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Enhancing Meat Analog Texture Using Wet-Spun Fibroin Protein Fibers: A Novel Approach to Mimic Whole-Muscle Meat

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ABSTRACT

The increasing demand for protein-rich, plant-based foods has driven the development of meat analogs that closely mimic the texture and mouthfeel of animal meat. While plant-based fibrils and electrospun silk fibroin fibers have been explored for texture enhancement and scaffolding in both meat analogs and cell-based meats, the use of wet-spun fibroin protein fibers as a food ingredient remains underexplored. This study investigates the potential of wet-spun recombinant fibroin fibers to enhance the textural properties of meat analogs. Short fibers, with varying tensile strengths and diameters, were incorporated into a commercial ground pork analog to create improved patty samples. The results showed that adding hydrophilic, 30 μm-diameter, 3-mm short protein fibers at 1% (w/w) significantly increased the springiness of the pork analog by 45%. Additionally, fiber sheets designed to mimic the endomysium structure of intramuscular connective tissue were integrated into the minced pork analog using a three-dimensional needle punching technique. This approach successfully recreated the interlacing endomysium structure found in whole-muscle pork, yielding a texture that closely matched the slice shear force, springiness, and cohesiveness of traditional pork. In conclusion, the incorporation of wet-spun protein fibers offers a promising strategy to enhance the textural qualities of meat analogs, making them more comparable to animal meat and potentially more appealing to consumers seeking high-quality plant-based alternatives.

1 | Introduction

As global population and meat consumption increase, meat analogs have emerged as environmentally friendly and ethical alternatives (Grundy et al. 2022). These typically consist of textured vegetable protein (TVP) to provide a meat-like texture (Baune et al. 2022). However, TVP's fibrous structure, produced by thermo-extrusion, differs significantly from animal meat due to larger fiber sizes and different physiochemical properties (Xiong 2023). Despite its fibrous nature, TVP cannot adequately mimic muscle meat because it lacks the complex structure of both muscle fibers and intramuscular connective tissues (He et al. 2021). To the best of the authors' knowledge, a "connective tissue-like" ingredient, though essential for the structure and chewy texture of whole muscle meat analogs (WMMA), is not yet available to the food industry (Sha and Xiong 2020).

Previous research has explored plant protein fibers to enhance meat analog texture, focusing on microscopic protein fibrils as texture modifiers (Chen, Jones, and Campanella 2021). While wet-spun fibers have been used in applications such as encapsulation and packaging, their use in meat analogs remains limited

(Aoyagi 2017). Commercial production of wet-spun soy protein fibers in the 1980s ceased due to high manufacturing and storage costs, as the brittle fibers required storage in either frozen or acid baths. Silkworm silk, another fibrous protein, has been studied for various food applications, including as a texture modifier in limited instances in Japan (Kazuko and Kiharu 2013; Osaki and Ichikawa 2012). Additionally, fibroin protein fibers have been used for muscle cell growth in in vitro meat applications (Bomkamp et al. 2022). However, while studies on silk protein and fibroin fibers provided valuable insights into their functionality in food systems, these findings cannot directly corroborate the feasibility or effectiveness of wet-spun recombinant fibroin-inspired protein fibers in meat analogs. The aforementioned studies focused on different applications and conditions, and further investigation is required to understand the specific functionality of wet-spun recombinant fibers in plant-based meat systems.

In this study, wet-spun recombinant fibroin-inspired protein fibers were used as a textural additive at low concentrations (approximately 1% by weight), allowing for precise control of physicochemical properties and mechanical characteristics. In contrast to soy protein fibers, which were used as bulk ingredients, the reduced material requirements of wet-spun recombinant fibroin-inspired fibers and their effectiveness at low concentrations potentially make them a more cost-effective and industrially scalable alternative. The primary aim of this study is to evaluate wet-spun recombinant fibroin-inspired protein fibers as a textural additive in meat analogs. By evaluating short fibers with various protein properties and fiber tensile strengths in burger patties, and reproduction of endomysium-like fiber sheets of varying fiber lengths and sheet densities in WMMA, the effectiveness of protein fibers in improving the texture of meat analogs was studied (Figure 1).

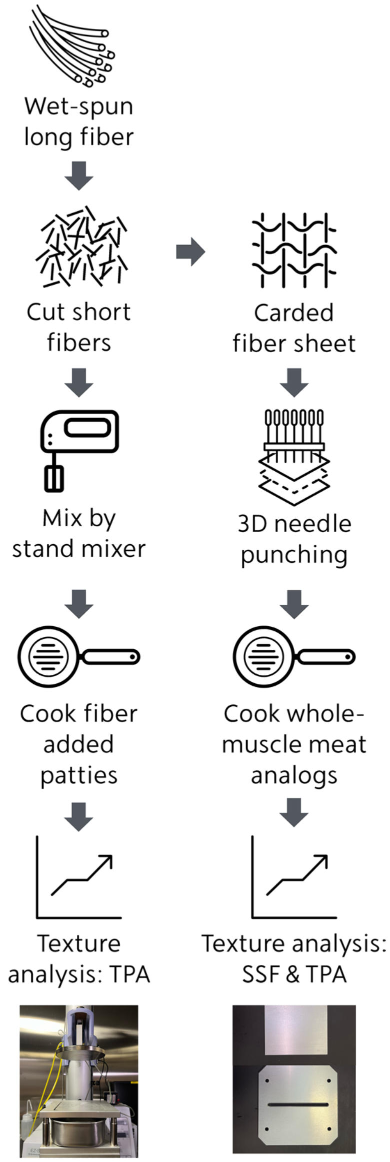

FIGURE 1 | Schematic diagram for sample preparation and analysis. In this study, the effectiveness of wet-spun recombinant fibroin-inspired protein fibers as a texture additive in meat analogs was evaluated in two ways: Short fibers in patty samples and carded fiber sheets in whole-muscle meat analog samples.

2 | Materials and Methods

2.1 | Materials

Three types of wet-spun recombinant fibroin-inspired protein fibers with varying combinations of fiber diameter and protein composition were used in this research: Hydrophobic_L (large diameter fiber prepared from hydrophobic artificial silk-inspired protein), Hydrophobic_S (small diameter fiber prepared from hydrophobic artificial silk-inspired protein), Hydrophilic_L (large diameter fiber prepared from hydrophilic artificial silk-inspired protein); all of the above were supplied by Spiber Inc. (Tsuruoka, Yamagata, Japan), while silkworm silk fibers were sourced from a local silk manufacturer. These wet-spun recombinant fibroin-inspired fibroin fibers were manufactured at kilogram-scale at Spiber Inc. from recombinant proteins design from spider silk protein motifs. The authors did not prepare the fibers used in this research, due to the extensive time required to make enough fiber at laboratory-scale for the purpose of this research. These fibers, composed entirely of protein with minimal impurities, were made with food-safe chemicals and no synthetic polymers. The fiber diameter and tensile properties were controlled during spinning through nozzle size and fiber extension. Commercial plant-based ground pork analog, OmniPork (OmniFoods International Ltd. Kowloon Bay, Hong Kong, ROC), and needle punching tools were online. Ground pork was purchased locally. OmniPork's ingredients included: vegetable protein (soybean protein, pea protein, rice protein, shiitake), yeast extract, maltodextrin, potato starch, sugar, salt, malt extract, glucose/thickener (methylcellulose), flavor, colorant (beetroot).

2.1.1 | Characterization of Protein Fibers

2.1.1.1 | Fiber Diameter Measurement. Optical microscope ZEISS AXIO Imager M1m (ZEISS, Oberkochen, Baden–Württemberg, Germany) and photo-documentation software NIS-Elements D (Nikon Corp. Minato City, Tokyo, Japan) were used to measure fiber diameter. Single filaments were first hand-mounted onto tensile testing paper then observed under the microscope at 500× magnification. A total of five fiber specimens were made for each protein fiber, and each specimen was measured at two points along the length of a single filament.

2.1.1.2 | Fiber Tensile Property Measurement. Tensile test was performed on single filaments that were mounted on tensile testing papers to obtain ultimate strength and failure strain. The specimen test length was 20 mm. An INSTRON 1195 machine (Illinois Tool Works, Glenview, Illinois, U.S.) was used with a 1 N load cell and 10 cm/s test speed. The diameter of single filaments obtained using the procedure described above was entered into the Instron Tensile Test program (Illinois Tool Works, Glenview, Illinois, U.S.) to calculate the cross-sectional area of a single filament.

2.1.2 | Cutting Short Fiber

Long multifilaments obtained from suppliers on bobbins were cut into short fiber of predetermined length using desktop short fiber cutting machine NP-300 (Intec Co. Ltd. Seoul, Korea).

2.1.3 | Preparation of Fiber Sheet

Short fibers prepared following the process detailed in 2.1.2 were crimped and carded using an industrial carding machine (INTEC Sample Roller Card, model: ISC-600 by Intec Co. Ltd. Seoul, Korea) to obtain protein sheets to be incorporated into WMMA samples. The sheets were prepared with short fibers of varying lengths at different sheet densities combinations: 52 mm_14 g/m^2, 52 mm_21g/m^2, 52 mm_27 g/m^2, 52 mm_30 g/m^2, 30 mm_24 g/m^2, 30 mm_25 g/m^2, 30 mm_30 g/m^2, 46 mm_25 g/m^2, 52 mm_25 g/m^2, 17 mm_30 g/m^2, 24 mm_30 g/m^2 (fiber sheet specs were denoted as "length_density"). The density of the protein sheets was controlled by adjusting the initial weight of fiber input into the carding machine.

Commercially available edible protein sheets prepared from gelatin and zein were purchased from Gelatex Technologies (Tallinn, Estonia) to have a customized sheet density of about 25 g/m^2. Figure 2 contains the microscopic images of the above-prepared and purchased protein sheets. The images were taken

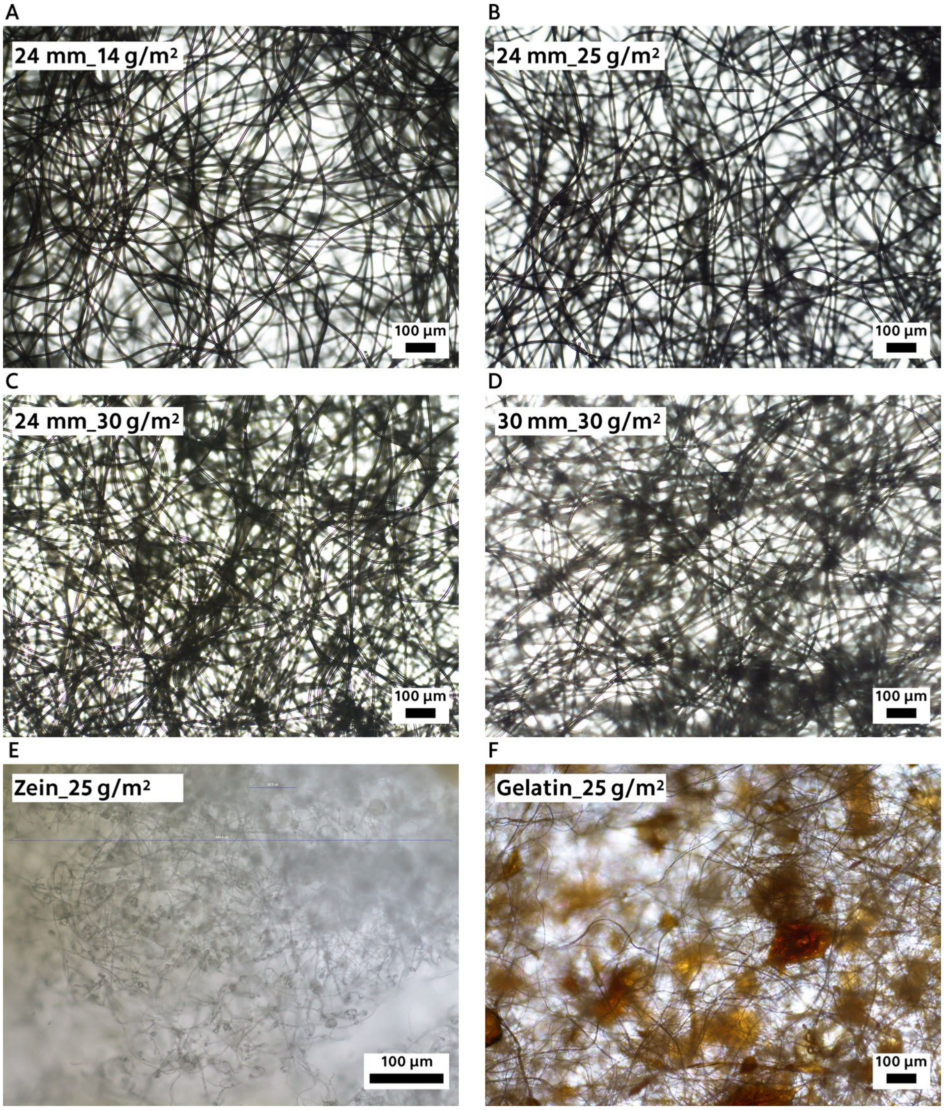

FIGURE 2 | Microscopic image of protein sheets. Protein sheets (A–D) were prepared from recombinant fibroin-inspired protein fiber Hydrophobic_S at the following fiber length and sheet density, (A): 24 mm_14 g/m^2, (B): 24 mm_25 g/m^2, (C): 24 mm_30 g/m^2, (D): 30 mm_30 g/m^2. Protein sheet (E–F) were commercially available zein and gelatin fiber sheets prepared at 25 g/m^2 density (irregular fiber length). (Scale bar: 100 µm).

on an optical microscope ZEISS AXIO Imager M1m (ZEISS, Oberkochen, Baden–Württemberg, Germany) and photo-documentation software NIS-Elements D (Nikon Corp. Minato City, Tokyo, Japan).

2.2 | Preparation and Characterization of Fiber-Added Meat Analogs

Two types of fiber-added meat analogs were prepared in this study using two formats of protein fibers: first, short protein fibers were combined with the meat base (minced pork analog or animal-based pork) by mixing then molding to form patties (a comminuted meat analog). Second, protein fiber sheets prepared according to 2.1.2 were needle punched into the meat base to form WMMA samples. The samples were prepared by directly combining the meat base with fibers, without any additional ingredients. In other words, the binding within the samples was a result of the added fibers and existing binders in the meat base (potato starch and methylcellulose in the minced pork analog, and hydrolyzed proteins in animal-based pork).

2.2.1 | Preparation of Short-Fiber-Added Patties

Frozen plant-based minced meat analog was thawed at 4°C overnight before use. Ground pork was purchased from a local supermarket the day before sample preparation and stored at 4°C until sample preparation. 3-mm short fibers were combined with the meat base by mixing with a spatula for 5 min according to the formulations specified in Table 1. A total of 100 g of the meat base and short fiber mixture was shaped in a circular mold with a diameter of 9 cm and a depth of 1.8 cm, covered by a cap and stored at 4°C overnight. Three samples were prepared for each treatment type. Samples were cooked on the next day on a piece of cooking sheet set on an electric grill at 200°C. Each sample was cooked for 7 min per side then evaluated for cooking loss, size reduction, and TPA immediately.

TABLE 1 | Formulation of short protein fiber-added patties.

Treatment	Meat base	Meat % weight	Fiber type	Fiber % weight
Control	Commercial minced pork analog	100	—	0
1% Hydrophobic_S		99	Hydrophobic_S	1
5% Hydrophobic_S		95	Hydrophobic_S	5
1% Hydrophilic_L		99	Hydrophilic_L	1
1% Hydrophobic_L		99	Hydrophobic_L	1
1% Silk		99	Silk	1
Pork	Pork	100	—	0

TABLE 2 | Formulation of protein fiber sheet-added whole muscle meat analogs.

Treatment name	Meat base	Protein fiber type	Staple fiber length (mm)	Sheet density (g/m^2)
52 mm_14 g/m^2	Commercial minced pork analog	Hydrophobic_S (10 µm)	52	14
52 mm_21 g/m^2				21
52 mm_27 g/m^2				27
52 mm_30 g/m^2				30
30 mm_24 g/m^2			30	24
30 mm_25 g/m^2				25
30 mm_30 g/m^2				30
46 mm_25 g/m^2			46	25
52 mm_25 g/m^2			52	
17 mm_30 g/m^2			17	30
24 mm_30 g/m^2			24	
Gelatin_25 g/m^2		4 µm gelatin	Unknown (irregular)	About 25
Zein_25 g/m^2		2 µm zein	Unknown (irregular)	About 25

2.2.2 | Preparation of Sheet-Added WMMAs

Sheet-added WMMAs were prepared according to the formulations outlined in Table 2. 160 g of meat base was rolled into a thin layer (20 cm × 30 cm), with a fiber sheet placed on top. The layers were cut into six 10 cm squares, stacked, and needle-punched with barbed needles. The sample was wrapped in plastic and stored at 4°C until cooking. Samples were cooked on an electric grill at 200°C for 7 min per side.

2.3 | Characterization of Fiber-Added Meat Analogs

2.3.1 | Cooking Loss and Size Reduction

2.3.1.1 | Cooking Loss. Cooking loss was determined by measuring the raw and cooked weight of three patties for each treatment and calculated by the following equation (Pang et al. 2020):

$$\text{Cooking loss}\,(\%) = \frac{\text{raw weight} - \text{cooked weight}}{\text{raw weight}} \times 100\,\%$$

2.3.1.2 | Diameter and Thickness Reduction. Changes in diameter or thickness of patties before and after cooking were determined by measuring the diameter or thickness of three patties for each treatment and calculated by the following equations, respectively (Guedes-Oliveira et al. 2019):

$$\text{Diameter reduction}\,(\%) = \frac{\text{raw diameter} - \text{cooked diameter}}{\text{raw diameter}} \times 100\,\%$$

$$\text{Thickness reduction}\,(\%) = \frac{\text{raw thickness} - \text{cooked thickness}}{\text{raw thickness}} \times 100\,\%$$

2.3.2 | Texture Profile Analysis of Fiber-Added Patties

The texture of fiber-added patties was evaluated using TPA to measure hardness, springiness, cohesiveness, and chewiness. Testing parameters were optimized from the original TPA protocol by Bourne 1968 to suit this study. Specifically, the strain level was reduced from 75% to 50% to address the lack of structural integrity of the commercial plant-based control samples (without the addition of wet-spun fibroin protein fibers). The textural integrity of the controls could not sustain the original strain level, leading to excessive deformation or sample failure during the first compression. Reducing the strain allowed for usable data collection from the control samples while maintaining the validity of comparisons with fiber-added samples (Rosenthal 2010). Additionally, the compression speed was increased from 0.83 to 3.3 mm/s to mimic human chewing speed, as suggested by literature (Nishinari, Fang, and Rosenthal 2019; Pons and Fiszman 1996). Speeds above 4 mm/s caused structural failure, while Rosenthal recommended a minimum of 2 mm/s. Thus, the final speed was set at 3.3 mm/s. TPA was conducted 6–9 times immediately after cooking using a Universal Testing Instrument EZ Test (Shimadzu Corp. Kyoto, Japan). Cooked samples were cut to predetermined sizes, and TPA parameters were obtained from force-time curves: hardness (first compression maximum force), springiness (time ratio for second and first compression), cohesiveness (work ratio for second and first compression), and chewiness (hardness × springiness × cohesiveness) (Trinh 2012).

2.3.3 | Texture Profile Analysis and Slice Shear Force Measurement of WMMAs

Since most texture analyses of meat analogs were conducted on comminuted products, an array of texture studies and methodologies for animal meats were referenced and modified to analyze the WMMA prepared in this study (Cardello et al. 1983; de Huidobro et al. 2005; Guzek et al. 2013; Lorenzen et al. 2010; Wheeler, Shackelford, and Koohmaraie n.d.). Compare to the TPA compression speed used to analyze fiber-added patties, the speed was further increased from 3.3 to 5 mm/s. This was because the WMMA samples were less crumbly than patty samples and could sustain a higher testing speed before structural failure. Additionally, in a standard slice shear force (SSF) test, samples would be cored using a hollow metal rod into cylinders and samples' fiber orientation would be set perpendicular to the SSF blade during testing (Shackelford, Wheeler, and Koohmaraie 1999). In this study, the WMMA samples were cut into cuboids (Table S1). This was because the main composition of the samples was TVP instead of muscle fibers. The TVP particles, even with the presence of binders and fiber sheets, could not have gone through coring without structural failure. Moreover, the samples did not have a fiber orientation, as the fibers in the sheets were orientated randomly like the endomysium in intracellular connective tissue (Purslow 2020). Therefore, the step where muscle fibers would be aligned perpendicularly to the testing blade was omitted. Instead, the cuboid samples were sheared in half along the width. SSF analysis of sheet-added WMMA samples was conducted four times immediately after cooking for each treatment using a Universal Testing Instrument EZ Test (Shimadzu Corp. Kyoto, Japan). Cooked samples were cut (4 × 2 cm × original height of the sample) and sliced using a shear blade of 3 mm thickness and 45° cutting edge attached to a 500 N load cell. The blade cut through the entire sample's thickness through the slit on the platform holding the sample. The crosshead speed was 300 mm/min. SSF (in Newton) is the maximum force required to cut through the sample.

Sensory evaluation was not conducted in this study due to the GMO-derived nature of the fiber ingredient, for which safety data is still being collected. Instrumental texture analysis was used as a predictor of sensory properties to provide initial insights into the effects of fiber length and density on the texture of the meat analog.

2.4 | Statistical Analysis

Three independent batches of patty samples were prepared for each treatment, with one patty sampled from each batch for cooking loss and size reduction measurements. Each batch was prepared separately to ensure independence between replicates, thereby accounting for batch-to-batch variability. The three patties were then cut into nine 2.5 cm square pieces for TPA measurements. For WMMA samples, two different samples were prepared for each treatment, then cut into six 2 cm square pieces for TPA, and four 2 × 4 cm rectangular pieces for SSF analysis. The mean and standard deviation calculations and analysis of variance (ANOVA and post hoc Tukey HSD test, using $p < 0.05$) of each property were conducted using JMP (Version 17. SAS Institute Inc. Cary, NC, 1989–2023).

3 | Results and Discussion

3.1 | Sample Space Development: Patties Containing Short Protein Fibers

In the first part of the study, four distinct fiber types, each featuring unique combinations of fiber diameter and protein hydrophilicity, were meticulously selected to elucidate the differential impacts these characteristics may impart as fiber-shaped texture modifiers in meat analogs at inclusion rates of either 1% or 5%. Concerning protein hydrophilicity, it is well-documented in the textile industry that fibers with higher hydrophilicity tend to exhibit greater shrinkage rates under conditions of moisture and heat (Célino et al. 2014; Mohammed et al. 2023). Building on this established knowledge, we posited that fibers with enhanced hydrophilicity (Hydrophilic_L) would demonstrate more pronounced shrinkage behaviors compared to their hydrophobic counterparts (Hydrophobic_L), potentially influencing the textural properties of meat analogs. Furthermore, we explored the implications of fiber diameter and tensile strength on the textural outcomes in meat analog samples. We hypothesized that fibers with larger diameters and superior tensile strength would more significantly alter the texture of meat analogs, contributing to a more robust and cohesive product. This hypothesis was based on the premise that thicker and stronger fibers could better withstand oral masticating conditions, thereby enhancing the overall textural experience of the meat analog sample.

3.2 | Patties Containing Short Protein Fibers: Cooking Loss Measurements

Four types of protein fibers with varying combinations of fiber diameter and protein composition were used in this research: Hydrophobic_L (large diameter fiber prepared from hydrophobic artificial silk-inspired protein), Hydrophobic_S (small diameter fiber prepared from hydrophobic artificial silk-inspired protein), Hydrophilic_L (large diameter fiber prepared from hydrophilic artificial silk-inspired protein) and Silk (natural silk fibers sourced from local silk manufacturer). Their fiber diameter and tensile strengths were characterized using methods described in Section 2.1.1, and the results were detailed in Table 3. Hydrophobic_L and Hydrophilic_L had fiber diameters and tensile strength values that were not statistically different. On the other hand, Hydrophobic_S and Silk had fiber diameters and tensile strain values that were not statistically different.

Cooking loss and size reduction were measured for short protein fiber-added patty samples listed in Table 1, and the results were summarized in Table 4: samples including Control, 1%Hydrophobic_S, 1%Hydrophilic_L, 1%Hydrophobic_L, 1%Silk had cooking loss, diameter and thickness reduction

TABLE 3 | List of protein fibers and their physical and mechanical properties.

Fiber name	Main protein composition	Fiber diameter (μm)	Tensile strength (%)	Tensile strain (%)	Source
Hydrophobic_L	Hydrophobic artificial silk-inspired protein	28.7 ± 1.7	117 ± 10	192 ± 45	Spiber Inc.
Hydrophobic_S	Hydrophobic artificial silk-inspired protein	10.7 ± 0.3	153 ± 13	62 ± 10	Spiber Inc.
Hydrophilic_L	Hydrophilic artificial silk-inspired protein	26.9 ± 1.0	100 ± 7	100 ± 11	Spiber Inc.
Silk	Silkworm silk fibroin	11.9 ± 1.8	310 ± 93	58 ± 19	Local silk manufacturer

TABLE 4 | Cooking loss and diameter reduction of protein fiber-added patties.

Treatment name	Cooking loss (%)	Diameter reduction (%)	Thickness reduction (%)
Control	$23.75 \pm 2.36^{b,c}$	11.11 ± 0.00	16.67 ± 0.00
1% Hydrophobic_S	$22.67 \pm 1.15^{b,c}$	11.11 ± 0.00	16.67 ± 0.00
5% Hydrophobic_S	26.67 ± 1.53^{a}	16.67 ± 0.00	16.67 ± 0.00
1% Hydrophilic_L	23.67 ± 1.53^{b}	11.11 ± 0.00	16.67 ± 0.00
1% Hydrophobic_L	$21.00 \pm 2.65^{b,c}$	11.11 ± 0.00	16.67 ± 0.00
1% Silk	$22.67 \pm 2.08^{b,c}$	11.11 ± 0.00	16.67 ± 0.00
Pork	24.00 ± 1.00^{b}	22.22 ± 0.00	2.78 ± 0.00

Note: ($n = 3$; means not sharing any superscript letter (a, b, c) are significantly different by Tukey's honest significant difference test at 95% confidence level).

values that were not statistically different. In other words, the addition of all types of protein fibers at 1% to plant-based meat did not affect cooking loss nor size reduction. The fiber content was further increased to 5% using Hydrophobic_S (sample 5%Hydrophobic_S): an increased cooking loss of 26.67% and diameter reduction of 16.67% were observed. The increase in fiber content from 1% to 5% using Hydrophobic_S did not affect reduction in thickness. The inclusion of protein fibers at 1% (w/w), regardless of fiber properties, did not affect the cooking loss or degree of sample size reduction as expected. Specifically, differences in cooking loss and sample size reduction (thickness and diameter) were not observed between 1%Hydrophilic_L and 1%Hydrophobic_L (difference in protein hydrophilicity), 1%Hydrophobic_S and 1%Hydrophobic_L (difference in fiber diameter), or artificial silk-inspired protein and silkworm silk fibroin (difference in protein source and tensile properties). With this result, the inclusion of protein fibers was increased from 1% to 5%. Notably, the cooking loss and diameter reduction of 5%Hydrophobic_S were significantly higher than the plant-based control. In general, the addition of fibers to materials like dental filling or concrete contribute to reduced shrinkage (Aghaee and Khayat 2021; Mangoush et al. 2021). In this research, 1% addition might not have been high enough to reduce shrinkage as observed in other fiber-reinforced composites; yet 5% was too high and might have resulted in micro-cracks around the fibers, increasing cooking loss and shrinkage. Alternatively, this could be the result of high tensile elongation and contraction property of protein fibers (Gu, Jiang, and Hu 2019), which is not common in plant fibers nor other man-made fibers commonly studied in fiber-reinforced composites (Ho et al. 2012; Shakir Abbood et al. 2021). Though further studies in the mechanisms were required, 5% Hydrophobic_S samples' prominent cooking loss and shrinkage behavior were similar to the shrinking behavior of animal-based pork and therefore desirable in the authors' opinion, because this allows the recreation of the same cooking experience for chefs and consumers when using plant-based samples that include protein fibers.

3.3 | Patties Containing Short Protein Fibers: Texture Profile Analysis

Texture profile analysis (TPA) was conducted to measure the hardness, springiness, cohesiveness, and chewiness of the short fiber-added patty samples. The results were summarized in Figure 2. The hardness values of samples containing 1% fibers (1% Hydrophobic_S, 1% Hydrophilic_L, 1% Hydrophobic_L, 1%Silk) were not statistically different from the Control. Sample containing 5% fiber (5% Hydrophobic_S) exhibited a 331% increase in hardness. However, when compared to pork samples, the hardness of 5% Hydrophobic_S was 28 N lower. Hydrophobic_L fiber was effective in improving the springiness of the sample at 1% addition; 1% Hydrophobic_L samples boasted a springiness as high as that of pork (around 0.8). 5% Hydrophobic_S samples also improved the springiness of the samples, though still lower than the springiness of pork and 1% Hydrophobic_L samples. 1% Hydrophobic_S and 1% Hydrophilic_L had springiness values not statistically different from that of control. In terms of cohesiveness, 5% Hydrophobic_S showed 39% increase in Cohesiveness when compared to the control, though still 21% lower than pork. No difference in cohesiveness was observed in 1% Hydrophobic_S and 1% Hydrophobic_L. Decrease in cohesiveness was observed in 1% Hydrophilic_L and 1% Silk. Chewiness of all samples containing 1% fibers (1% Hydrophobic_S, 1% Hydrophilic_L, 1% Hydrophobic_L, 1% Silk) were not statistically different from the Control, and much lower than Pork. 5% Hydrophobic_S showed 629% increase in Chewiness when compared to the control, though still 64% lower than pork. The texture profiles obtained indicated that the addition of short protein fibers at a 1% (w/w) inclusion rate generally failed to enhance the textural parameters of the plant-based control (Figure 3). An exception was noted with 1% Hydrophobic_L, which significantly increased the springiness of the commercial minced pork analog by 45%. This increase is likely attributable to the higher tensile strain of Hydrophobic_L, which was at least 92% greater than that of all other protein fibers used, indicating a superior elasticity that contributed to the enhanced springiness of the sample. The improvement was so marked that the springiness of Hydrophobic_L samples matched that of pork patties.

When the inclusion rate was increased to 5% (w/w), the effects on the texture profiles of the meat analog were more pronounced. Hydrophobic_S fibers at this higher concentration further improved the sample's TPA parameters across all measures. Additionally, the inclusion of 5% (w/w) silk notably increased hardness by 147%, although it did not significantly impact other textural parameters (Figure S1). In terms of TPA hardness, it was observed that the higher the tensile properties of the fibers, the greater the TPA hardness. This phenomenon is commonly seen in fiber-reinforced composites where robust fiber materials are integrated into composites to enhance toughness.

Hydrophilic_L recorded the lowest hardness value, as its higher hydrophilicity led to diminished tensile properties when the fibers interacted with water within the meat analogs. Cohesiveness was negatively impacted in samples containing Hydrophobic_L and Hydrophilic_L (larger diameter fibers), potentially due to the larger diameter creating more significant micro-cracks around the fibers, thereby reducing cohesiveness. It remains unclear why Silk, which also has a small diameter, similarly, resulted in reduced cohesiveness. Conversely, the springiness of samples was reduced to below control levels when Hydrophobic_L concentration was increased from 1% to 5% (w/w), possibly due to fiber overcrowding leading to matrix disruption and a decrease in sample elasticity.

Notably, the addition of all protein fibers at a 5% (w/w) concentration resulted in an unpleasant mouthfeel, and the samples were deemed unacceptable as foodstuffs by taste volunteers. Consequently, the authors conclude that while a 5% fiber addition proved effective in TPA tests, it is not a suitable method for improving the texture of meat analogs.

3.4 | Sample Space Development: WMMAs Containing Protein Sheets

Protein sheets were prepared using short fibers of different lengths at various sheet densities and incorporated into a commercial minced meat analog to study their textural implications. Specifically, the fiber sheet specs were as follows (denoted as

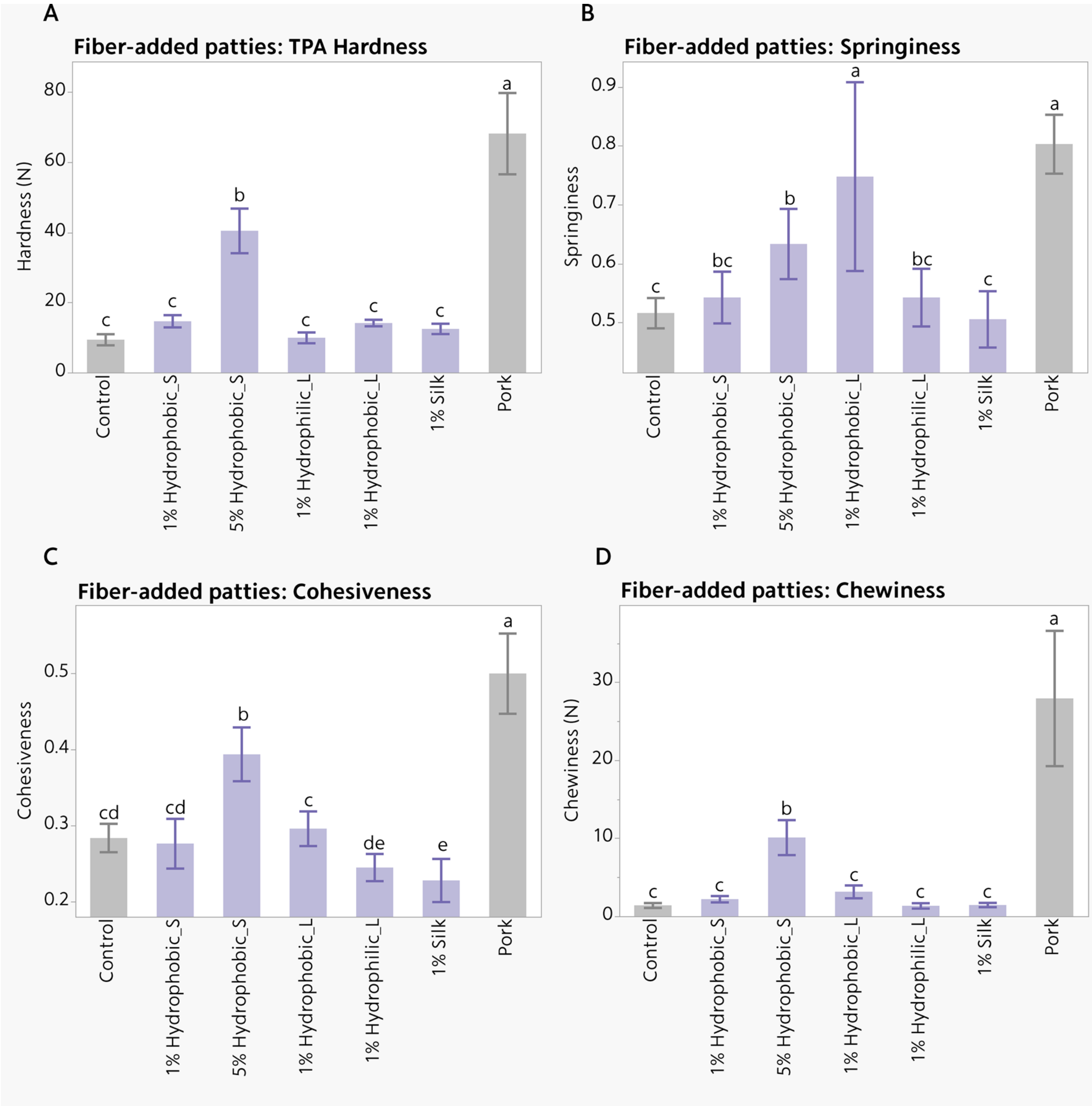

FIGURE 3 | Texture profile analysis result of fiber-added patties. (A–D) represent the mean value of the following texture profile parameters of each treatment, respectively: TPA hardness, springiness, cohesiveness, and chewiness. ($n = 9$; error bar is one standard deviation calculated by unbiased estimation; means not sharing any letter are significantly different by Tukey's honest significant difference test at 95% confidence level. Gray bars represent plant-based and animal-based controls. Purple bars represent treated plant-based samples).

"length_density"): 52 mm_14 g/m^2, 52 mm_21/m^2, 52 mm_27 g/m^2, 52 mm_30 g/m^2, 30 mm_24 g/m^2, 30 mm_25 g/m^2, 30 mm_30 g/m^2, 46 mm_25 g/m^2, 52 mm_25 g/m^2, 17 mm_30 g/m^2, 24mm_30g/m^2. The relationship between fiber length/sheet density and texture, were investigated and the results were discussed in this section. Furthermore, 3D-needle-punching techniques were applied to fiber sheet incorporation to create an interlacing structure like endomysium in the WMMA samples. Textile technologies had been adapted into the manufacturing process of various materials for increased mechanical strengths (Akbari et al. 2016; Miura, Ishida, and Shinya 2021). In composite engineering, hook fitted needles were used to transfer fibers between fiber layers laid out in composite materials. sheets that sandwiched the composite material, resulting in improved mechanical properties of the composite (Chen et al. 2016). In this study, protein fiber sheets were place in layers between the minced pork analog then needle punched with barbed needles to transferred fibers from the above layers, introducing interlacement and a tougher texture in WMMA.

3.4.1 | Protein Sheets of Varying Density

The effect of protein sheet on TPA and SSF hardness at fixed fiber lengths and varying sheet density was summarized in Figure 4A,B, respectively. Fiber sheet-added WMMA samples had TPA hardness values that were not significantly different between varying protein sheet densities (30 mm_25 g/m^2 vs. 30 mm_30 g/m^2, and 52 mm_14 g/m^2 vs. 52 mm_21/m^2 vs. 52 mm_27 g/m^2 vs. 52 mm_30 g/m^2). On average, the TPA hardness of sheet-added samples was 45 N, which was more than 7 times higher than that of the control (6 N). However, the average TPA hardness of sheet-added WMMAs was still lower than that of whole-muscle pork, which was 94 N on average, by 52%. Even though the addition of protein sheet did not reproduce the TPA hardness of pork (leg and loin), the SSF hardness of all samples was in the same range or higher than that of pork controls. Specifically, samples containing 30 mm fiber sheets (30 mm_25 g/m^2, 30 mm_30 g/m^2) both had SSF hardness higher than that of pork controls. 52 mm-fiber-sheet-added samples had SSF hardness that was not statistically different from that of pork. This means the "first bite experience" of the 52 mm samples was indistinguishable from that of its animal counterpart, which matched the sensory data collected from volunteers who tasted the samples. A stepwise increase in SSF hardness was also observed in sample sets with the same fiber length. This could be understood as the SSF testing blade cutting through more protein fibers in higher density sheets during slice shearing, resulting in higher SSF hardness.

Next, the effect of protein sheet on springiness and cohesiveness at varying sheet density and fixed fiber lengths was summarized in Figure 4C,D, respectively. All samples containing fiber sheets, other than 52 mm_14 g/m^2, had significantly higher springiness compared to the control. Samples 30 mm_25 g/m^2, 30 mm_30 g/m^2 and 52 mm_30 g/m^2 had springiness values not significantly different from that of pork leg. The springiness of the treated samples increased in stepwise fashion with increasing sheet density when the fiber length was fixed at either 30 mm or 52 mm. Only 30 mm_25 g/m^2 and 52 mm_30 g/m^2 samples' cohesiveness values were statistically different from the control. Nonetheless, all fiber-sheet-added WMMA samples had cohesiveness values not significantly different from that of pork loin and leg.

3.4.2 | Protein Sheets of Varying Fiber Length

The effect of protein sheet on TPA and SSF hardness at varying fiber length and fixed sheet density was summarized in Figure 5A,B, respectively. Trends very similar to the set of samples discussed in 3.2 were observed. WMMA samples had TPA hardness values that were not significantly different between varying fiber length (30 mm_25 g/m^2 vs. 46 mm_25 g/m^2 vs.

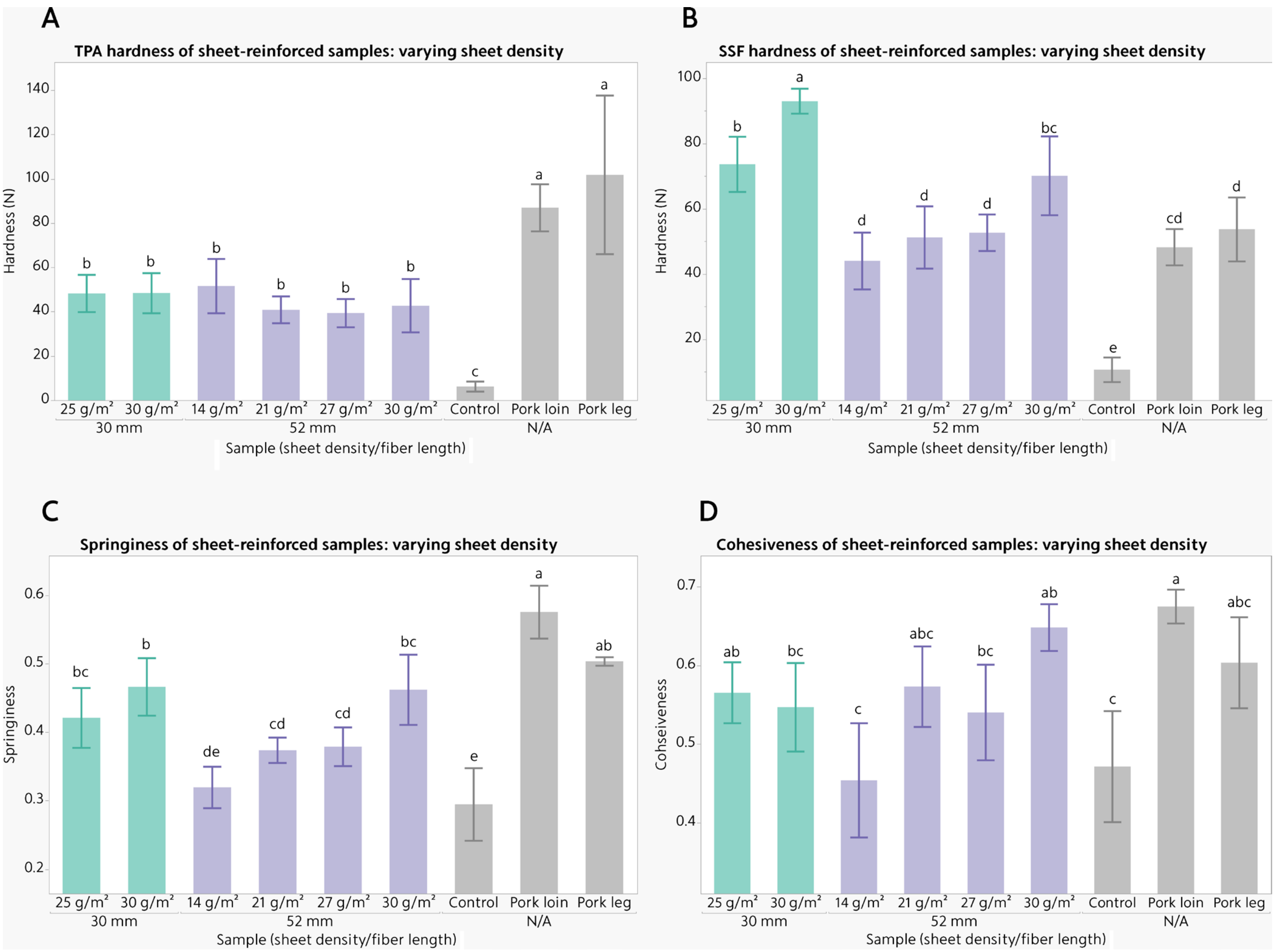

FIGURE 4 | Texture profile analysis (TPA) and Slice shear force (SSF) analysis result of sheet-reinforced whole-muscle meat analog samples with varying textile density. (A–D) represent the mean value of the following texture parameters of each treatment, respectively: TPA hardness, SSF hardness, springiness, and cohesiveness. ($n=6$ for TPA and $n=4$ for SSF; error bar is one standard deviation calculated by unbiased estimation; means not sharing any letter are significantly different by Tukey's honest significant difference test at 95% confidence level. Green bars represent samples containing 30 mm fiber sheets at varying sheet density. Purple bars represent samples containing 52 mm fiber sheets at varying sheet density. Gray bars represent plant-based and animal-based controls).

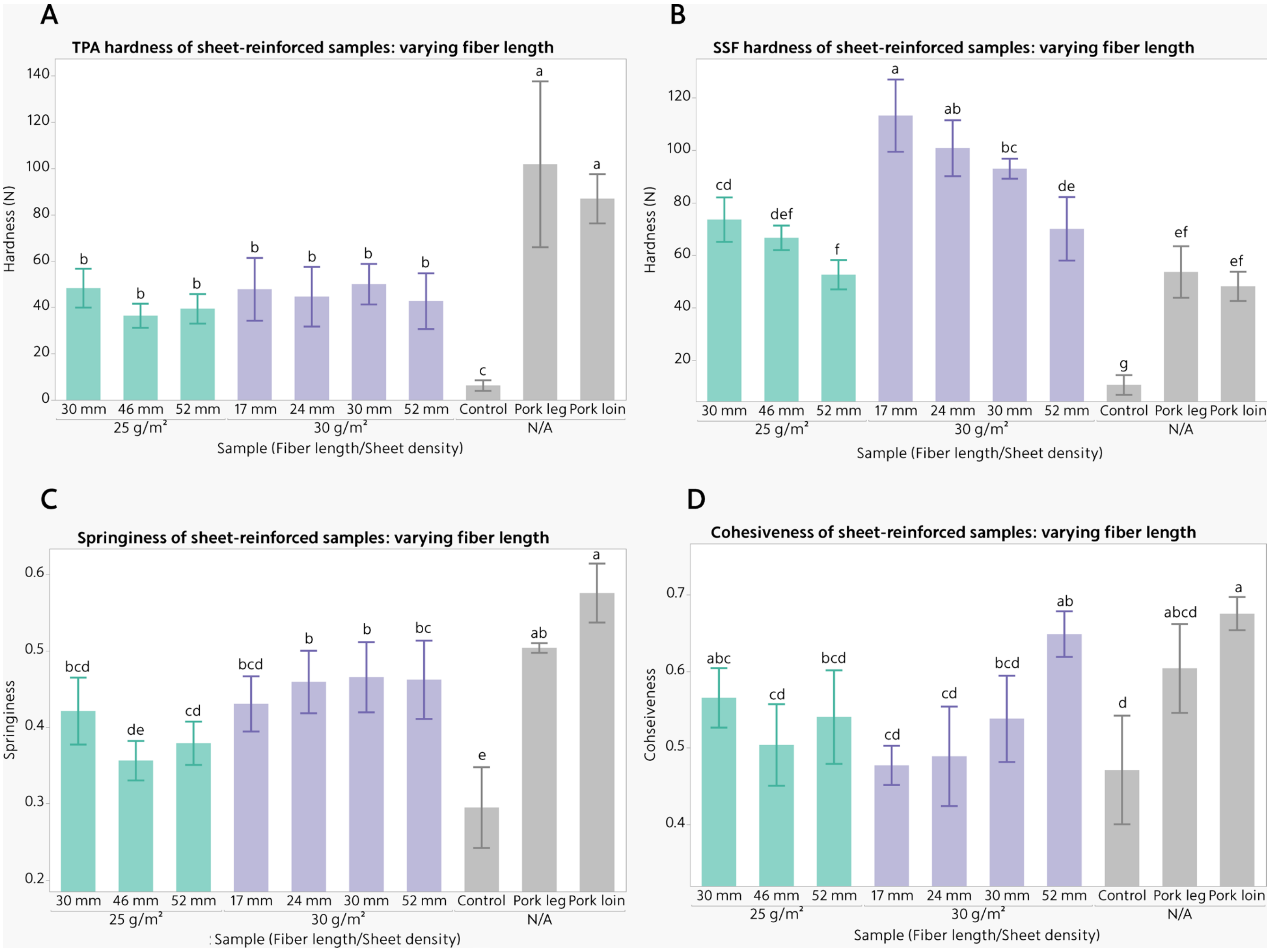

FIGURE 5 | Texture profile analysis (TPA) and slice shear force (SSF) analysis result of sheet-reinforced whole-muscle meat analog samples with varying staple fiber length. (A–D) represent the mean value of the following texture parameters of each treatment, respectively: TPA hardness, SSF hardness, springiness, and cohesiveness. ($n = 6$ for TPA and $n = 4$ for SSF; error bar is one standard deviation calculated by unbiased estimation; means not sharing any letter are significantly different by Tukey's honest significant difference test at 95% confidence level. Green bars represent samples containing 25 g/m^2 fiber sheets at varying fiber length. Purple bars represent samples containing 30 g/m^2 fiber sheets at varying fiber length. Gray bars represent plant-based and animal-based controls).

52 mm__25 g/m^2 and 17 mm_30 g/m^2 vs. 24 mm_30 g/m^2 vs. 30 mm_30 g/m^2 vs. 52 mm_30 g/m^2). This set of WMMA samples had an average TPA hardness 44 N, which was also at least seven times higher than that of the control (6 N). The TPA hardness of sheet-added WMMAs was 53% lower on average than that of whole-muscle pork (94 N on average). The SSF hardness values of all samples were again in the same range or higher than that of pork controls. Specifically, samples with longer fiber sheets added (46 mm_25 g/m^2, 52 mm__25 g/m^2, 52 mm_30 g/m^2) had SSF hardness values not statistically different from that of pork controls. Samples of shorter fiber lengths (30 mm_25 g/m^2, 17 mm_30 g/m^2, 24 mm_30 g/m^2) had SSF hardness values higher than that of pork leg and loin. A stepwise decrease in SSF hardness was observed in sample sets with the same sheet density (25 g/m^{2}or 30 g/m^2). This could be explained by the following: with the weight of the protein fiber added to the meat analog samples fixed at 1% (w/w), the samples prepared with shorter fibers had more pieces of fibers per sample than those prepared with longer fibers. This meant the SSF testing blade was cutting through more protein fibers during slice shearing, resulting in higher SSF hardness.

Next, the effect of protein sheet on springiness and cohesiveness at varying fiber length and fixed sheet densities was summarized in Figure 5C,D, respectively. All treated samples, other than 46 mm_25 g/m^2, had significantly higher springiness compared to the control. Samples 30 mm_25 g/m^2 and all 30 g/m^2 samples had springiness values not significantly different from that of pork leg. We also observed that at fixed sheet density, the springiness of the samples was not significantly different despite varying fiber lengths. Combined with the springiness result from 3.2, we concluded that springiness was a textural parameter that was manipulated by the density of the fiber sheet, rather than the fiber length. Only 30 mm_25 g/m^2 and 52 mm_30 g/m^2 samples' cohesiveness values were statistically different from the control. No clear trends were observed when the fiber length was increased from 30 mm to 52 at 25 g/m^2 fixed density, nor when the fiber length was increased from 17 mm to 52 mm at

30 g/m^2 density. All WMMA samples had cohesiveness values not significantly different from that of pork loin and leg.

3.4.3 | Protein Sheets of Varying Protein Type

Lastly, commercially available gelatin and zein protein fiber sheets were evaluated for their textural effect in WMMAs alongside 30 mm_25 g/m^2 Hydrophobic_S (referred to Hydrophobic_S for simplicity in this section) samples, ground pork, pork leg and loin; the results are summarized in Figure 6. The sheet density of the purchased protein sheets were custom-made to be 25 g/m^2 sheet samples. Due to the nature of the spinning process adopted by the manufacturer (spray spinning), the fiber length and diameter could not be customized. Therefore, the purchased protein fiber sheets had different fiber length and diameter from the protein sheets prepared in this study (detailed in Table 2), though cannot be used for direct comparisons.

TPA results indicated that the TPA hardness of samples prepared using commercial zein (11.6 N) and gelatin (11.8 N) sheets was not significantly different from that of ground pork and was almost 8 times lower than whole-muscle pork (91.8 N). On the contrary, Hydrophobic_S samples had a hardness of 39.6 N, distinguishing it from ground pork and samples containing zein and gelatin fiber sheets. In terms of SSF hardness, zein and gelatin sheet-added samples had significantly lower values than both ground pork and whole-muscle pork. Hydrophobic_S had SSF values not statistically different from ground pork, which was almost 3 times lower than whole-muscle pork. In previous research, electrospun zein fibers (EZF) with a diameter ranging between 0.5–1 µm were applied to tofu-style soy gels by Mattice and Marangoni. It was found that just 0.05% (w/w) incorporation of EZF was enough to significantly increase the TPA hardness and chewiness of the gels. On the other hand, the springiness and resilience were not impacted by the addition of EZF (Mattice and Marangoni 2020). In this study, purchased zein fiber sheets were incorporated into the commercial pork

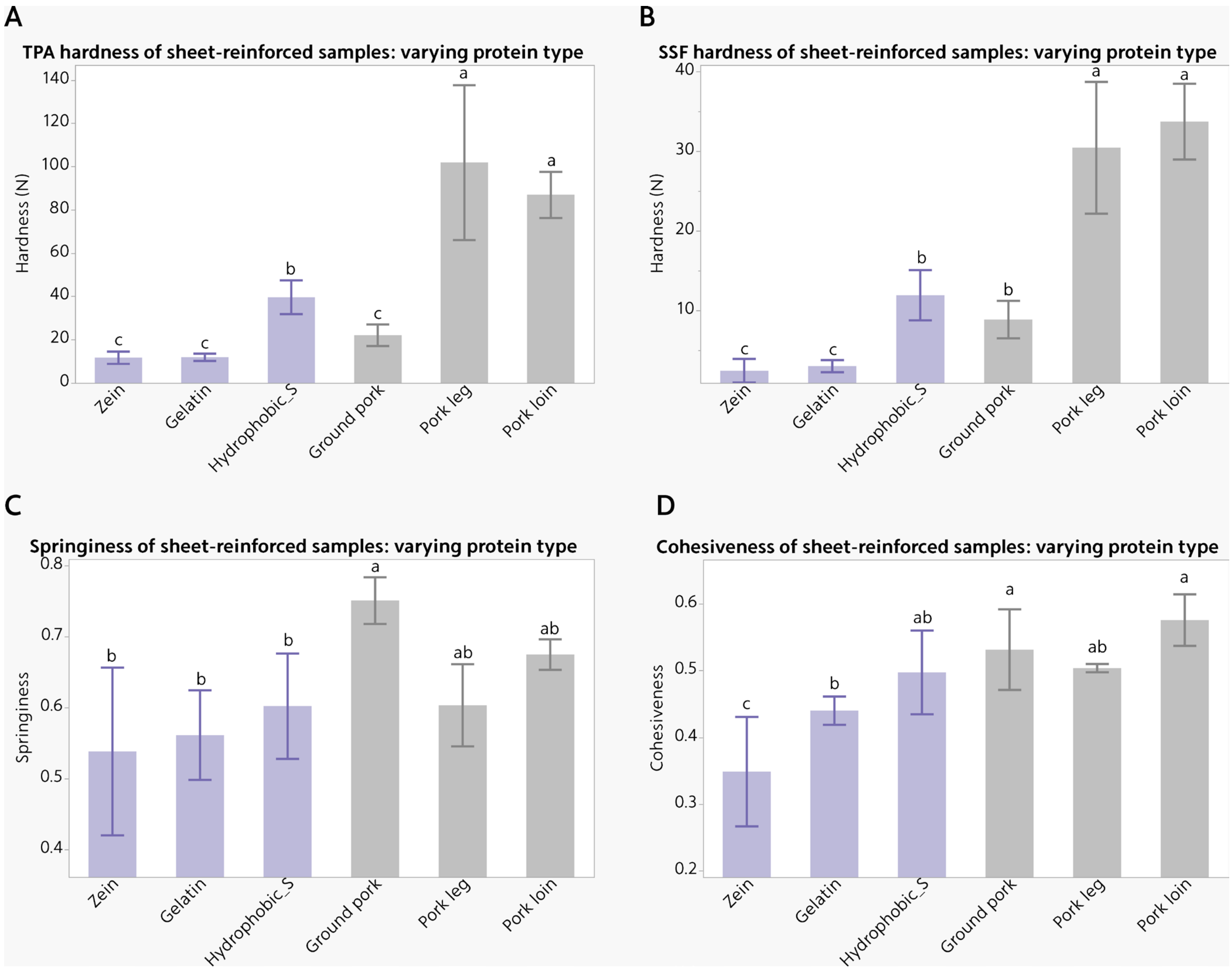

FIGURE 6 | Texture profile analysis (TPA) and slice shear force (SSF) analysis result of sheet-reinforced whole-muscle meat analog samples with varying protein type. (A–D) represent the mean value of the following texture parameters of each treatment, respectively: TPA hardness, SSF hardness, springiness, and cohesiveness. ($n = 6$ for TPA and $n = 4$ for SSF; error bar is one standard deviation calculated by unbiased estimation; means not sharing any letter are significantly different by Tukey's honest significant difference test at 95% confidence level. Purple bars represent treated plant-based samples. Gray bars represent animal-based controls).

analog at 0.94% (w/w), however, it did not improve the textural properties compare to the control. The authors believed this was because zein fibers had higher mechanical properties than the tofu-style gel matrix in Mattice and Marangoni's study, but lower mechanical properties than the TVP matrix of commercial pork analog. This showed the advantage of using a commercial meat analog product to conduct experiments, instead of model gels. Nevertheless, both studies agreed on the great potential for fibers to modify texture of meat analogs. Additionally, wet-spun protein fibers were reported to have higher mechanical properties and larger fiber diameters compared to electrospun protein fibers, which gave them a greater potential to improve meat analog texture (Wang et al. 2023). It was shown in our study that Hydrophobic_S sheet-treated samples had a higher TPA hardness than the ground pork control, though not high enough to be in the range of whole-muscle pork leg or loin. SSF analysis results indicated that samples prepared with Hydrophobic_S protein sheet had a SSF hardness in the same range as ground pork, which is higher than that of both zein and gelatin sheet-treated samples.

All the sheet-reinforced samples, regardless of protein type, had springiness values that were not statistically different from each other. In addition, these values were within similar range with whole-muscle pork samples. Notably, ground pork had the highest springiness values out of all samples (including whole-muscle pork) and were statistically higher from sheet-reinforced samples. In terms of cohesiveness, zein sheet-reinforced samples had the lowest values. Gelatin and Hydrophobic_S samples had values that were statistically similar. However, Hydrophobic_S had a higher mean value than gelatin samples and was statistically similar to the cohesiveness of all pork samples (ground, leg, loin). All fiber sheet-treated samples contained protein sheets of the same density (around 25 g/m^2), and it was once again observed that their springiness values were not significantly different. This once again confirmed that springiness was manipulated by protein sheet density, instead of fiber length or protein type. In terms of cohesiveness, gelatin sheet-treated samples had a higher value compared to zein sheet-treated samples. Since gelatin is an excellent binding agent in its powder form (Chandra and Shamasundar 2015), the increase in cohesiveness could be attributed to the intrinsic binding strength of gelatin proteins, instead of the fiber form of the protein acting as a textural component. In addition, we observed that gelatin fiber sheets' structural integrity and mechanical properties decreased due to moisture in the sample. A gelatin fiber sheet of higher mechanical property (e.g., higher sheet density or higher fiber diameter) could improve the textural properties of gelatin-fiber-sheet-treated samples.

4 | Conclusions

Meat analog is a category of food that is gaining popularity due to its various benefits like lower environmental impact, ethicality, and healthiness. This study demonstrates that the addition of wet-spun recombinant protein fibers/fiber sheets is effective in reproducing certain textural properties of animal meat. Specifically, the addition of hydrophilic, 30 μm-diameter, 3-mm short protein fibers at 1% (w/w) to plant-based meat analog increase its springiness by 45%. The resulting springiness value matched to that of pork patties. Furthermore, all textural properties of meat analog samples were improved with the incorporation of endomysium-like fiber sheets via 3D needle punching, with SSF hardness, springiness, and cohesiveness values statistically indistinguishable from those of animal-based whole-muscle meat. The new WMMA prototypes introduce a new category of meat analog to consumers. The authors believe finetuning of the tensile properties of protein fibers can further diversity the product profile of meat analogs, recreating the delicate texture of various types of animal meat. Reformulations of minced meat with appropriate additives that interact well with protein fibers can also further improve the quality of the final WMMA and suit different consumer preferences.

Nomenclature

SSF slice shear force

TPA texture profile analysis

TVP textured vegetable protein

WMMA whole-muscle meat analog

Author Contributions

Rita Chuang: conceptualization, investigation, funding acquisition, writing – original draft, methodology, validation, visualization, formal analysis, project administration, data curation, supervision, resources. **Arin Naidu:** conceptualization, investigation, methodology, validation, visualization, writing – review and editing, formal analysis, data curation. **Josephine Galipon:** writing – review and editing, supervision.

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Ethics Statement

The authors have nothing to report.

Consent

The authors have nothing to report.

Conflicts of Interest

Rita Chuang and Arin Naidu were employees of Spiber Inc. a biomanufacturing company for protein-based materials using precision fermentation, at the time this experiment was conducted. Josephine Galipon declares no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.