Integrated Biochemical, Thermal and Microstructural Assessment of High Hydrostatic Pressure Effects on Bovine Intramuscular Collagen

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ABSTRACT: *High hydrostatic pressure (HHP) is a promising non-thermal technology that can alter meat structure while preserving its nutritional and sensory attributes. This study examined pressure-induced modifications in bovine endomysial and perimysial collagen through biochemical, thermal, structural, and microstructural analyses. Beef samples were subjected to treatments at 200, 400, and 600 MPa for 10 and 20 minutes. Collagen changes were evaluated by hydroxyproline quantification, soluble and insoluble collagen determination, SDS–PAGE electrophoresis, differential scanning calorimetry (DSC), scanning electron microscopy (SEM), and statistical modeling. Results revealed significant effects of pressure and pressure × time interactions ($p < 0.05$) in both connective tissue compartments. Endomysial collagen showed greater susceptibility to solubilization, whereas perimysial collagen exhibited a heterogeneous response, likely due to its organized supramolecular architecture. SDS–PAGE confirmed that the primary collagen structure remained largely intact, while DSC thermograms indicated shifts in thermal transitions. SEM micrographs demonstrated increased interfibrillar spacing and partial unraveling of connective tissue ultrastructure under higher pressures. Regression and correlation analyses identified pressure as the main driver of collagen modification, particularly within the endomysium. Overall, findings suggest that HHP alters bovine intramuscular connective tissue primarily by disrupting supramolecular organization rather than cleaving collagen peptides, underscoring its potential for meat processing applications.*

KEYWORDS: *Collagen remodeling; Intramuscular connective tissue; Endomysium; Perimysium; Hydroxyproline; Meat tenderness*

I. INTRODUCTION

Meat tenderness is one of the most important quality traits shaping consumer acceptance, market value, and repeat purchase of beef products. Among the structural factors responsible for meat texture, intramuscular connective tissue (IMCT) — particularly collagen — plays a central role in determining background toughness and overall palatability [1,2]. Collagen's physicochemical properties, including its concentration, fibrillar organization, intermolecular cross-linking, and thermal stability, strongly affect how meat behaves during both mastication and thermal processing [2,3]. Muscles subjected to greater locomotive activity tend to contain more highly cross-linked collagen, which translates into lower solubility and reduced tenderness [3]. Improving the quality of connective tissue-rich cuts therefore remains an

important technological challenge for the meat industry.

Collagen in skeletal muscle is hierarchically organized into specialized connective tissue compartments, mainly the endomysium and perimysium, which differ substantially in both structure and function [4,5]. Endomysial collagen forms a delicate fibrillar network surrounding individual muscle fibers, while perimysial collagen forms thicker, mechanically resistant bundles around groups of fibers [4]. These two fractions differ not only in fibrillar arrangement but also in collagen concentration, cross-link density, hydration behavior, and thermal stability [5,6]. It follows that endomysial and perimysial collagen are likely to respond differently to technological processes aimed at modifying meat texture, which is why understanding these compartment-specific responses is essential for clarifying the mechanisms behind connective tissue modification and meat tenderization.

Traditional strategies for improving tenderness include aging, enzymatic tenderization, mechanical disruption, electrical stimulation, and thermal processing [1,7]. While effective under specific conditions, these approaches can come with drawbacks: long processing times, uneven treatment distribution, excessive protein degradation, cooking losses, or undesirable sensory changes [7,8]. In recent years, non-thermal and mild-processing technologies have drawn growing interest as alternatives that can modify muscle structure while preserving nutritional and sensory quality [8,9].

High-pressure processing (HPP), also known as high hydrostatic pressure (HHP), is one such technology and has emerged as a promising tool for modifying meat proteins and connective tissue architecture [9,10]. HHP typically applies hydrostatic pressures of 100–600 MPa under controlled temperature conditions [10]. Unlike thermal treatments, pressure processing acts mainly on non-covalent interactions — hydrogen bonding, hydrophobic interactions, and electrostatic forces — while largely sparing covalent peptide bonds [11,12]. As a result, HHP can induce conformational changes, hydration rearrangements, aggregation, and shifts in protein supramolecular organization without necessarily causing extensive molecular degradation [11,13].

Pressure-induced structural changes in meat systems have been studied extensively for myofibrillar proteins and for microbial inactivation [9,10]. The response of intramuscular connective tissue collagen, however — and particularly how endomysial and perimysial networks behave differently under pressure — has received comparatively little attention [6,14]. Earlier studies have shown that HHP can alter collagen's thermal behavior, including shifts in denaturation and thermal shrinkage temperatures as measured by differential scanning calorimetry (DSC) [14,15]. These investigations found that pressures above roughly 500 MPa significantly affect collagen thermal stability, especially in endomysial fractions exposed to longer pressurization times [15]. Such findings point to HHP's potential to affect connective tissue organization and collagen functionality through changes in molecular packing and fibrillar interactions.

Even so, most of this prior work has focused mainly on collagen's thermal transitions, leaving the broader picture — how collagen solubility, hydroxyproline extractability, protein profile, thermal stability, and microstructural organization relate to one another under high-pressure conditions — still poorly understood [14–16]. In particular, little is known about how endomysial and perimysial collagen differ in their susceptibility to pressure-induced supramolecular rearrangement, or how these structural responses connect to connective tissue functionality and potential tenderization mechanisms [5,16].

Recent work on connective tissue has emphasized that changes in collagen architecture don't occur through molecular degradation alone, but also through shifts in fibrillar organization, intermolecular interactions, hydration dynamics, and hierarchical structural packing [11,13,17]. Under pressure, these effects may partially loosen collagen networks and alter extractability and thermal behavior without extensively cleaving collagen polypeptide chains [13,17]. Untangling this requires an integrated analytical approach capable of

capturing the mechanistic basis of pressure-induced connective tissue modification.

The present study therefore set out to investigate the effects of high hydrostatic pressure on bovine intramuscular connective tissue, using an integrated biochemical, structural, thermal, and microstructural approach with particular focus on endomysial and perimysial collagen fractions. Collagen modifications were assessed through hydroxyproline quantification, soluble and insoluble collagen determination, SDS–PAGE electrophoresis, differential scanning calorimetry (DSC), scanning electron microscopy (SEM), and statistical modeling based on a factorial experimental design with correlation analysis. The goal was to clarify how each collagen compartment responds to pressure treatment and to offer mechanistic insight into the supramolecular rearrangements underlying connective tissue modification and potential improvements in meat tenderness.

II. MATERIALS AND METHODS

II.1. Raw Material

Beef muscle samples were obtained from a commercial abattoir and transported under refrigeration. Visible fat and connective tissue were trimmed away, and the muscle was cut into standardized portions (approximately 5 × 4 × 3 cm). Samples were vacuum-packed in polypropylene bags and stored at −18 °C until processing and analysis, following common practice for meat structure studies [6].

II.2. High-Pressure Processing (HPP)

High-pressure processing was carried out in a laboratory-scale high-pressure unit (Stansted Fluid Power, model S-FL-850-9-W; Stansted, UK). Vacuum-packed samples were subjected to pressures of 200, 400, or 600 MPa for 10 or 20 min, with non-pressurized samples serving as controls [7–9]. Processing temperature was monitored throughout and did not exceed 45 °C. After pressurization, samples were rapidly cooled and stored at −18 °C until further analysis. The pressure–time combinations and temperature control applied here were consistent with established HPP protocols for meat systems [10–12].

II.3. Isolation of Intramuscular Connective Tissue

Collagen-rich fractions corresponding to the endomysium and perimysium were isolated from both pressurized and control samples using a calcium chloride extraction procedure based on Stanton and Light, with subsequent modifications reported in the literature [13]. Briefly, partially thawed meat was homogenized in 0.5 M $CaCl_2$ under refrigeration, filtered through nylon mesh, and washed repeatedly to separate the connective tissue fractions. Endomysial and perimysial residues were collected, purified, and prepared for subsequent analyses.

II.4. Protein Profile Analysis (SDS-PAGE)

Protein profiles of the perimysial collagen extracts were analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) using 10% polyacrylamide gels under reducing conditions. Sample preparation, electrophoretic conditions, and protein visualization followed standard procedures commonly used for collagen and connective tissue proteins [27,28]. Molecular weight markers were used to estimate protein band distribution and to assess pressure-induced changes in protein profiles.

II.5. Chromatographic Analysis

Hydroxyproline was quantified by high-performance liquid chromatography (Waters Alliance, model 2695) using a Waters C18 column (3.9 × 150 mm), a 10 µL injection volume, a Waters 474 fluorescence detector, and data acquisition via Empower software. Pre-column derivatization was performed with 6-aminoquinolyl-N-succinimidyl carbamate (AQC), as described by Cohen and Michaud [1993]. The hydroxyproline standard (trans-4-hydroxy-L-proline, SigmaUltra) was purchased from Sigma. Under these conditions, the hydroxyproline standard eluted at a retention time of approximately 12 minutes, consistent with the

method described by Abreu, Godoy, Santos, Pacheco, and Souza [2011].

A four-point calibration curve was constructed based on the mass of standard injected at each volume, yielding a coefficient of determination (R^2) of 0.9971. For sample preparation, lyophilized perimysium was ground in a blender for 30 s, and 500 mg portions were weighed into glass ampoules. The ampoules were sealed and hydrolyzed under vacuum at 110 °C for 24 h, following AOAC procedures [1995]. After hydrolysis, samples were reconstituted and two aliquots were taken — one for hydroxyproline analysis and one for the remaining amino acids. Aliquots were transferred to 50 µL glass vials and dried under vacuum for 24 h. Once reconstituted and homogenized, samples were derivatized with AQC, borate buffer, and the accompanying reagent kit, then incubated in a sand bath at 55 °C for 10 min. A 5 µL aliquot of each derivatized sample was injected into the chromatograph. Amino acid content was quantified from the resulting chromatograms using the calibration curve in the Empower software.

II.6. Collagen Content and Solubility

Collagen content in the endomysial and perimysial fractions was determined indirectly through hydroxyproline quantification following acid hydrolysis, using a colorimetric method widely adopted in meat and connective tissue research [29,30]. Results were expressed as collagen equivalents and used to evaluate pressure-induced changes in collagen solubility relative to untreated controls.

II.7. Thermal Analysis (DSC)

The thermal properties of perimysial collagen were evaluated by differential scanning calorimetry (DSC). Samples were sealed in aluminum pans and scanned at a constant heating rate over an appropriate temperature range. Onset, peak, and endset temperatures of collagen denaturation, along with denaturation enthalpy (ΔH), were calculated following established protocols for collagen thermal characterization [31,32].

II.8. Microstructural Analysis

The microstructure of perimysial collagen was examined by scanning electron microscopy (SEM). Freeze-dried samples were mounted on aluminum stubs, sputter-coated with gold, and observed at an appropriate accelerating voltage. Micrographs were assessed qualitatively to evaluate pressure-induced changes in collagen fibrillar organization, following conventional sample preparation and imaging procedures [33].

II.9. Experimental Design and Statistical Analysis

A factorial experimental design was used to evaluate the effects of pressure level and processing time. Data were analyzed by analysis of variance (ANOVA), and regression models were applied to describe treatment effects. Statistical significance was set at $p < 0.05$, following standard statistical practice for food science experiments [34]. Table 1 summarizes the treatments used.

Tabla 1. Description of treatments used

Treatments	Time (min.)	Pressure (MPa)
TC (control)	0	0
T2	10	200
T4	10	400
T6	10	600
T8	20	200
T10	20	400
T12	20	600

III. RESULTS AND DISCUSSION

III.1. Effects of High-Pressure Processing on Collagen Protein Profile (SDS–PAGE)

The electrophoretic profiles of perimysial collagen fractions subjected to high hydrostatic pressure (HHP) are shown in Figure 1. Across the evaluated pressure range (200–600 MPa) and processing times (10 and 20 min), the major collagen-associated protein bands remained largely preserved, indicating that the applied HHP conditions did not promote extensive cleavage of collagen polypeptide chains. The predominant bands in the molecular weight region characteristic of collagenous proteins — particularly those corresponding to α-chains and associated high-molecular-weight components — remained detectable in all treatments, suggesting that collagen's primary structure was preserved.

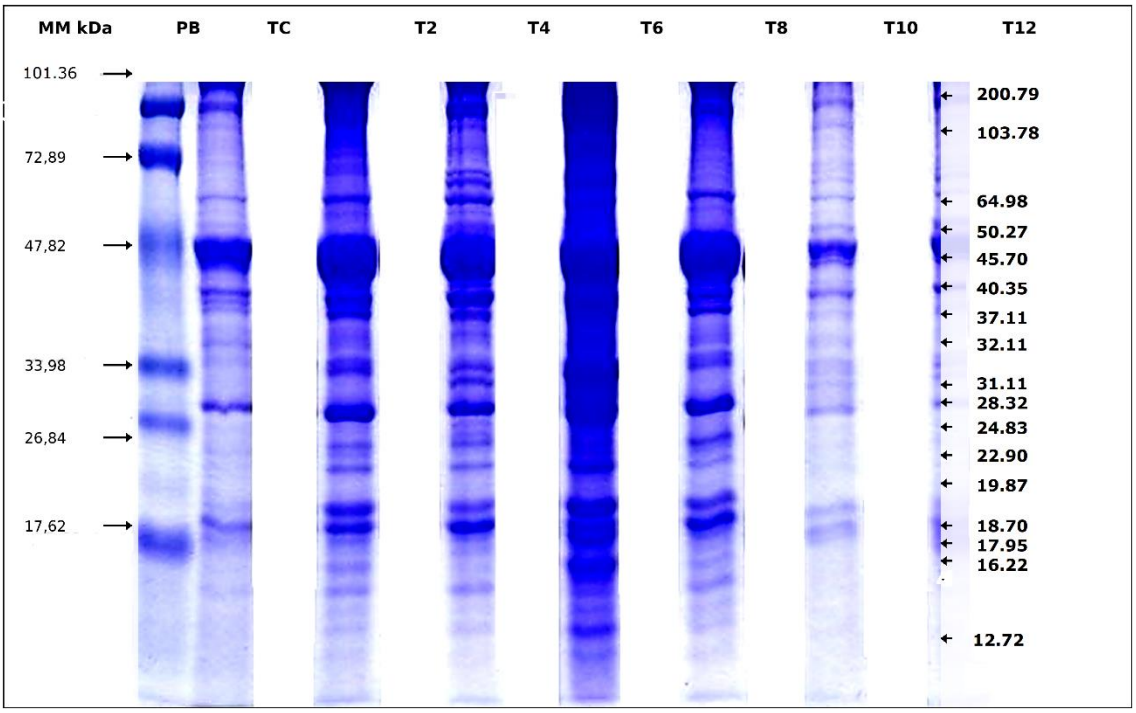

Figure 1. SDS–PAGE electrophoretic profiles of bovine collagen extracted from perimysial connective tissue subjected to high hydrostatic pressure treatments.

While the overall electrophoretic pattern stayed relatively stable, qualitative differences in band intensity and distribution emerged among treatments, especially at higher pressure levels. Treatments T6 and T12 (600 MPa) showed noticeable changes in band definition and staining intensity relative to the control (TC), pointing to modifications in protein extractability and supramolecular organization rather than outright molecular degradation. Treatment T4 (400 MPa/10 min), by contrast, produced a denser, more compact electrophoretic profile, which may reflect pressure-induced aggregation or reduced solubilization of collagen-associated proteins during extraction.

Notably, no accumulation of low-molecular-weight fragments was observed in the lower region of the gels, reinforcing the interpretation that HHP did not induce extensive hydrolysis of collagen molecules. This is consistent with how pressure acts on proteins more generally: hydrostatic pressure preferentially disrupts non-covalent interactions — hydrogen bonding, hydrophobic interactions, electrostatic associations — while covalent peptide bonds remain comparatively stable under the pressure–temperature conditions typical of food processing [4,6]. The modifications observed here are therefore more likely tied to conformational rearrangements, fibrillar dissociation, hydration changes, and pressure-induced shifts in intermolecular interactions within the collagen network than to peptide bond cleavage.

These findings align with earlier work on connective tissue collagen under high-pressure conditions. Chang et al. (2013) found that HHP significantly

altered the thermal behavior of endomysial and perimysial collagen without evidence of severe protein degradation, suggesting that pressure modifies collagen stability mainly through structural rearrangement rather than peptide cleavage. Other studies on pressure-treated bovine connective tissue have similarly reported that collagen retains much of its electrophoretic integrity even as substantial changes occur in thermal stability, fibrillar organization, and connective tissue functionality — reinforcing the idea that HHP acts predominantly at the supramolecular level of collagen organization.

The differential behavior observed among treatments may also reflect the intrinsic structural complexity of perimysial collagen. Perimysium is built from thicker, highly cross-linked collagen fibrils arranged in compact bundles that give muscle tissue its mechanical resistance. This dense, hierarchical organization likely confers greater resistance to pressure-induced disruption than is seen in more delicate connective tissue fractions such as the endomysium [1]. As a result, pressure treatment may initially promote only partial loosening of interfibrillar interactions and shifts in hydration dynamics, with more pronounced structural disorganization requiring higher pressure or longer exposure.

The electrophoretic profiles also reveal an interesting contrast between connective tissue proteins and myofibrillar proteins under pressure. Myofibrillar proteins are highly susceptible to pressure-induced denaturation and aggregation, whereas collagen showed comparatively greater structural stability across the full HHP range tested. This distinction underscores how heterogeneously muscle components respond to hydrostatic pressure, and it highlights why connective tissue fractions deserve specific attention in studies of meat tenderization mechanisms [4,8].

Taken together, the SDS–PAGE results strongly suggest that high hydrostatic pressure modifies bovine perimysial collagen mainly through supramolecular and conformational rearrangements rather than extensive molecular degradation. The preservation of collagen electrophoretic integrity, combined with the qualitative changes in extractability and band organization observed at higher pressures, indicates that HHP remodels the connective tissue matrix structurally while leaving the fundamental collagen molecular backbone intact. These findings provide mechanistic grounding for the thermal, microstructural, and solubility analyses discussed in the following sections.

III.2. Effects of High-Pressure Processing on Collagen Solubility and Hydroxyproline Distribution in Endomysial and Perimysial Fractions

The effects of high hydrostatic pressure (HHP) on collagen organization were evaluated through hydroxyproline quantification and determination of soluble and insoluble collagen fractions from endomysial and perimysial connective tissues (Table 2). Unlike previous studies, which focused predominantly on collagen thermal transitions, the present results show that HHP also modulates collagen behavior through changes in extractability and supramolecular organization — revealing distinct, compartment-specific responses within bovine intramuscular connective tissue [14–15].

Table 2. Collagen content of endomysial and perimysial fractions of pressurized and non-pressurized meat. *(Hydroxyproline values converted to collagen using the AOAC (1995) correction factor)*

Time	Treatment	Hydroxyproline (Soluble)	Collagen (mg/100 g)	Hydroxyproline (Insoluble)	Collagen (mg/100 g)
0 min	Control (TC)	802.20	6417.60c ± 5.9	2375.30	19002.40ab ± 412.38
10 min	T2 (200 MPa)	799.55	6396.40c ± 1.63	2691.55	21532.40ab ± 302.85
10 min	T4 (400 MPa)	765.00	6120.00d ± 0.28	2130.75	17046b ± 94.68
10 min	T6 (600 MPa)	825.25	6602.00b ± 11.10	3218.50	25748a ± 101.68

20 min	T8 (200 MPa)	791.75	6334.00c ± 3.61	885.15	7081.20c ± 199.90
20 min	T10 (400 MPa)	798.55	6388.40c ± 0.64	2446.95	19575.60ab ± 81.67
20 min	T12 (600 MPa)	868.40	6947.20a ± 4.81	3261.40	26091.20a ± 160.51

Values followed by different letters in the same column differ significantly ($p < 0.05$) according to Tukey's test.

Hydroxyproline quantification served as an indirect indicator of collagen content, since hydroxyproline is an amino acid closely associated with collagen structure [16]. Variations in hydroxyproline concentration among treatments therefore reflect changes in collagen extractability, fibrillar organization, and intermolecular interactions rather than extensive molecular degradation — an interpretation strongly supported by the SDS–PAGE profiles discussed earlier, which showed preservation of the major collagen-associated bands after pressure treatment [4–12].

Significant differences ($p < 0.05$) were found among treatments for both the soluble and insoluble collagen fractions (Table 2). In the soluble fraction, associated predominantly with endomysial collagen, treatment T4 (400 MPa/10 min) showed the lowest collagen content (6120 mg/100 g), while T12 (600 MPa/20 min) showed the highest (6947.2 mg/100 g). This progressive increase under the most severe pressure–time combination suggests that higher pressure combined with longer holding time promotes greater collagen extractability, likely through pressure-induced loosening of the connective tissue matrix [13,17].

This pattern indicates that HHP affects the hierarchical organization of collagen fibrils by weakening the non-covalent interactions that stabilize the supramolecular collagen network. Because endomysial collagen has a finer fibrillar arrangement and a lower density of mature intermolecular cross-links, this compartment appears more susceptible to pressure-induced structural rearrangement [1,2] — and indeed showed greater responsiveness to treatment, particularly at 600 MPa.

One notable finding is that pressure level alone does not explain the observed behavior: treatments at the same pressure but with different holding times produced distinct responses. T6 (600 MPa/10 min) and T12 (600 MPa/20 min), for instance, differed significantly, with T12 showing markedly higher soluble collagen content. This points to exposure time as a critical factor driving progressive hydration changes, fibrillar relaxation, and collagen network dissociation under pressure [11,13].

The insoluble fraction, associated predominantly with perimysial connective tissue, showed a more heterogeneous response. The control sample contained 19,002.4 mg/100 g of insoluble collagen, while T12 reached the highest value (26,091.2 mg/100 g). Interestingly, T8 (200 MPa/20 min) showed a pronounced drop in insoluble collagen (7081.2 mg/100 g), suggesting that moderate pressure combined with prolonged treatment can destabilize the structure enough to shift collagen between extractable and non-extractable fractions.

This contrast between endomysial and perimysial behavior underscores the importance of accounting for connective tissue compartmentalization when evaluating pressure-induced modifications in meat systems [4–6]. Perimysial collagen forms thicker, mechanically resistant fibrillar bundles with higher collagen concentration and greater cross-link density [1,3], a structural organization that likely confers greater resistance to pressure-induced dissociation than the more delicate endomysial network [2,3]. As a result, pressure treatment may initially act on hydration and interfibrillar interactions within the perimysium before driving more extensive structural disorganization.

The chromatographic profiles obtained during hydroxyproline analysis (Figure 2) confirmed well-defined hydroxyproline peaks at approximately 12 min retention time, supporting the analytical consistency of the quantification method. Variations in peak intensity across treatments further reinforce the interpretation that HHP modifies collagen extractability and connective tissue organization in a pressure-dependent manner [29,30].

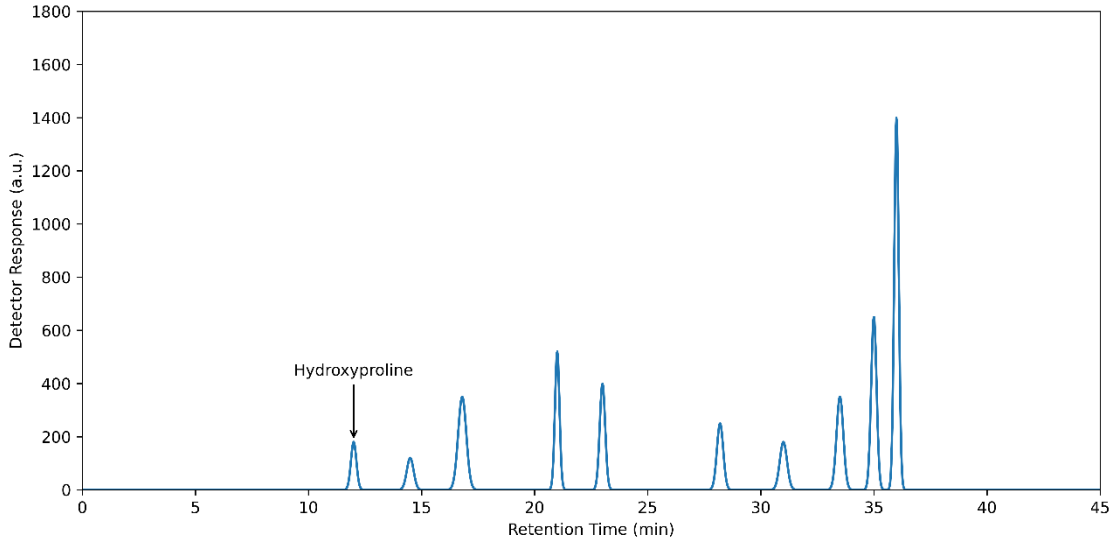

Figure 2. Representative chromatographic profile obtained during hydroxyproline analysis used for collagen quantification. The x-axis represents the retention time (min), and the y-axis corresponds to detector response (arbitrary units). The hydroxyproline peak, used as an indicator of collagen content, appears at approximately 12 min.

These findings indicate that HHP-induced modifications in connective tissue are not primarily driven by molecular degradation of collagen, but rather by changes in its supramolecular architecture. The preserved electrophoretic integrity observed in SDS–PAGE, together with the variations in hydroxyproline extractability and collagen solubility reported here, strongly suggests that hydrostatic pressure acts mainly by rearranging fibrillar packing, hydration dynamics, and interfibrillar interactions [4–12].

These observations are consistent with earlier reports showing that pressure treatment alters connective tissue functionality through partial dissociation of collagen fibrils and changes in thermal stability, rather than extensive peptide cleavage [14,15,12]. Chang et al. (2013) found that pressures above 500 MPa significantly altered the thermal shrinkage behavior of endomysial and perimysial collagen, pointing to pressure-induced changes in connective tissue stability [15]. Similarly, Ichinoseki et al. [11] reported that high-pressure treatment increased collagen solubilization through fibrillar dissociation, without extensive molecular breakdown. Suzuki et al. [13] observed reductions in meat hardness linked to pressure-induced connective tissue modifications, while structural elasticity was preserved. Unlike these earlier studies, which focused mainly on DSC-derived thermal transitions, the present results provide integrated evidence connecting collagen extractability, compartment-specific structural responses, and pressure-induced supramolecular remodeling — substantially broadening the mechanistic picture of how HHP affects intramuscular connective tissue.

The experimental hydroxyproline values for the endomysial and perimysial fractions (Table 3) further support the differential susceptibility of these compartments to pressure treatment. Endomysial hydroxyproline values increased progressively with pressure, particularly at 600 MPa, whereas perimysial collagen showed greater variability and a less linear response. This reflects the structural complexity of perimysial collagen and its stronger resistance to pressure-induced dissociation [1,3].

Table 3. Experimental values of hydroxyproline in Endomysium and Perimysium

Run	Pressure (MPa)	Time (min)	Coded Pressure (X1)	Coded Time (X2)	Hydroxyproline – Endomysium (mg/100 g)	Hydroxyproline – Perimysium (mg/100 g)
1	200	10	-1	-1	798.4	2477.4
2	600	10	1	-1	833.1	3146.6
3	200	20	-1	1	789.2	1026.5
4	600	20	1	1	871.8	3147.9
5	200	10	-1	-1	800.7	2905.7
6	600	10	1	-1	817.4	3290.4
7	200	20	-1	1	794.3	743.8
8	600	20	1	1	865.0	3374.9

Notes: X1 = Pressure factor (MPa); X2 = Time factor (min). Endomysium corresponds to the soluble collagen fraction, while perimysium corresponds to the insoluble collagen fraction.

From a technological standpoint, these findings matter because endomysial collagen is associated mainly with connective tissue flexibility and localized tenderness perception, whereas perimysial collagen contributes more directly to background toughness in connective tissue-rich muscles [2,5]. Selectively modifying these fractions through pressure may therefore represent an important mechanism by which HHP improves meat texture while preserving structural integrity.

Taken together, these results show that high hydrostatic pressure drives compartment-specific modifications in bovine intramuscular connective tissue, acting mainly through supramolecular rearrangement rather than extensive molecular degradation. The combined evidence from hydroxyproline quantification, collagen solubility, electrophoretic profiling, and the thermal and microstructural analyses that follow strongly supports HHP as a structure-modulating technology capable of reorganizing collagen architecture and connective tissue functionality in meat systems [11,13,17].

III.3. Effects of High-Pressure Processing on Thermal Behavior and Supramolecular Stability of Perimysial Collagen (DSC)

Differential scanning calorimetry (DSC) revealed that high hydrostatic pressure (HHP) induced notable changes in the thermal behavior of perimysial collagen, reflecting pressure-driven shifts in connective tissue supramolecular organization (Figure 3). Unlike earlier studies that focused primarily on thermal shrinkage temperature alone, the present work integrates DSC data with electrophoretic, solubility, hydroxyproline, and microstructural analyses, allowing for a broader mechanistic interpretation of pressure-induced collagen remodeling.

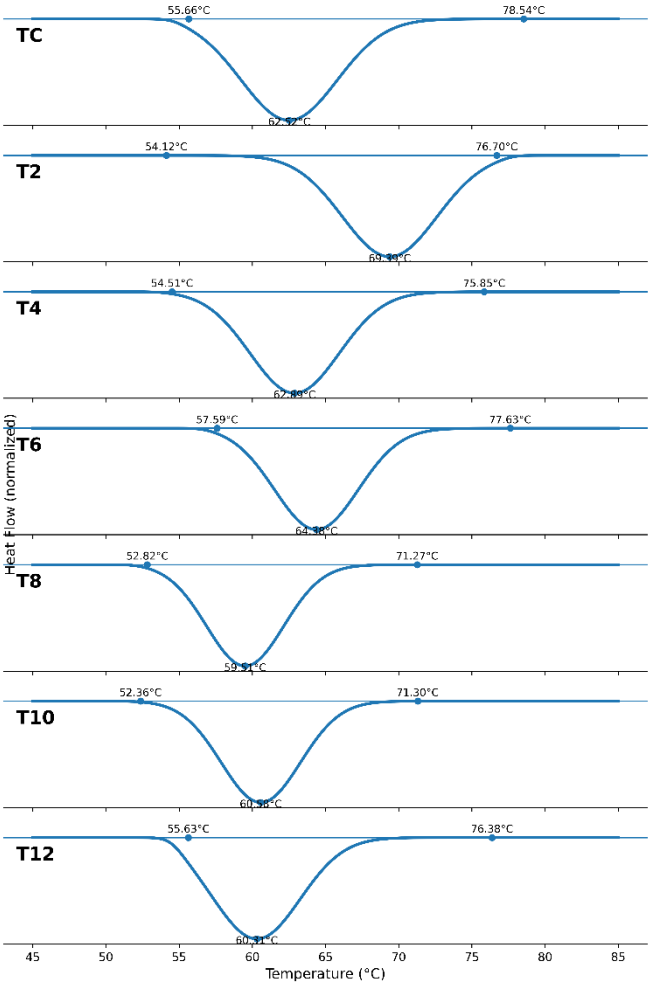

Figure 3. Differential scanning calorimetry (DSC) thermograms of perimysial collagen extracted from beef subjected to high hydrostatic pressure treatments (200–600 MPa; 10 and 20 min). The curves show onset (To), peak (Tp), and final (Tf) temperatures of collagen denaturation, highlighting pressure-induced modifications in the thermal stability of perimysial collagen.

The thermograms showed clear shifts in onset (To), peak (Tp), and final denaturation temperatures (Tf) as a function of pressure intensity and processing time. The control (TC) displayed a typical collagen denaturation profile with higher thermal stability, while pressure-treated samples showed distinct changes in the shape and position of the thermal transition peaks. These shifts indicate that HHP altered the physicochemical stability of the collagen fibrillar network by modifying the intermolecular interactions that maintain its hierarchical organization [11,12].

Notably, the pressure treatments did not produce a simple linear thermal response — collagen stability instead followed a heterogeneous, pressure-dependent pattern. Moderate pressure treatments, particularly T8 and T10, reduced thermal transition temperatures, suggesting partial destabilization and loosening of the collagen fibrillar arrangement. Treatments at higher pressure levels, especially T6 and T12 (600 MPa), showed partial recovery or maintenance of thermal stability, suggesting that severe pressure conditions may simultaneously trigger structural relaxation and localized molecular reorganization.

This pattern supports the idea that HHP acts mainly through changes in non-covalent interactions rather than irreversible cleavage of collagen molecules.

Under hydrostatic pressure, hydrogen bonding, hydrophobic interactions, electrostatic associations, and water–protein interactions are destabilized, leading to rearrangement of fibrillar packing and hydration dynamics [4,11,13]. As a result, collagen thermal behavior becomes strongly dependent on the balance between fibrillar dissociation and pressure-induced aggregation.

The variations in denaturation enthalpy (ΔH) further support this interpretation. Reductions in ΔH are commonly linked to decreased molecular order and partial weakening of collagen's supramolecular packing, while localized increases may reflect pressure-induced stabilization or reorganization of collagen domains [13,14]. The coexistence of these distinct thermal behaviors across treatments suggests that collagen responds to HHP through complex structural adaptation rather than a single, uniform denaturation pathway.

These findings matter because they show that HHP-induced thermal changes are tied to connective tissue architecture rather than to extensive collagen degradation — an interpretation strongly supported by the preserved electrophoretic profile observed in SDS–PAGE. Even under severe pressure conditions, collagen-associated bands remained largely intact, confirming that pressure altered thermal behavior without extensive hydrolysis of collagen polypeptide chains [4–12].

The present results also help clarify some differences relative to earlier reports on pressure-treated collagen. Chang et al. (2013) found that HHP significantly altered thermal shrinkage temperatures of endomysial and perimysial collagen, especially above 500 MPa [15], but that work focused predominantly on DSC-derived thermal transitions alone. The integrated results obtained here show that thermal changes are only one manifestation of broader supramolecular rearrangements involving fibrillar dissociation, hydration changes, extractability shifts, and microstructural remodeling.

The distinct thermal responses observed among treatments relate closely to the structural complexity of perimysial collagen. Perimysium consists of thick collagen bundles stabilized by extensive intermolecular cross-linking and organized into mechanically resistant fibrillar networks [1,3]. Because of this dense hierarchical structure, pressure treatment may initially promote partial interfibrillar loosening before driving more pronounced destabilization of collagen packing — which helps explain why some treatments showed reduced thermal stability while others showed signs of partial structural reorganization.

It is also worth noting that the observed thermal changes occurred at processing temperatures well below collagen's thermal denaturation point. The modifications detected in the DSC thermograms can therefore be attributed mainly to pressure effects rather than heat-induced denaturation [11,12], reinforcing HHP's classification as a non-thermal, structure-modulating technology capable of altering connective tissue functionality while largely preserving collagen's molecular integrity.

From a technological standpoint, pressure-induced reductions in collagen thermal stability may have practical implications for meat tenderness and subsequent cooking. Partial destabilization of collagen fibrils could facilitate connective tissue softening during cooking by reducing resistance to thermal shrinkage and deformation. HHP may therefore improve the tenderness of connective tissue-rich beef cuts without causing excessive protein degradation or severe structural damage [2,5].

Overall, the DSC results show that high hydrostatic pressure modifies bovine perimysial collagen mainly through supramolecular rearrangement and fibrillar reorganization. Interpreted alongside the hydroxyproline, solubility, and SDS–PAGE data, the thermal analyses provide strong evidence that HHP modulates connective tissue functionality through structural remodeling rather than molecular destruction.

III.4. Microstructural Rearrangement of Perimysial Collagen Induced by High Hydrostatic Pressure (SEM)

Scanning electron microscopy (SEM) provided direct visual evidence of pressure-induced changes in the architecture of bovine perimysial collagen (Figure 4). The micrographs clearly showed that high hydrostatic pressure altered both collagen fibril organization and interfibrillar interactions,

reinforcing the hypothesis that HHP acts mainly through supramolecular remodeling of connective tissue structure [18].

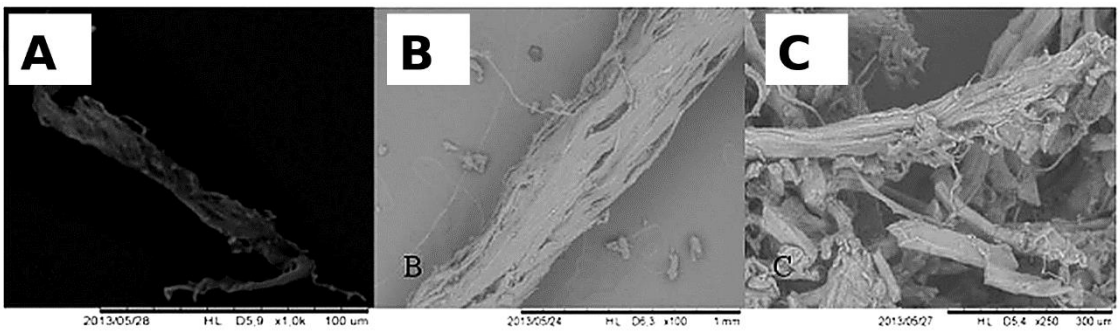

Figure 4. Scanning electron microscopy (SEM) images of bovine perimysial collagen structure. (A) Control sample showing compact collagen fiber bundles. (B) Collagen fibrils exhibiting organized fiber alignment. (C) Pressure-treated sample showing partial disruption and increased interfibrillar spacing. Scale bars: 100 µm (A), 1 mm (B), and 300 µm (C).

The control sample (Figure 4A) showed the typical morphology of intact perimysial connective tissue — compact, continuous collagen bundles arranged in an organized, highly cohesive fibrillar network. The fibers appeared densely packed, with limited interfibrillar spacing and strong structural continuity, reflecting perimysium's mechanical role in maintaining muscle integrity and resistance to deformation [1,3].

Figure 4B showed organized fibrillar alignment with well-preserved bundle orientation, characteristic of structurally stable connective tissue. Pressure-treated samples (Figure 4C), by contrast, displayed substantial microstructural changes: partial fibrillar disorganization, irregular alignment, localized disruption of collagen bundles, and increased interfibrillar spacing. These changes indicate that HHP weakened the non-covalent interactions responsible for collagen fibrillar cohesion.

Despite this visible disorganization, the overall integrity of the collagen fibers remained intact — there was no evidence of complete fibril rupture or extensive structural collapse, which is fully consistent with the SDS–PAGE results showing preserved collagen molecular profiles after pressure treatment. Taken together, this combination of preserved molecular integrity and altered fibrillar organization strongly supports the view that HHP modifies connective tissue mainly at the supramolecular level [4–12].

The increased interfibrillar spacing in pressure-treated samples suggests that HHP promoted hydration-mediated expansion and partial dissociation of collagen fibrillar packing. Pressure-induced redistribution of water within the connective tissue matrix may weaken hydrophobic interactions and hydrogen bonding between fibrils, facilitating partial loosening of the collagen network [9,11,13] — a mechanism consistent with the increases in collagen extractability and hydroxyproline solubilization reported in the previous sections.

At higher pressure levels, more pronounced structural disorganization became evident, including irregular fibrillar orientation and localized porous regions within the perimysial matrix. These features point to progressive destabilization of the hierarchical collagen organization under severe pressure. Still, the absence of extensive fibrillar fragmentation suggests that HHP drove controlled structural remodeling rather than destructive degradation of connective tissue architecture.

The SEM observations correlate closely with the DSC results discussed earlier: treatments showing altered thermal stability also showed less organized fibrillar structure and greater interfibrillar separation. This correspondence indicates that pressure-induced thermal changes are directly tied to shifts in collagen supramolecular organization and fibrillar packing [11,13].

These microstructural findings substantially extend the interpretation proposed in earlier work. Chang et al. (2013) reported changes in collagen thermal stability after HHP treatment but did not pair those observations with direct microstructural evidence of fibrillar rearrangement [15]. Here, the combination of SEM, SDS–PAGE, hydroxyproline quantification, collagen solubility, and DSC analyses shows that thermal changes are part of a broader process of connective tissue remodeling driven by hydrostatic pressure.

From a functional standpoint, partial disorganization of the perimysial collagen network may reduce connective tissue rigidity and facilitate deformation during mastication and cooking. Because perimysial collagen contributes heavily to background toughness in beef muscles, pressure-induced loosening of this fibrillar network may play a major role in improving tenderness without extensive degradation of muscle proteins [2,5].

Taken together, the SEM observations offer compelling evidence that high hydrostatic pressure works as a structure-modulating technology, capable of reorganizing collagen architecture through controlled supramolecular rearrangement. The preservation of collagen molecular integrity alongside substantial microstructural remodeling shows that HHP modifies connective tissue functionality through mechanisms fundamentally different from conventional thermal denaturation.

Integrated Structural Interpretation

The combined results from SDS–PAGE, hydroxyproline quantification, collagen solubility, DSC, and SEM analyses show that high hydrostatic pressure modifies bovine intramuscular connective tissue mainly through supramolecular remodeling rather than extensive molecular degradation. The distinct responses observed between endomysial and perimysial collagen underscore the importance of connective tissue compartmentalization in shaping pressure-induced structural behavior.

Together, the data indicate that HHP weakens the non-covalent interactions stabilizing collagen fibrillar organization, driving changes in hydration dynamics, interfibrillar spacing, collagen extractability, and thermal stability, while preserving much of the collagen molecular backbone. These findings substantially extend earlier interpretations based mainly on thermal transitions, showing that pressure-induced thermal changes are directly linked to broader hierarchical rearrangements within the connective tissue matrix.

The integrated analytical approach adopted in this study thus offers a more comprehensive mechanistic understanding of how hydrostatic pressure modulates collagen functionality and connective tissue architecture. From a technological standpoint, these results highlight HHP's potential as a controlled structure-modulating strategy for improving the quality and tenderness of connective tissue-rich beef cuts while preserving overall protein integrity and product quality [4,8,9].

III.5. Statistical Modeling and Compartment-Specific Response of Collagen Fractions to High Hydrostatic Pressure

To further clarify the mechanisms governing pressure-induced connective tissue modification, statistical analyses were performed using hydroxyproline content as an indirect indicator of collagen structural response in the endomysial and perimysial fractions subjected to high hydrostatic pressure (HHP). Unlike earlier work focused mainly on descriptive thermal behavior, the present study incorporated factorial experimental design, regression modeling, interaction analysis, and response surface interpretation to establish predictive relationships between processing variables and collagen structural change.

The experimental design considered two independent variables: pressure level (200 and 600 MPa) and pressurization time (10 and 20 min), [19]. Hydroxyproline values for the soluble (endomysial) and insoluble (perimysial) fractions showed markedly different response patterns between the two compartments, underscoring the heterogeneous nature of intramuscular connective tissue organization [20].

The endomysial fraction ranged from 789.2 to 871.8 mg/100 g, whereas the perimysial fraction showed substantially greater variability, ranging from 743.8 to 3374.9 mg/100 g. This reflects fundamental structural differences between the two networks:

endomysial collagen forms a finer, less cross-linked fibrillar matrix surrounding individual muscle fibers, while perimysial collagen forms thicker, mechanically resistant bundles surrounding fiber groups and contributes more directly to background toughness [1,2,3].

Analysis of variance (ANOVA) showed that pressure significantly influenced hydroxyproline extractability in the endomysial fraction ($p < 0.05$), and a significant pressure–time interaction was also observed, indicating that collagen's structural response depended on both pressure intensity and exposure duration. This supports the view that HHP-induced changes involve progressive supramolecular rearrangement, including hydration shifts, fibrillar relaxation, and pressure-mediated destabilization of non-covalent interactions [4,11,13, 20].

For the perimysial fraction, both pressure and processing time significantly affected hydroxyproline levels ($p < 0.05$), as did their interaction. However, perimysial collagen behaved quite differently from endomysial collagen statistically: while the endomysium showed a relatively progressive increase in extractability under severe pressure, the perimysium followed a more heterogeneous, non-linear pattern. This suggests that the highly organized perimysial network responds to pressure through more complex adaptation, involving both fibrillar dissociation and localized structural stabilization [21,22].

Effect analysis confirmed pressure as the dominant factor governing collagen modification in both fractions. In the endomysium, pressure had the strongest positive effect, followed by the pressure–time interaction, with time alone contributing comparatively little. In the perimysium, by contrast, processing time carried a negative coefficient, indicating that prolonged exposure under certain conditions may promote partial structural reorganization or stabilization of the fibrillar matrix rather than continuous dissociation[23].

Regression analysis produced predictive models describing compartment-specific collagen behavior as a function of pressure and time. For the endomysial fraction:

$$Y=819.98-0.06A-3.33B+0.01AB$$

Where Y represents the endomysial collagen response, A corresponds to pressure level, and B corresponds to pressurization time.

For the perimysial fraction, the predictive model was:

$$Y=5159.12-3.31A-273.10B+0.46AB$$

The regression coefficients point to important mechanistic differences between the two compartments. In the endomysium, the relatively moderate coefficients suggest progressive, controlled pressure-induced modification, consistent with its less cross-linked fibrillar organization. The larger coefficients in the perimysial model, by contrast, reflect the greater structural complexity and sensitivity of this collagen-rich network to pressure-induced supramolecular rearrangement.

Notably, the significant interaction terms in both models show that collagen behavior under HHP cannot be explained by pressure or time effects in isolation. Instead, the connective tissue response emerges from the combined influence of pressure intensity and exposure duration on collagen hydration, fibrillar packing, and intermolecular stability — an interpretation that substantially broadens earlier work focused mainly on isolated thermal responses.

Response surface analysis further clarified this compartment-specific behavior. The endomysial fraction showed the greatest collagen modification under severe pressure combined with longer processing times, consistent with progressive destabilization and increased extractability of this finer fibrillar network. The perimysial fraction, in contrast, showed greater structural sensitivity at moderate pressure–time combinations, while more severe conditions appeared to favor partial reorganization or stabilization of collagen architecture.

These findings support the hypothesis that HHP modifies connective tissue mainly through supramolecular remodeling rather than extensive molecular degradation, fully consistent with the SDS–PAGE profiles, which showed preserved collagen molecular integrity despite substantial changes in extractability, thermal behavior, and microstructure [4–12]. The observed hydroxyproline variations are therefore best interpreted as markers of pressure-induced fibrillar dissociation, hydration change, and rearrangement of collagen's hierarchical organization, rather than degradation.

These results also build on previous work on pressure-treated connective tissue. Riley et al. (2007) showed that insoluble collagen content is one of the primary determinants of meat toughness, underscoring the technological importance of connective tissue modification [5]. Ichinoseki et al. (2006) reported increased hydroxyproline extractability after pressure treatment, consistent with partial fibrillar dissociation rather than molecular degradation [11]. Suzuki et al. (2006) similarly observed reductions in meat hardness alongside preserved elasticity, indicating that pressure-induced changes occur mainly at the structural rather than molecular level [13]. Unlike these studies, which focused predominantly on isolated texture or thermal parameters, the present work integrates statistical modeling with biochemical, electrophoretic, thermal, and microstructural analyses, showing that hydroxyproline distribution patterns are directly tied to compartment-specific supramolecular remodeling of collagen under pressure.

From a technological standpoint, the differential response of endomysial and perimysial collagen has important implications for pressure-assisted tenderization strategies. Because endomysial collagen was more susceptible to pressure-induced extractability, HHP may preferentially affect connective tissue flexibility and local structural compliance, while controlled modification of perimysial collagen may help reduce background toughness without compromising overall connective tissue integrity.

Overall, the statistical analyses offer strong quantitative evidence that high hydrostatic pressure acts as a structure-modulating technology, reorganizing bovine intramuscular connective tissue through compartment-specific supramolecular mechanisms. The predictive models and interaction effects obtained here advance the mechanistic understanding of collagen behavior under HHP and reinforce the value of integrated analytical approaches for studying connective tissue functionality in pressure-processed meat systems.

III.6. Correlation Analysis and Multivariate Interpretation of Pressure-Induced Collagen Remodeling

To build a more integrated picture of how HHP modifies connective tissue, correlation and multivariate analyses were performed using hydroxyproline data from the endomysial and perimysial fractions together with the processing variables (Figure 5). Unlike earlier studies, which focused mainly on isolated thermal or textural parameters, this multivariate approach allowed compartment-specific relationships between pressure intensity, processing time, and collagen structural response to be identified.

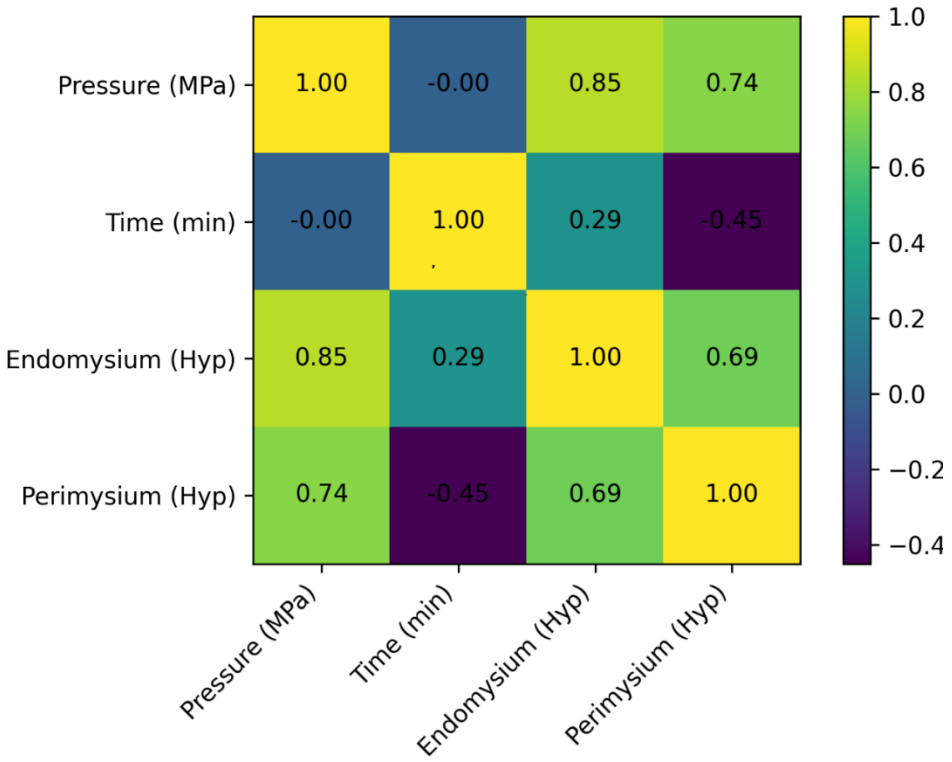

Figure 5. Correlation heatmap illustrating the relationships between processing parameters (pressure and time) and hydroxyproline content in endomysial (soluble collagen) and perimysial (insoluble collagen) fractions of beef subjected to high hydrostatic pressure (HHP).

The heatmap showed pressure as the dominant variable linked to collagen modification in both compartments. A strong positive correlation between pressure and hydroxyproline extractability was found in the endomysial fraction (r = 0.85), indicating that increasing pressure intensity progressively drove collagen solubilization and structural destabilization of the endomysial network. This supports the hypothesis that hydrostatic pressure weakens the non-covalent interactions maintaining collagen's supramolecular organization, facilitating greater extractability of collagen-associated components [4,11,13].

The perimysial fraction showed a more complex pattern. Pressure was again positively associated with hydroxyproline (r = 0.74), though more weakly than in the endomysium. More notably, processing time was negatively correlated with perimysial collagen (r = −0.45), suggesting that prolonged pressure exposure may drive structural rearrangements that reduce extractability or partially stabilize the fibrillar matrix. This points to a key mechanistic distinction between the two compartments: endomysial collagen, with its finer fibrillar organization and lower cross-link density, responded mainly through progressive dissociation and increased extractability, while the perimysium — built from highly organized, mechanically resistant collagen bundles — responded through a dynamic balance of fibrillar destabilization, hydration change, and localized structural reorganization [1,2,3].

A positive correlation between endomysial and perimysial hydroxyproline values (r = 0.69) was also observed, indicating that both compartments responded to pressure treatment simultaneously, though through distinct structural pathways. This reinforces the view that HHP acts on connective tissue architecture globally, while compartment-specific behavior is still shaped by fibrillar organization and cross-link density.

These multivariate relationships were highly consistent with the thermal and microstructural findings discussed earlier: treatments associated with increased endomysial extractability also showed reduced collagen thermal stability and partial fibrillar loosening by SEM. This strengthens the integrated interpretation that pressure-induced collagen modification stems mainly from supramolecular remodeling of connective tissue architecture rather than molecular degradation.

The negative relationship between processing time and perimysial extractability offers further evidence that collagen behavior under pressure is non-linear. Under prolonged pressurization, connective tissue may undergo partial structural reorganization or pressure-assisted stabilization, possibly tied to rearrangement of fibrillar packing and redistribution of hydration within the collagen matrix — which helps explain why severe pressure conditions did not always produce proportional increases in collagen dissociation.

These findings substantially extend earlier reports on pressure-treated connective tissue. Previous studies showed that HHP modifies collagen thermal stability and connective tissue functionality [11,14,15], but few examined the integrated statistical relationships between collagen extractability, compartment-specific response, and structural reorganization. The present results show that hydroxyproline distribution patterns can serve as sensitive indicators of hierarchical collagen remodeling under pressure.

Principal component analysis (PCA) further reinforced the multivariate nature of collagen's response to HHP (Figure 6). The first principal component (PC1), accounting for 63.1% of total variance, was strongly associated with pressure intensity and clearly separated low-pressure (200 MPa) from high-pressure (600 MPa) treatments, confirming pressure as the primary driver of collagen structural modification in bovine connective tissue.

The second principal component (PC2), explaining 32.2% of total variance, was associated mainly with processing time and with interaction effects between pressure and compartment-specific response. The separation observed along PC2 indicates that exposure duration modulated collagen's structural behavior differently depending on the connective tissue compartment involved.

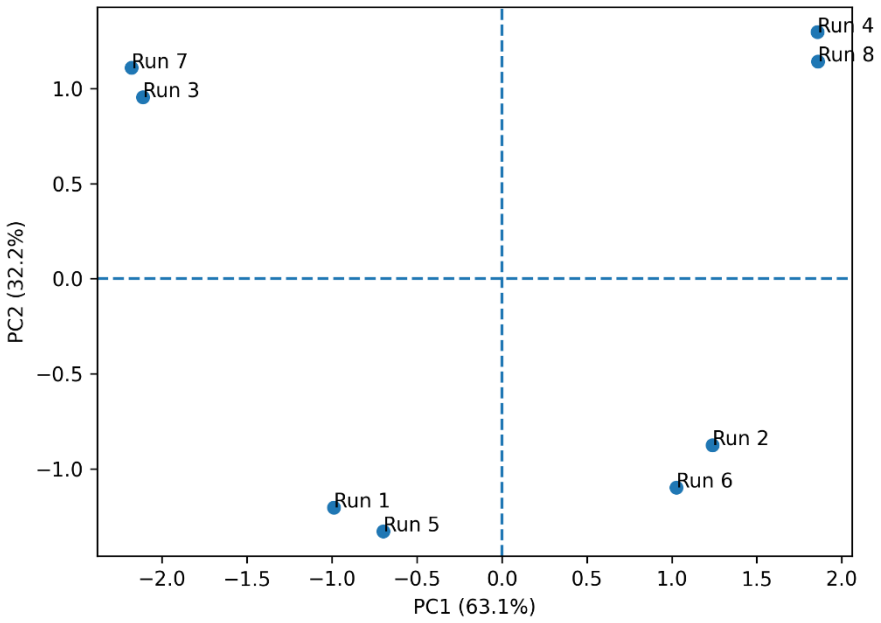

Figure 6. Principal component analysis (PCA) score plot showing the multivariate relationships between processing variables (pressure and time) and collagen-related responses (endomysial and perimysial hydroxyproline content) in beef subjected to high hydrostatic pressure.

Treatments combining high pressure with longer processing times (Runs 4 and 8) grouped in the positive region of both PC1 and PC2, indicating greater overall collagen modification — these were associated with increased hydroxyproline extractability, altered thermal stability, and more pronounced fibrillar disorganization. Low-pressure treatments (Runs 1, 3, 5, and 7), by contrast, clustered in the negative region of PC1, reflecting reduced structural impact on connective tissue architecture.

Interestingly, intermediate treatments positioned near the transition zones of the PCA plot show that collagen's response to HHP is not binary, but rather follows a continuous structural adaptation process governed by pressure intensity and exposure duration. This distribution supports the interpretation that connective tissue remodeling under pressure results from the interplay of multiple mechanisms — hydration dynamics, fibrillar dissociation, and supramolecular reorganization.

The PCA also highlighted compartment-specific sensitivity: endomysial collagen was more strongly tied to pressure-driven extractability changes, while perimysial collagen depended more heavily on interaction effects between pressure and time. These findings reinforce the conclusion that connective tissue compartmentalization plays a decisive role in determining collagen's structural response to hydrostatic pressure.

From a mechanistic standpoint, these multivariate analyses provide compelling evidence that HHP modifies connective tissue mainly through structural remodeling rather than molecular destruction. The strong statistical associations among pressure, hydroxyproline extractability, thermal behavior, and microstructural disorganization occurred without extensive collagen fragmentation, as confirmed by SDS–PAGE. The observed modifications are therefore best understood as pressure-induced rearrangements in fibrillar architecture and intermolecular interactions [4–12].

Technological Implications for Pressure-Assisted Meat Processing

From a technological standpoint, the integrated results of this study show that high hydrostatic pressure is a highly promising non-thermal strategy for controlled modification of bovine intramuscular connective tissue. Unlike conventional thermal treatments, which often cause extensive protein denaturation and quality loss, HHP promoted selective remodeling of collagen's supramolecular organization while largely preserving its molecular integrity.

The compartment-specific response observed between endomysial and perimysial collagen suggests that pressure intensity and processing time can be strategically tailored to the targeted connective tissue modification. Endomysial collagen showed greater susceptibility to pressure-induced extractability and fibrillar loosening, while perimysial collagen was more resistant, though still modifiable. This differential behavior opens opportunities for designing pressure-assisted processing strategies that improve tenderness while preserving the structural stability of the muscle matrix.

Together, the biochemical, electrophoretic, thermal, microstructural, and multivariate analyses show that HHP drives connective tissue remodeling mainly through supramolecular dissociation, hydration-mediated fibrillar rearrangement, and weakening of non-covalent interactions. These mechanisms may facilitate connective tissue softening during cooking and mastication without extensive degradation of collagen molecules or significant loss of product integrity.

From an industrial standpoint, these findings are highly relevant for the valorization of connective tissue-rich beef cuts, which are traditionally associated with lower tenderness and reduced commercial value. By selectively modulating collagen architecture, HHP may improve textural quality and consumer acceptance while preserving the nutritional and structural characteristics of meat products.

Most importantly, this study advances current understanding of pressure-assisted connective tissue modification by showing that collagen behavior under HHP is governed by compartment-specific supramolecular mechanisms rather than simple thermal destabilization. This integrated mechanistic interpretation distinguishes the present work from earlier studies centered mainly on thermal

transitions and establishes a broader framework for understanding collagen functionality in pressure-processed meat systems [4,8,9,14,15, 22].

IV. CONCLUSIONS

This study showed that high hydrostatic pressure modifies bovine intramuscular connective tissue mainly through structural and physicochemical rearrangements at the supramolecular level, rather than through extensive molecular degradation of collagen. The integrated results from hydroxyproline quantification, collagen solubility, SDS–PAGE, DSC, SEM, regression modeling, correlation analysis, and PCA consistently showed that pressure affected collagen extractability, thermal behavior, and fibrillar organization while largely preserving the electrophoretic integrity of collagen-associated proteins.

Endomysial and perimysial collagen responded differently to HHP, confirming that connective tissue compartmentalization is a decisive factor in pressure-induced collagen modification. Endomysial collagen — finer and less cross-linked — was more susceptible to pressure-induced solubilization and showed greater hydroxyproline extractability, especially under higher pressure–time combinations. Perimysial collagen, by contrast, showed a more heterogeneous and resistant response, reflecting its dense, highly organized, mechanically reinforced fibrillar architecture.

The thermal and microstructural results indicated that pressure altered collagen stability by modifying fibrillar packing, hydration dynamics, and non-covalent interactions within the connective tissue matrix. DSC thermograms revealed shifts in collagen denaturation behavior, while SEM images confirmed partial fibrillar disorganization, increased interfibrillar spacing, and loosening of collagen bundles — changes that occurred without evidence of extensive peptide cleavage, reinforcing the view that HHP acts as a controlled structure-modulating technology.

Statistical modeling and multivariate analyses further confirmed pressure as the dominant factor governing collagen modification, with processing time modulating the response depending on the connective tissue fraction. The positive association between pressure and hydroxyproline extractability, together with the PCA separation of treatments by processing intensity, supports the conclusion that HHP-induced collagen remodeling follows a compartment-specific, non-linear physicochemical pathway.

Overall, these findings provide an integrated mechanistic framework showing that HHP modifies bovine collagen through supramolecular reorganization, altered extractability, thermal destabilization or reorganization, and microstructural remodeling. This structural–physicochemical interpretation sets the present work apart from studies focused mainly on collagen thermal transitions, and points to HHP's potential as a targeted strategy for modulating connective tissue functionality, improving the technological value of collagen-rich beef cuts, and guiding future optimization of pressure-assisted meat processing.

Practical Applications

High hydrostatic pressure (HHP) showed significant potential as a non-thermal technology for the controlled modification of bovine connective tissue structure. The results indicate that HHP promotes collagen remodeling mainly through supramolecular rearrangement rather than extensive protein degradation, preserving collagen's molecular integrity while altering fibrillar organization, extractability, and thermal behavior. These structural changes may contribute to improved tenderness in connective tissue-rich beef cuts, which are commonly associated with lower commercial value. The distinct responses observed between endomysial and perimysial collagen suggest that pressure intensity and processing time can be strategically tailored to target specific connective tissue fractions according to the desired textural outcome. HHP therefore represents a promising technological alternative for the meat industry, with potential to improve meat quality, enhance the value of tougher cuts, and support the development of innovative processing strategies that reduce thermal damage while preserving nutritional and structural properties.

Author Contributions:

L.M.F. performed the investigation and formal analyses and wrote the original draft of the manuscript; J.L.R.A. and A.R. provided support for

conducting the methodologies and the logistics of the experiment (project administration, funding acquisition, management of resources, and use of equipment), and performed the investigation and formal analyses, wrote and conceptualized the main manuscript text, and prepared the visualizations of figures and tables. All authors reviewed and edited the main manuscript. All authors have read and agreed to the published version of the manuscript.

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ABSTRACT GRAPHIC