of the extrudate; B represents the macroscopic photo images, which is torn down from the middle to allow observation of the fiber structure.

(Fig. 4**A8 & B8**), which is caused by the protein denaturation and structural contraction that occur in chicken during cooking (Shaarani, et al., 2006). In contrast, the microstructure of meat analogues made with either HWG or WG additions displayed short filamentous fibers between the long fibers, connecting them to form a porous structure (Fig. 4**B5 & B7**).

Color analysis

Fig. 3**F** displays the L* values, which indicate the brightness of the color (0 = black, 100 = white), for both meat analogues and chicken meat (Fang, et al., 2014). The Maillard reaction is the main factor influencing the color of meat analogues, which forms protein–sugar adducts and highly colored insoluble polymers called melanoidins

during extrusion cooking (Fang, et al., 2014). However, the Maillard reaction is considered to be complex and can be affected by various factors, like the type of protein and sugar, temperature and moisture. In this study, the addition of HWG significantly whitened the meat analogues ($p < 0.05$), likely due to its lower sugar content compared to SPC. Conversely, adding WG obviously increased the brightness of the meat analogues compared to HWG, possibly because WG contain fewer available amino groups for the early Maillard reaction, but hydrolysis of wheat proteins promotes the Maillard reaction to proceed (Lee, et al., 2012). Furthermore, chicken meat had the highest L* value, reflecting its different composition.

Nutritional and functional properties of meat analogues

Antioxidant capacity analysis

Antioxidant capacity is crucial in assessing food functionality, typically measured by the DPPH and $ABTS^{\bullet+}$ radical scavenging rates, which are commonly used and effective. Fig. 5**A & B** demonstrate that the addition of HWG to SPC significantly improves the DPPH and $ABTS^{\bullet+}$ radical scavenging rates of meat analogues, with its antioxidant capacity increasing with increasing HWG addition. This is due to the strong antioxidant capacity of HWG, confirmed by previous studies (Cheng, et al., 2006) and attributed to its lower molecular weight and specific peptides with strong radical scavenging activity (Karami, et al., 2019). On the other hand, meat analogues with WG addition, having a high molecular weight, exhibited relatively weak antioxidant capacity. In addition, the $ABTS^{\bullet+}$ radical scavenging rate of meat analogues with WG addition was lower than that of SPC alone, possibly due to chemical cross-linking of wheat protein and soy protein during extrusion, resulting in a denser structure and hindering the free radical scavenging reaction.

Protein digestion

The protein digestibility of the meat analogues increased as the

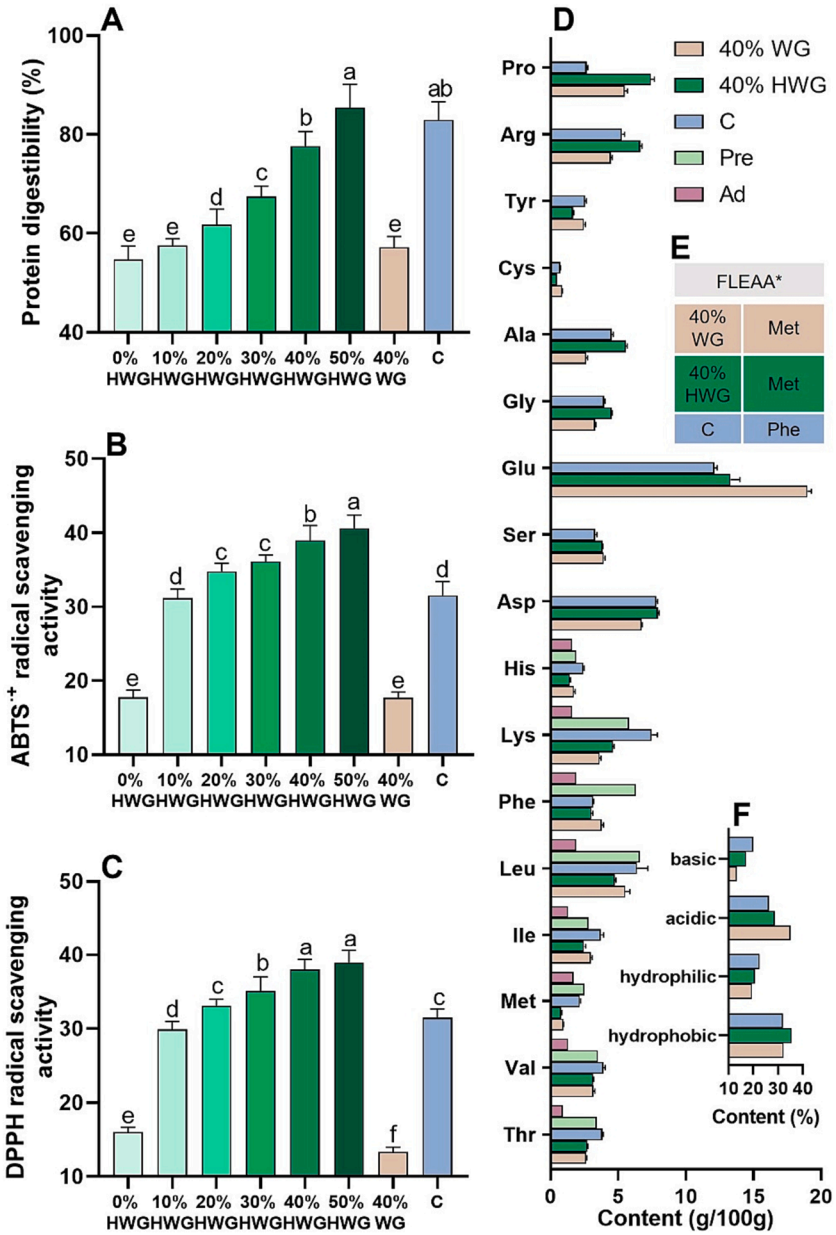

Fig. 5. A represents protein digestibility; B and C represent $ABTS^{\bullet+}$ and DPPH radical scavenging, respectively; D represents amino acid levels of 40 % HWG, 40 % WG extrudates, and chicken, where Pre and Ad represent FAO/WHO recommended pattern of pre-school child (2–5 years) and adult, respectively; E represents their first limiting essential amid acid (FLEAA*), F represents their amino acid distribution.

proportion of HWG increased gradually (Fig. 5**C**). This result was anticipated because HWG primarily consists of small molecule peptides known for their high solubility, a characteristic that promotes a favorable protein digestion process. Moreover, the increase in protein digestibility may also be related to the texture of the meat analogues. Previous research has shown that a compact and smooth gel structure in meat analogues can reduce protein contact with enzymes, thereby reducing protein bioavailability (Chen, et al., 2021). In contrast, meat analogues that exhibit a higher fibrous degree and lower hardness (achieved by increasing the proportion of HWG) could enhance protein-enzyme reactions, thus improving protein digestibility. However, the meat analogues with WG addition had poor protein digestibility despite their abundant fibrous structure, which may be due to the lower solubility of WG and the compact structure resulting from its excellent adhesion. In comparison, the protein digestibility of chicken meat is generally higher than that of meat analogues. Plant proteins typically have lower digestibility than animal proteins, and among animal proteins, chicken meat has higher digestibility than others (Carbonaro, et al., 2012). Myosin contained in chicken breast, which is different from the two major storage globulins (7S and 11S) in soy protein, is considered one of the main factors contributing to the high digestibility of chicken protein. The preparation process may induce partial denaturation of both HWG and SPC, potentially resulting in an overall reduction in protein digestibility (Carbonaro et al., 2005). Additionally, the processing method used for chicken meat, such as cooking in hot water, can improve the digestibility of myosin heavy chains by denaturation. However, the protein digestibility of the meat analogue with 50 % HWG addition exceeded that of chicken meat due to the higher content of small molecule peptides in HWG and the flabbier texture structure of the meat analogue at this proportion. At 40 % HWG addition, the protein digestibility of the meat analogue was no longer significantly different from that of chicken meat.

Amino acid composition

The amino acid composition (AAC) gives an indication of the quality of the protein in meat, which should have an appropriate balance of essential to non-essential amino acids for good protein quality (Kim, et al., 2013). Knowing the AAC of meat analogues is crucial, and the AAC of the 40 % WG addition meat analog, 40 % HWG addition meat analogue, and chicken meat were summarized (Fig. 5**D**). However, tryptophan could not be determined due to the acid hydrolysis method used in the experiment. Fig. 5**F** shows that the AAC of the 40 % WG addition meat analog was dominated by acidic amino acids, while the 40 % HWG addition meat analogue and chicken meat were dominated by hydrophobic amino acids. Additionally, the AAC of the 40 % HWG addition meat analogue and chicken meat had similar amino acid distributions. Animal proteins have a nutritional structure and amino acid composition similar to that of humans and are considered a high-quality protein source. Therefore, the nutritional value of 40 % HWG addition meat analogues for the human body is comparable to that of animal proteins. The FAO/WHO recommended essential amino acid amounts for children aged 2–5 years revealed that methionine was the first limiting essential amino acid (FLEAA) in the two meat analogues (Fig. 5**E**), while phenylalanine was the FLEAA in chicken meat. This is because the major component in the meat analogues is soy protein, which is usually deficient in methionine (Young & Pellett, 1994). In general, both meat analogues contain sufficient amino acids for human nutritional requirements, except for methionine. However, the content of arginine in the 40 % HWG addition meat analogue was significantly higher than that in the 40 % WG addition meat analogue and chicken meat, reaching 6.62 g/100 g. Arginine is not an essential dietary amino acid for healthy adults due to endogenous synthesis, but it is classified as a semi-essential or conditionally essential amino acid for infants, growing children, adults under catabolic stress, or those with small intestinal or renal dysfunction (Flynn, et al., 2002). Arginine plays an important role in the urea cycle, energy metabolism, immune function, and improvement of cardiovascular function. Therefore, 40 % HWG addition meat analogue has the potential to provide health benefits to humans as part of a balanced food intake.

Conclusion

In conclusion, this work successfully demonstrated the potential of using hydrolyzed wheat protein and soy protein concentrate to produce fiber-rich meat analogues. The addition of 40 % HWG was found to improve the texture of the meat analogue, resulting in a softer and easier to chew product compared to SPC-WG. Moreover, the meat analogues containing HWG showed high antioxidant capacity, high protein digestibility, and an amino acid composition that is more closely aligned with human nutritional requirements. These results demonstrate that the great potential of HWG as a texture modifier and nutritional enhancer in the development of meat analogues, providing consumers with a healthy and sustainable alternative to traditional meat products.

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.

Data availability

Data will be made available on request.

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