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Author	Seon-Min Oh[1], Jae Hoon Lee[1,2], Tae-Kyung Kim[1], Jinryong Park[1], Min-Cheol Kang[1], Min Kyung Park[1], Yun-Sang Choi[1*]
Affiliation	[1]Researcher, Research Group of Food Processing, Korea Food Research Institute, Wanju, 55365, Republic of Korea [2]Department of Food Science and Technology, Jeonbuk National University, Jeonju, 54896, Republic of Korea
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ORCID (All authors must have ORCID) **https://orcid.org**	Seon-Min Oh (https://orcid.org/0000-0002-4257-4649) Jae Hoon Lee (http://orcid.org/0000-0002-7440-6842) Tae-Kyung Kim (http://orcid.org/0000-0002-6349-4314) Jinryong Park (http://orcid.org/0000-0002-1298-2828) Min-Cheol Kang (http://orcid.org/0000-0002-9658-9045) Min Kyung Park (http://orcid.org/0000-0002-3619-9491) Yun-Sang Choi (http://orcid.org/0000-0001-8060-6237)
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5 **CORRESPONDING AUTHOR CONTACT INFORMATION**

For the corresponding author (responsible for correspondence, proofreading, and reprints)	**Fill in information in each box below**
First name, middle initial, last name	Yun-Sang Choi
Email address – this is where your proofs will be sent	kcys0517@kfri.re.kr
Secondary Email address	
Postal address	Research Group of Food Processing, Korea Food Research Institute, 245, Nongsaengmyeong-ro, Wanju-gun 55365, Korea
Cell phone number	+82-10-4713-5623
Office phone number	+82-63-219-9387
Fax number	+82-63-219-9076

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Evaluation of Scaffold Properties for Cell-Cultured Food Based on Protein Sources and Their Mixtures

Abstract

For cultured meat applications, the development of protein-based scaffolds is essential to produce sustainable edible materials with suitable textures and functionalities. In this study, scaffolds were fabricated using various plant-based protein sources, including soybean (GSP), pea (GPP), and faba bean (GFP), and mixed protein formulations (S1–S4), and their physicochemical, mechanical, and biological properties were evaluated. All the scaffolds exhibited a pale yellow color and porous surface morphology. Water absorption analysis revealed that GSP exhibited the highest uptake among the single-protein scaffolds. Notably, the partial substitution of pea or faba bean proteins with other plant proteins, such as soy, significantly improved the water absorption capacity compared to that of GPP and GFP. The degradation rate of plant protein-based scaffolds remained below 10% during the early incubation stages, but increased markedly after 12 h. Mixed-protein scaffolds exhibited over 20% degradation at 48 h, whereas single-protein scaffolds showed less degradation. Texture profile analysis demonstrated that mixed-protein scaffolds had significantly higher hardness and chewiness than single-protein scaffolds, likely due to enhanced protein–protein interactions and network formation. However, cell proliferation analysis indicated that single-protein scaffolds supported better cell attachment and proliferation, with scaffolds prepared using faba bean proteins showing the highest proliferation rate. These results suggest that plant-based protein scaffolds can be tailored based on protein composition to optimize both their physicochemical and biological properties, thereby offering promising strategies for the development of edible scaffolds for cultured meat production.

Keywords: cell-cultured meat, scaffold, plant-based protein, physicochemical properties, cell proliferation

1. Introduction

As global meat consumption rises, traditional food production faces limitations due to environmental and public health concerns (Lee et al., 2024; Kim et al., 2022). Cultured meat has emerged as a promising alternative, offering ethical and sustainable benefits by cultivating animal cells without slaughter (Post et al., 2020; Siegrist and Hartmann, 2020). Its development integrates technologies such as cell line establishment, bioreactor cultivation, and scaffold engineering (Lee et al., 2024). A key challenge in cultured meat production is replicating the texture of real meat. This requires three-dimensional (3D) scaffolds that support cell attachment, proliferation, and differentiation (Xiang et al., 2022). Unlike 2D systems, 3D scaffolds promote meat-like tissue formation and improve sensory qualities (Seah et al., 2022; Wang et al., 2023). Common scaffold materials include collagen, gelatin, fibrin, and silk fibroin, valued for their biocompatibility and mechanical strength (Seah et al., 2022; Tang et al., 2024). Among them, collagen and gelatin have been most studied (Yu et al., 2022). However, scaffold performance can vary depending on protein composition, crosslinking, pore structure, and mechanical properties, all of which influence cell behavior (Ben-Arye et al., 2020; Bomkamp et al., 2022). Thus, careful scaffold design is essential to support efficient cell growth and tissue development in cultured meat systems (Levi et al., 2022; Mariano Jr et al., 2024a).

Plant-derived proteins offer economic advantages over animal-based or synthetic scaffolds due to their lower production costs, scalability, and existing food-grade status. Furthermore, addressing sensory properties such as texture and edibility can help improve consumer acceptance, especially for hybrid or fully edible cultured meat systems. Recent studies have investigated the potential of food-grade proteins, such as soy and whey proteins, as alternative scaffold materials for cultured media (Charron et al., 2024; Mariano Jr et al., 2024b). The structural and chemical properties of plant-based proteins vary depending on their

composition and processing methods, which can significantly affect their effectiveness as scaffolds in cellular agriculture. In addition to soy, legumes such as peas and faba beans are rich in globulin-type storage proteins, which contain functional groups that can promote cell adhesion. Pea protein is highly digestible, biocompatible, and exhibits excellent water absorption, creating a moist environment favorable for cell viability (Ge et al., 2020). Moreover, its low allergenic potential makes it suitable for edible applications. Faba bean protein offers additional advantages, including high protein content, thermal stability, and strong gelling ability, which enhance scaffold integrity and may further promote cell attachment (Shen et al., 2025)

However, while previous studies have evaluated single plant-based proteins such as soy or pea individually, limited research has systematically investigated the combined use of multiple plant proteins in scaffold design. This study addresses this gap by investigating the synergistic effects of mixed plant-based protein scaffolds (soy, pea, and faba bean) and their influence on structural, mechanical, and biological properties. Understanding the functional interplay among these proteins offers new insights into compositional tuning strategies for optimizing scaffold properties in cultured meat applications. The incorporation of food-derived proteins into the scaffold design is a crucial step toward ensuring the safety and regulatory compliance of cultured meat products for commercial applications. Therefore, this study aimed to evaluate the structural and functional characteristics of various plant-based protein scaffolds with a particular focus on their physicochemical properties.

2. Material and Method

2.1. Material

In this study, protein materials including soy protein (ES Food Ingredients, Republic of Korea), pea protein (Baekse Food, Republic of Korea), and faba bean protein (Chamgoods, Republic of Korea), were used for scaffold preparation. Potato starch (Ever Healthcare Food, Republic of Korea) and gellan gum (ES Food Ingredients) were also used.

2.2. Preparation of scaffolds

The scaffold was prepared using the method described by Lin et al. (2022), with some modifications. The mixing ratios of the protein-polysaccharide scaffolds are presented in Table 1. To investigate potential interactions and synergistic effects among plant-based protein components, both equal and varied ratios were used in the formulations. These combinations were determined based on preliminary experiments that evaluated gel formation, structural stability, and handling properties. In addition, relevant literature was consulted to support the selection of functionally compatible protein blends suitable for scaffold fabrication (Wollschlaeger et al., 2022; Ikuse et al., 2024). Each mixture was incubated in a water bath at 50 °C for 30 min. The prepared solution was then poured into petri dishes (30 mm × 15 mm) to a height of 7.5 mm and frozen at -20 °C for 24 h. The frozen samples were then immersed in a precooled (4℃) 8% (w/w) $CaCl_2$ in 90% ethanol solution and maintained at –20℃ for an additional 24 h to induce ionic cross-linking. After freezing, the scaffolds were cut into uniform sizes using an 8 mm punch. The cut scaffolds were then washed with 70% ethanol and triple-distilled water, followed by freeze-drying operated at -80℃ for 48 h. The freeze-dried scaffolds were hydrated in muscle cell culture media and incubated for three days to assess their structural stability.

2.3. Microscopy

To investigate the surface porosity of the scaffolds, the samples were coated with platinum and mounted on carbon tape. Scanning electron microscopy (SEM; EM-30, Coxem, Daejeon, Republic of Korea) was used to capture images. The pore sizes on the scaffold surface were analyzed using ImageJ software (NIH, Bethesda, MD, USA).

2.4. Water absorption

The water absorption rate of the fabricated scaffolds was measured by immersing the samples in distilled water and incubating them in a water bath at 37 °C. After 12, 24, 48, and 72 h, the scaffolds were removed, the excess surface water was blotted with filter paper, and the scaffolds were weighed (Su et al., 2024). The water absorption rate was calculated at each time point using the following equation:

$$Water\ absorption\ rate\ (\%) = \frac{Wet\ scaffold\ weight - Initial\ scaffold\ weight}{Initial\ scaffold\ weight} \times 100$$

2.5. Scaffold degradation

To assess the degradation rate of the scaffolds, samples were immersed in phosphate-buffered saline (PBS, pH 7.4) at 37 °C for up to 72 h. After immersion, the scaffolds were gently rinsed with distilled water to remove residual salts and were subsequently freeze-dried. The dry weight of the scaffolds was measured and the degradation rate was calculated using the following equation:

$$Scaffold\ degradation\ rate\ (\%) = (W_1/W_0) \times 100$$

where W_1 is the dry weight of the scaffold after immersion for a specific time point, and W_0 is the initial dry weight of the scaffold before immersion.

2.6. Texture analysis

To evaluate the hardness and chewiness of the scaffolds, texture analysis was conducted using the method described by Song et al. (2022) with modifications. A texture analyzer (TA.XT2 plus, Stable Micro Systems, UK) was used to assess the mechanical properties of the samples. Prior to the analysis, the scaffolds were immersed in distilled water for 24 h. A cylindrical probe with a 5 mm diameter was employed, and the test parameters were set as follows: pre-test speed of 2 mm/s, test speed of 1 mm/s, and post-test speed of 2 mm/s. A force of 5 g was applied to evaluate the hardness and chewiness of the scaffolds.

2.7. Cell culturing and seeding on scaffolds

The growth medium consisted of 20% fetal bovine serum (Gibco, Grand Island, NY, USA) and 1% penicillin-streptomycin antibiotics (Gibco, Grand Island, NY, USA) in DMEM/F-12 (1:1) medium (Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12, Gibco). C2C12 myoblasts were cultured in growth medium at 37 °C in 5% CO_2 and humidified air (95%) for 3 days. Cells were harvested when confluence reached 70% and cell suspensions were prepared at a concentration of 5×10^6 cells/mL. A 20 μL cell suspension was seeded onto each scaffold, resulting in a final density of 1×10^5 cells per scaffold. After seeding, the scaffolds were incubated at 37 °C in a 5% CO_2 environment for 1 h to allow cell attachment. Following the attachment phase, 1 mL of growth medium was added to each scaffold, and the samples were incubated on a shaker for 3 days.

148 2.8. Cell proliferation assay

149 Cell viability was assessed using the water-soluble tetrazolium salt (WST) assay. At
150 the end of the incubation period, 100 μL of WST solution (EZ-Cytox, DoGen, Republic of
151 Korea) was added to each well and incubated at 37 °C in 5% CO_2 and humidified air (95%).
152 After 4 h, 100 μL of the supernatant was transferred to a 96-well plate, and the absorbance was
153 measured at 450 nm using a microplate reader (SpectraMax Plus 384, Molecular Devices, San
154 Jose, CA, USA).

155

156 2.9. Statistical analysis

157 The results are expressed as mean values ± standard deviation. Statistical analyses were
158 performed using one-way analysis of variance (ANOVA) in SPSS Ver. 20.0 (SPSS Inc., Chicago,
159 USA). Pairwise comparisons of means between samples were conducted using Tukey's multiple
160 comparison test, with statistical significance set at $p < 0.05$.

161

3. Results and Discussion

3.1. Appearance and surface morphology of scaffolds

The appearance and surface characteristics of the scaffolds manufactured with plant-based protein materials at various mixing ratios are shown in Fig. 1. All the scaffolds exhibited a light yellow color, regardless of the protein composition. Although the surface roughness increased after freezing and thawing, no major differences in the overall appearance were observed across the samples. Additionally, soybean protein, pea protein, faba bean protein, and protein-material-blended scaffolds maintained a diameter of approximately 8 mm after freeze drying, indicating minimal tissue shrinkage during freeze drying. According to Lin et al. (2022), scaffolds containing soybean protein exhibit wrinkles on the surface and an ivory color, which intensifies with increasing protein content. Although the scaffolds generally exhibited a light-yellow hue, this trend was also observed in our study.

Scanning electron microscopy (SEM) analysis revealed clear differences in the microstructures of the scaffold surfaces depending on the protein material. GSP exhibited a higher number of surface pores than the other protein-based scaffolds, whereas GPP displayed fewer pores and a smoother surface. GFP also exhibited a porous structure, but it was less pronounced than the soybean protein-based scaffold, with slight bulging of the tissue around the pores, showing distinct surface characteristics. Furthermore, the mixed protein scaffolds (S1-S4) exhibited varying pore sizes and densities depending on the mixing ratio. S2, in which some pea proteins replaced soybean and chickpea proteins, showed an increase in porosity, whereas S3 showed a decrease in porosity. The average pore size of the GSP was 117.8 μm, compared to 72.59 μm for GPP, and 94.80 μm for GFP. Among the mixed protein scaffolds, S2 exhibited the largest pores (113.5 μm), followed by S1 (107.1 μm), S3 (89.9 μm), and S4 (79.67

μm), suggesting that the microstructure of the scaffold is influenced by the protein composition and mixing ratio.

Pore size and distribution are critical factors in cell culture. Smaller pores can aid cell attachment and migration, whereas larger pores enhance nutrient and oxygen diffusion and promote cell proliferation and viability (Carletti et al., 2011; Singh et al., 2023). O'Brien et al. (2005) reported a linear relationship between cell attachment and the specific surface area, suggesting that cell viability is influenced by pore size. The ideal scaffold porosity is considered to be between 30–90%, with an optimal pore size range of 50–150 μm (Alam et al., 2024a), aligning with the pore sizes observed in both the single and mixed protein scaffolds in this study. These findings indicate that the scaffolds used in this study have potential applications in cell culture. However, they exhibited non-uniform pore sizes, likely due to uncontrolled ice crystal formation during the freezing process. According to Chen et al. (2024), scaffolds frozen at -30°C and -80°C exhibited pores with irregular polygonal shapes, whereas those treated with liquid nitrogen developed narrower pores characterized by elongated grooves. Xia et al. (2025) reported that in soybean protein–carrageenan–sodium alginate mixtures, high Ca^{2+} concentrations induced rapid external gelation, hindering diffusion and resulting in irregular pore formation. In contrast, crosslinking with a $CaCl_2$/KCl mixture allowed K^+ to stabilize the network, promoting uniform crosslinking, more regular pore structures, and improved cell growth. This suggests that optimizing the freezing process or developing pore-controlling technologies is necessary to ensure a uniform pore distribution in the scaffolds.

3.2. Water absorption

The water absorption rate is a key indicator that reflects the ability of a scaffold to absorb water over a certain period and is essential for evaluating its physical stability and shape retention (Fig. 2A). The water absorption behavior of scaffolds made from different plant protein sources was investigated. The GSP scaffold, composed of soybean protein, absorbed more than 600% of its initial weight after 12 h of immersion. In contrast, the GPP and GFP scaffolds, derived from pea and chickpea proteins, exhibited absorption rates of 480–580%, indicating lower water uptake than that of GSP. This difference is attributed to the varying porous structures formed by different protein materials. A previous study also demonstrated that scaffolds containing soy protein exhibited significantly higher water absorption (2300–2500%) over 7 days, compared to those containing pea protein (1100–1200%), highlighting the strong water-binding capacity of soy-based materials (Kim et al., 2024).

Excessive water absorption may compromise the structural integrity of the scaffolds, leading to deformation, whereas insufficient absorption can hinder cell attachment, which is a critical factor in scaffold functionality. Therefore, selecting scaffolds with appropriate water absorption capacity is essential to support both cell attachment and proliferation (Chen et al. 2023). The absorption rate is influenced by the protein type and composition. For instance, scaffolds containing glutenin, a major wheat protein, show water absorption rates ranging from 700% to 1500%, depending on the glutenin content (Xiang et al., 2022). Additionally, the scaffold fabrication method significantly affects water absorption. Scaffolds made from soy, mung bean, and chickpea proteins via extrusion exhibit lower water uptake, whereas those made from wheat protein display higher absorption rates (Ikuse et al., 2024). Fang et al. (2025) reported that scaffolds formed by dietary fiber–protein mixtures exhibit decreased water absorption upon crosslinking with $CaCl_2$ or transglutaminase (TGase) owing to reduced

porosity. The water absorption rate of uncrosslinked samples reached 413.94%, whereas cross-linked samples absorbed only approximately 300%.

For the mixed protein samples (S1–S4) composed of soybean, pea, and chickpea proteins, water absorption exceeded 500% after 12 h and surpassed 600% after 24 h in all samples except S1. This reduction compared to GSP is likely due to lower soybean protein content. However, partial substitution with pea or chickpea proteins enhanced the overall water absorption capacity, suggesting that hydration properties can be optimized by adjusting the protein composition. Consistent with our findings, Lin et al. (2022) reported that scaffolds with higher protein contents exhibited enhanced water absorption owing to the presence of hydrophilic functional groups, even when the porosity was reduced. These results highlight the critical role of protein composition in determining the water absorption behavior of the scaffolds.

3.3. Degradation

Understanding scaffold degradation is essential to evaluate the structural stability and functional lifespan of scaffolds under cell culture conditions. In cultured meat production, the degree of scaffold degradation varies depending on the intended application. In some cases, complete degradation may be desirable to avoid residual materials in the final product, particularly in fully edible systems. In other instances, partial or minimal degradation may be preferable for maintaining structural support throughout the culture period. Degradation is typically assessed by changes in physical properties, such as weight or morphology, and can be influenced by factors such as medium composition, pH, osmotic pressure, and enzymatic activity (Seah et al., 2022).

The degradation rate of the scaffolds over time is presented in Fig. 2B. At 12 h, all samples exhibited relatively low and comparable degradation rates, ranging from 3.42% to 7.91%, with no statistically significant differences observed among the groups. However, at 24 h and 48 h, distinct degradation patterns emerged depending on the scaffold composition. Scaffolds composed of mixed plant proteins (S1–S4) generally exhibited higher degradation rates than those composed of single proteins. Among them, S2 demonstrated the highest degradation rate, reaching 24.54% at 24 h, and 25.05% at 48 h. S1 and S3 also showed relatively high degradation levels, exceeding 21% at 48 h. In contrast, single-protein scaffolds, such as GSP, GPP, and GFP, exhibited lower degradation rates, ranging from 17.76% to 20.71% at the final time point. Scaffolds composed of mixed plant proteins (S1–S4) generally exhibited higher degradation rates than those composed of single proteins. These differences in degradation behavior may not be solely attributed to water permeability, as the single-protein scaffold exhibited higher water permeability but also showed a lower degradation rate than the mixed-protein scaffolds.

This apparent contradiction may be attributed to the reduced structural cohesion observed in mixed-protein scaffolds. The incorporation of proteins with differing conformational and electrostatic properties likely disrupts the formation of uniform and compact networks, thereby weakening intermolecular interactions and increasing susceptibility to hydrolytic degradation. Prior studies have demonstrated that heterogeneous protein matrices often exhibit diminished mechanical integrity and lower resistance to enzymatic or aqueous breakdown (Ianovici et al., 2022). In contrast, single-protein scaffolds tend to form more homogenous and densely entangled networks, stabilized by stronger intramolecular bonding, which may account for their greater structural stability even under conditions of higher water absorption. This suggests that factors beyond water diffusion, such as network cohesiveness,

protein–protein compatibility, and intrinsic structural stability, play critical roles in determining degradation resistance. These interactions may prevent the formation of tightly entangled scaffolds, rendering them more susceptible to hydrolytic degradation over time (Liu et al., 2019). In contrast, single-protein systems, such as GSP, may benefit from more uniform aggregation and stronger intramolecular interactions, forming a more stable network despite higher water uptake.

The structural stability of the scaffolds can also be significantly influenced by the composition, concentration, and processing methods of the raw materials. According to Chien and Shah (2012), enzyme-treated soy protein scaffolds (3% and 5% SPI) exhibited delayed degradation compared to untreated controls, especially under cell-seeded conditions, highlighting the role of enzyme-mediated crosslinking in enhancing scaffold stability. Measurement of weight loss in hydrogels with varying agar compositions revealed that hydrogels with less than 0.5% agar content completely degraded, exhibited very low stability. However, when the agar ratio increased to 3%, the hydrogels showed minimal degradation, which may hinder cell diffusion and proliferation in this platform (Lee et al., 2022). Meanwhile, Ianovici et al. (2022) reported that a higher protein content or enhanced crosslinking, such as in soy-alginate scaffolds, may improve structural resilience but also result in slower degradation rates. In conclusion, the degradation behavior of plant protein-based scaffolds was significantly influenced by the scaffold composition, water permeability, and structural integrity. These results emphasize the importance of balancing the degradation rates with functional stability for specific applications, particularly in cultured meat production.

297 3.4. Texture analysis

298 When considering scaffolds as edible materials, mechanical properties, such as
299 hardness, chewiness, and elasticity, are critical factors that determine the overall texture and
300 consumer acceptability of the final product. In this study, the textural properties of scaffolds
301 fabricated from different plant protein sources were evaluated (Fig. 2C). The single-protein
302 scaffolds (GSP, GPP, and GFP) exhibited hardness values of 60.48 g, 60.67 g, and 58.95 g,
303 respectively, with no statistically significant differences observed among them. Pea proteins
304 demonstrated slightly higher hardness and chewiness than soy and chickpea proteins, which
305 may be attributed to their lower porosity and distinctive hydrogel-forming properties. In
306 contrast, the mixed-protein scaffolds (S1–S4), which incorporated varying ratios of soy, pea,
307 and faba bean proteins, displayed distinct mechanical characteristics. Notably, scaffolds S1, S2,
308 and S3 exhibited a marked increase in hardness compared to the single-protein scaffolds, with
309 values of 92.10 g, 107.15 g, and 75.79 g, respectively. These increases suggest that certain
310 combinations of plant proteins may induce synergistic effects, potentially enhancing protein–
311 protein interactions and forming a denser, more crosslinked gel network. However, scaffold S4,
312 prepared with equal proportions (1:1:1) of the three protein sources, showed a hardness of
313 63.5 g—comparable to that of the single-protein scaffolds and not significantly different from
314 GSP, GPP, or GFP. This finding suggests that evenly balanced protein mixtures may not offer
315 the same reinforcing effect, possibly because of interference among the gelation behaviors of
316 each protein component, which could disrupt optimal network formation.

317 Chewiness, another key mechanical property, exhibited more pronounced differences
318 than hardness. The GSP, GPP, and GFP scaffolds showed chewiness values in the range of 52–
319 70, indicating relatively soft and elastic textures without significant variation. In contrast, the
320 mixed-protein scaffolds demonstrated considerable enhancement in chewiness. Among them,

scaffold S2 exhibited the highest chewiness value of 184.6, which was nearly three times greater than that of the single-protein scaffolds. Scaffolds S1 and S3 also recorded chewiness values exceeding 100, indicating that specific combinations, particularly those with a higher proportion of peas or faba bean proteins, significantly contributed to enhanced mechanical resistance during mastication.

These changes in texture are likely driven by differences in protein–protein and protein–water interactions, gelation behavior, and the formation of three-dimensional gel networks (Tang et al., 2025; Zheng et al., 2022). Zhang et al. (2023) demonstrated that the combination of peanut protein and wheat gluten improved the fiberization and elasticity of plant-based matrices through complementary structural roles and intermolecular interactions. These findings align with the current results, where mixed-protein scaffolds such as S2 and S3 exhibited enhanced chewiness, likely owing to synergistic interactions and optimized network formation. In contrast, S4, which incorporated equal proportions of soy, pea, and faba bean proteins, did not show similar improvements, suggesting that uniformly blended protein systems hinder cohesive gelation because of competitive or antagonistic interactions. From this result, in single-protein scaffolds, homogeneous network formation is favored due to uniform gelation behavior, which leads to consistent crosslinking density and stable hydrogel networks. In contrast, mixed-protein scaffolds involve heterotypic interactions among proteins with distinct isoelectric points and structural flexibility, potentially disrupting the uniformity of gelation. This can result in heterogeneous pore formation and variable water uptake. Certain protein combinations, such as those in S2 and S3, may still achieve favorable network structures due to synergistic interactions that enhance intermolecular bonding and matrix stability.

Previous studies have reported that the hardness and chewiness values of pork neck meat are approximately 154.16 g and 87.05, respectively (Yang et al., 2010). Compared with

these values, our S2 scaffold, which showed a hardness of 107.15 g and chewiness of 184.6, demonstrated comparable firmness and even superior chewiness. These findings suggest that the scaffold developed in this study indicates the potential of plant-based proteins to replicate the mechanical characteristics of actual meat. However, as textural transformation during cooking was not assessed in this study, further investigation is needed to evaluate the structural integrity and mechanical behavior of the scaffolds after thermal processing, which is critical for their practical application in real meat analogs.

These mechanical properties are not only important structurally but also influence consumer perception of cultured meat. Scaffolds like S2 and S3, with higher chewiness and moderate hardness, may better replicate the fibrous texture of cuts like flank or thigh meat, while softer scaffolds such as GFP or S4 may suit tenderloin-type or processed products. Since consumers expect plant- or cell-based alternatives to mimic both taste and texture, including bite and mouthfeel, the tunable textural profiles of our scaffolds suggest their potential for various cultured meat applications (Alam et al., 2024b).

3.5. Cell proliferation

The cell proliferation on each scaffold was evaluated after 4 days of culture using the WST assay (Fig. 3A). The results showed clear differences in cell growth depending on the scaffold composition. Among the single-protein scaffolds, GFP supported the highest cell proliferation (122.94%), followed by GPP (96.60%), and GSP (62.86%). In contrast, the mixed-protein scaffolds exhibited lower proliferation rates: S1 (84.02%), S3 (64.86%), S2 (36.78%), and S4 (30.71%). These results indicated that single-protein scaffolds, particularly GFP, were more favorable for cell growth than mixed-protein formulations. According to Lin et al. (2022), the viability of L929 cells cultured on starch-soybean protein-gellan gum composite scaffolds increased with higher soy protein content. These findings highlight the potential of plant-based proteins in enhancing cell proliferation, suggesting their promising applications in tissue engineering and cellular agriculture. Previous findings further support our results and reinforce the applicability of plant-based proteins for cell growth in scaffold systems. However, conflicting results have been reported in previous studies, where protein-blended hydrogels did not show significant improvements in cell adhesion or proliferation compared with pure polysaccharide gels, indicating the limited supportive effects of proteins under unmodified conditions (Wollschlaeger et al., 2022). Therefore, it is important to consider not only the type of protein, but also the composition of materials, such as polysaccharides, in scaffold design and optimization.

These quantitative findings are supported by the SEM images shown in Fig. 3B, which depict the morphology and distribution of the cells on each scaffold. Cells on the GFP and GPP surfaces were more abundant and evenly distributed, showing spread-out morphologies (yellow arrows) indicative of active attachment and proliferation. In particular, GFP presented a dense and relatively homogeneous surface where cells adhered well across the structure. In contrast,

the S2 and S4 scaffolds displayed fewer adhered cells with mostly rounded morphologies, suggesting lower cell–scaffold interactions and biocompatibility. GSP S1 and S3 exhibited intermediate cell densities. Although cell attachment was observed (yellow arrows), the distribution was either more localized or less extensive than that of GFP or GPP. Scaffold composition and structure are known to affect cell viability and proliferation, with factors such as surface chemistry, porosity, and mechanical stiffness influencing cell–matrix interactions (Wu et al., 2024; Xiang et al., 2022).

Among these factors, porosity and pore size play a particularly important role in modulating cellular responses by facilitating nutrient and oxygen diffusion, waste removal, and providing surface area for cell attachment. Previous studies have shown that scaffolds with interconnected pores ranging from 50 to 150 μm offer optimal conditions for cell infiltration and proliferation, whereas irregular or excessively large pores may impair cell–matrix interactions and reduce structural stability (O'Brien et al., 2005). In the present study, the relatively uniform and moderate pore sizes of GFP and GPP scaffolds, combined with appropriate stiffness and protein–protein interactions, likely contributed to enhanced cell proliferation by offering a structurally favorable and biocompatible microenvironment (Chen et al., 2024; Kong et al., 2024).

4. Conclusion

This study demonstrated the feasibility of using plant-based proteins as edible scaffolds for cultured meat applications. Scaffolds made from a single protein source, particularly faba bean protein, support superior cell adhesion and proliferation, highlighting their potential as viable edible scaffold materials. Mixed-protein scaffolds exhibit enhanced mechanical properties and water absorption, suggesting that compositional tuning can optimize scaffold functionality. Although the degradation rates increased over time, the overall structural stability remained within a usable range for short-term cell culture. These findings indicate that the strategic combination of plant proteins can enhance both the biological performance and textural properties, offering a promising approach for developing sustainable, functional scaffolds in cellular agriculture. By replacing animal-derived components with cost-effective plant proteins, this strategy also contributes to improved sustainability and economic feasibility in cultured meat production.

Conflict of interest

The authors declare no potential conflicts of interest.

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538

539 **Table caption**

540 **Table 1.** Designation of scaffolds based on various protein sources and mixture ratios

541

542 **Figure caption**

543 **Fig. 1.** Appearance and surface characteristics of scaffolds based on protein sources and mixing

544 ratios

545 **Fig. 2.** Water absorption (A), texture characteristics (B), and degradation properties (C) of

546 scaffolds according to protein sources and mixing ratios

547 **Fig. 3.** Cell proliferation (A) after 4 days and cell adhesion properties (B) of scaffolds based on

548 protein sources and mixing ratios

549 **Table 1.** Designation of scaffolds based on various protein sources and mixture ratios

Sample	Soy protein (mg/mL)	Pea protein (mg/mL)	Faba bean protein (mg/mL)	Potato starch (mg/mL)	Gellan gum (mg/mL)
GSP	100	0	0	20	10
GPP	0	100	0	20	10
GFP	0	0	100	20	10
S1	60	20	20	20	10
S2	20	60	20	20	10
S3	20	20	60	20	10
S4	33.33	33.33	33.33	20	10

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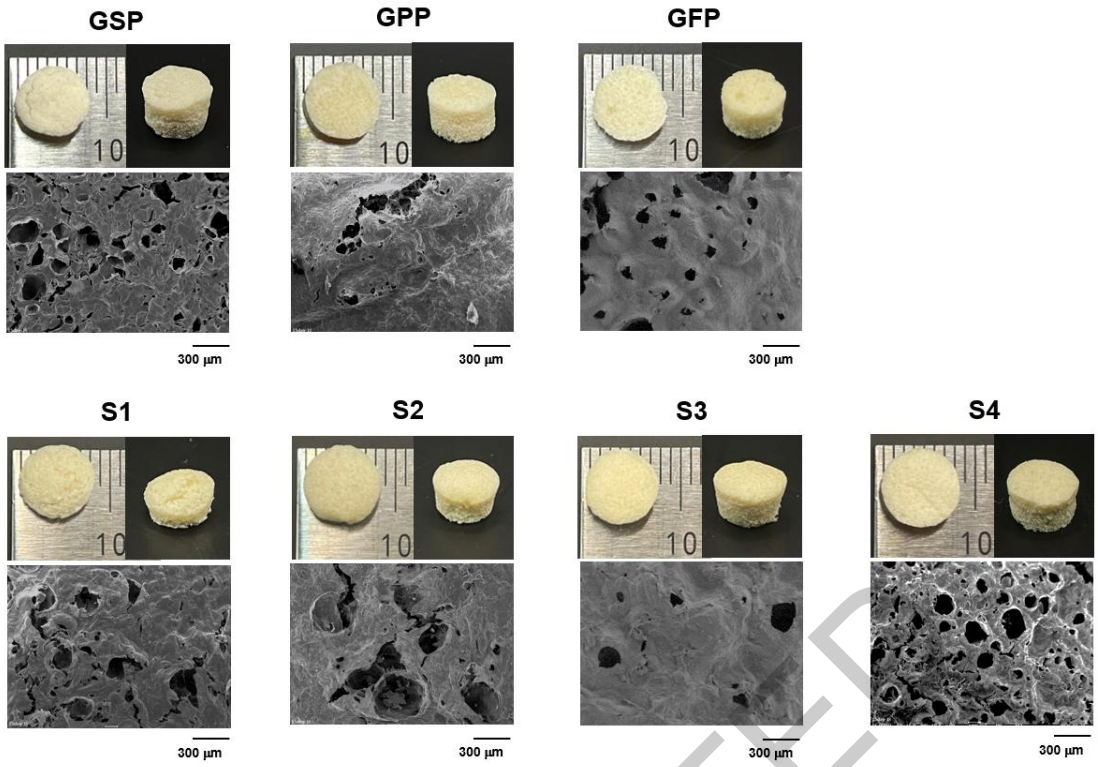
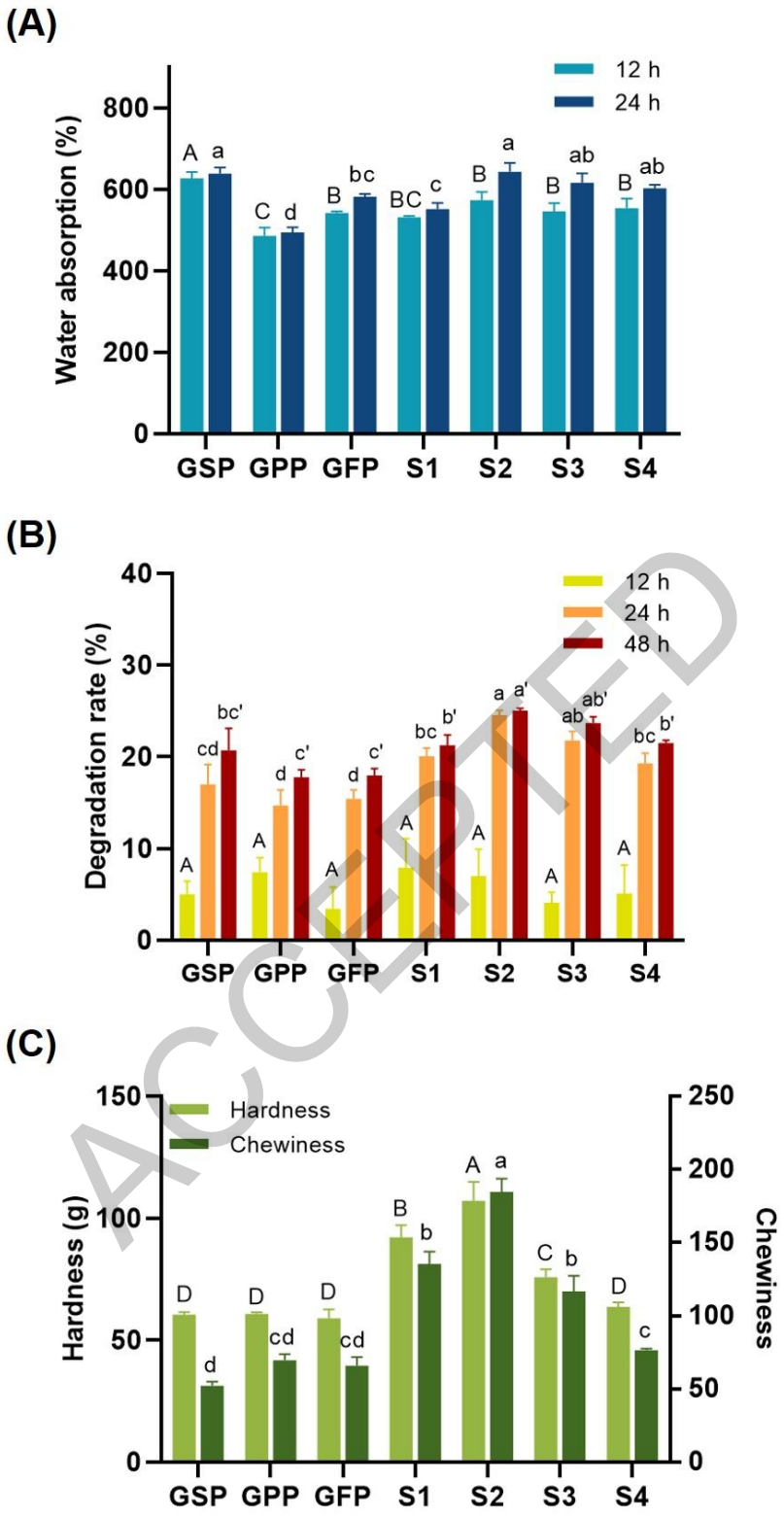


551

552 **Fig. 1.** Appearance and surface characteristics of scaffolds based on protein sources and mixing

553 ratios

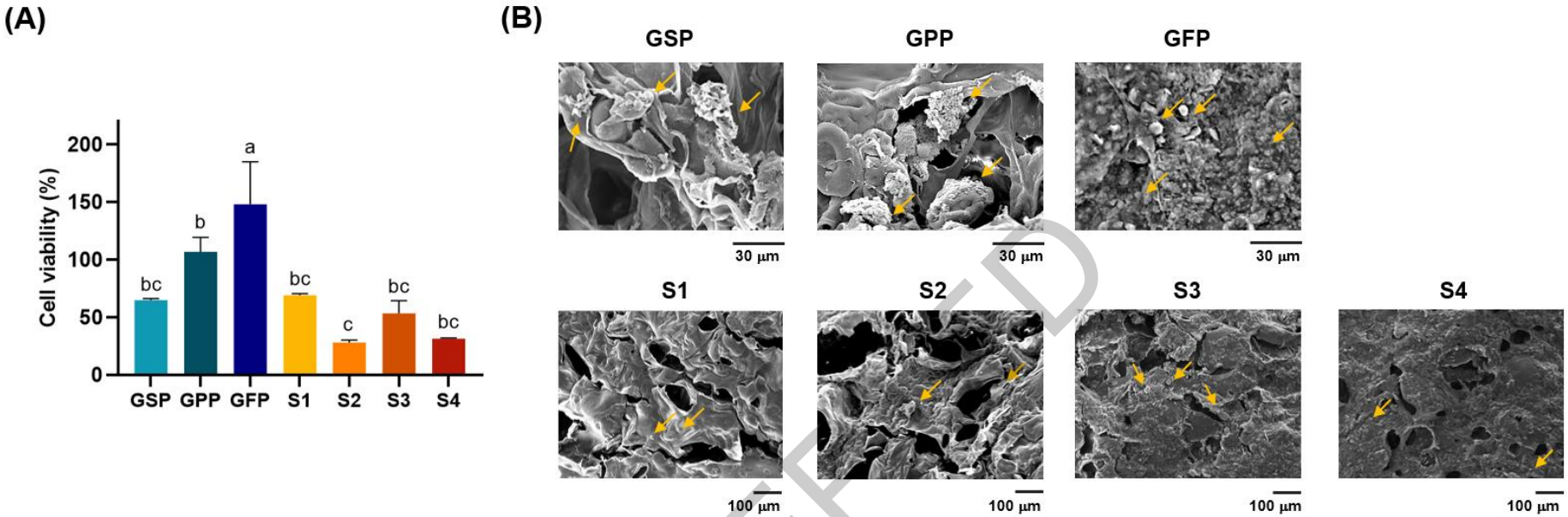
554



555

556 **Fig. 2.** Water absorption (A), degradation properties (B), and texture characteristics (C) of

557 scaffolds based on protein sources and mixing ratios



558

559 **Fig. 3.** Cell proliferation after 4 days (A) and cell adhesion properties (B) of scaffolds based on protein sources and mixing ratios

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