Meat and Muscle Biology™

Beef Tenderizing Potential of *Solanum dubium* (Gubbain) protease

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Abstract: This study aimed to evaluate the efficacy of *Solanum dubium* protease extract injection in tenderizing beef *m. longissimus thoracis et lumborum* (LTL). A total of 12 LTL muscles were obtained from 6 carcasses (from both left and right sides) at 24 h postmortem, and each muscle was divided into 2 equal samples to yield 24 samples ($n = 12$/treatment). Samples were then randomized to receive either an injection with *S. dubium* protease extract (at 10% of muscle weight) or no injection (control). After 24 h of incubation, the muscle was analyzed for color, sarcomere length (SL), myofibril fragment length (MFL), Warner-Bratzler shear force (WBSF), collagen solubility, quantification of meat degradation, and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The injected muscles with the *S. dubium* protease extract yielded lower ($P < .001$) WBSF values with a better L^* ($P < .05$) compared with samples of the control muscles. The results of MFL and the quantification of meat degradation reflected the great ability of the protease extract to degrade myofibrillar proteins. Collagen was hydrolyzed significantly by the protease extract. SDS-PAGE showed the presence of several new lower molecular weight bands after treating muscle with the protease extract. WBSF was significantly related to SL, MFL and collagen solubility. The protease enzyme from *S. dubium* seeds may be a novel and promising option as a meat tenderizer.

Key words: tenderization, *Solanum dubium* protease, microstructural, collagen, *m. longissimus thoracis et lumborum*
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Introduction

Meat tenderness is a critical quality trait that influences consumer acceptance, satisfaction, repeat purchase, and willingness to pay premium prices, remaining one of the most important attributes of meat quality (Warner et al., 2021; Warner et al., 2022). Postmortem proteolytic degradation of myofibrillar and connective tissue (collagen) proteins plays a major role in meat tenderization. The endogenous calpain protease enzyme contributes considerably to tenderization during aging. However, the endogenous proteolytic enzymes do not break down the muscle fiber structure or degrade the connective tissue collagen protein sufficiently (Bekhit, 2017; Koohmaraie and Geesink, 2006). The injection of meat with exogenous protease enzymes extracted from plant, bacterial, and fungal sources is a popular method to improve meat tenderness (Arshad et al., 2016). Several plant protease enzymes have been used for the tenderization of meat, such as papain, bromelain, ficin, actinidin, and zingibain. Protease tenderizers derived from microbes such as *Aspergillus oryzae* and *Bacillus subtilis* are also widely used (Ashie et al., 2002; Bekhit et al., 2014; Arshad et al., 2016). The identification of novel plant protease enzymes from unconventional sources

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has the potential to significantly contribute to the future supply of commercial exogenous proteolytic enzymes in the meat industry (Mohd Azmi et al., 2023). Gubbain (*Solanum dubium*) seeds contain Dubiumin serine protease, which is a good source of the exogenous proteolytic enzyme for food industries (Ahmed et al., 2009b). The initial study by Biraima and Webb (2018) showed that protease extract from the seeds of *S. dubium* improved tenderness considerably, without any negative impact on meat color or sensory properties in the *longissimus* muscle of Sudanese Baggara cattle.

Gubbein (*S. dubium*) is a recognized wild plant in Sudan that grows during the rainy season and is known as "Gubbein." The seeds are usually used by dairy farmers as a traditional protease for clotting milk and manufacturing white soft cheese. Recently, several studies have been cited on the application of proteolytic enzyme from the seeds of Gubbein (*S. dubium*) in dairy technology (Yousif et al., 1996; Abdalla et al., 2010; Ahmed, et al., 2009a; Ahmed et al., 2009b; Talib et al., 2009; El Owni et al., 2011; Kheir et al., 2011; Talib et al., 2011). It has been reported that the seeds of the *S. dubium* plant are nontoxic for humans (Mohamed et al., 2016). Despite its well-documented proteolytic activity, the application of *S. dubium* as a meat tenderizer remains largely unexplored. The literature thus supports the notion of both safe and excessive proteolytic activity in *S. dubium* seeds in addition to the availability of raw materials. Therefore, the Dubiumin enzyme could offer substantial utility in food applications, including meat processing. However, research examining its specific effects on meat quality, collagen solubility, and muscle protein degradation remains limited. Addressing this gap is critical for expanding the range of natural enzymatic tenderizers available to the meat industry.

This study aimed to evaluate the effects of protease extract from the seeds of the *S. dubium* plant on meat color and texture, collagen solubility, quantification of meat degradation, and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) pattern of beef *m. longissimus thoracis et lumborum* (LTL). This study also examined the association among histologic characteristics, collagen solubility, and meat tenderness, considering several key components that influence tenderness, such as sarcomere length (SL), postmortem changes, and proteolytic degradation of myofibrillar and connective tissue proteins (Arshad et al., 2016; Gagaoua et al., 2021).

Materials and Methods

Sampling

Animal ethics approval was granted by the Animal Ethical Committee of the University of Pretoria, South Africa (approval number: EC076-17). Six Afrikaner × Bonsmara crossbred steers of 12 mo old and an average weight of 386 ± 10 kg were used in this study. The steers were slaughtered according to standard procedures at the abattoir of the Agricultural Research Council–Animal Production Institute (ARC-API) in Irene, Gauteng, South Africa. No electrical stimulation was used, and, after dressing, the carcasses were placed directly in a chiller at 4°C for 24 h before sampling. A total of 12 muscles of the LTL were obtained from both carcass sides at 24 h postmortem. Each LTL muscle was considered an experimental unit. Muscles from each animal (left and right sides) were cut into 2 equal samples perpendicular to the longitudinal axis, resulting in 12 muscle samples per treatment group ($n = 6$). These samples were then randomized to receive either the injection treatment with *S. dubium* protease extract or no injection (control).

Sample preparation and injection treatment

The visible fat and connective tissue were trimmed from the muscle samples. Dry yellow fruits of *S. dubium* were collected in January 2018 from the rangeland of North Kordofan State, Sudan. The yellow coats of the Gubbein (*S. dubium*) fruits were removed by hand to obtain the seeds, which were powdered using an electric grinder. The powdered seeds were packed in plastic bags and placed in a container, then transported to ARC-API in Irene, Gauteng, South Africa for analysis. The importation of powdered seeds of *S. dubium* from Sudan was reviewed, and the Department of Agriculture, Forestry and Fisheries in South Africa issued a letter confirming that a permit under the Agricultural Pests Act, 1983 (Act No. 36 of 1983) was not required for the importation of plant products in powdered form. No phytosanitary certificate was required.

The extraction of the protease from *S. dubium* seeds was performed as described by El Owni et al. (2011) with some modifications. The dry powdered seeds of the *S. dubium* (83.33 g) were mixed with 500 mL of distilled water and stirred for 30 min using a magnetic stirrer (5 g/30 mL), resulting in a concentration of 16.67% w/v (weight per volume). The extract was filtered using a nylon mesh strainer and

centrifuged at 6000 rpm for 10 min. Then the supernatant was used for the injections. The protease enzyme of the final aqueous extract was confirmed by adding 5 mL of the extract to 50 mL of heated cow milk (60°C). Milk clotting was then seen after about 1 min. About 2 L of fresh aqueous protease extract was prepared before injection. The muscles were manually injected with 10% aqueous protease extract (muscle weight basis) perpendicular to their muscle fiber orientations using a syringe with a single needle (Ilian et al., 2004; Han et al., 2009; Liu et al., 2011; Biraima and Webb, 2018). The entire volume of the aqueous extract was uniformly injected into different portions of the whole muscle mass. The samples (both injected and control) were then vacuum packed, labeled, and stored for 24 h at 4°C to allow the proteases to degrade the muscle proteins (Biraima and Webb, 2018). After the storage period, meat samples of about 50 g each were cut perpendicular to the fibers from the middle of the muscle sections and used to evaluate instrumental color and SL. Samples for Warner-Bratzler shear force (WBSF) and collagen solubility were vacuum packed, labeled, and kept frozen at −20°C until processing, while samples for myofibril fragment length (MFL), quantification of meat degradation, and the SDS-PAGE pattern were frozen in liquid nitrogen and stored at −80°C until they were processed.

Meat color

Beef samples (15-mm thickness) were bloomed at 18°C (room temperature) for 1 h before measuring the internal cross-cut section color. The color readings (Commission Internationale de l'Éclairage L^*, a^*, b^*, chroma, and hue-angle) were taken with a Konica Minolta CM-600d/CM-700d spectrophotometer using illuminant D65 at 10° observer angle, measurement aperture 8 mm. The Konica Minolta was calibrated before the readings, following the manufacturer's instructions. Three random recordings were taken on each muscle sample (Pophiwa et al., 2016).

Sarcomere length measurements

The SL of the injected and the control muscles were measured at 48 h postmortem (after 24 h of incubation). Muscle tissue (2 g) was homogenized in distilled water (Dreyer et al., 1979; Hegarty and Naudé, 1970). A small drop of each homogenate was placed on a microscope slide and covered with a cover glass. Excess water was dried, and the slide was cleaned with a paper towel. Five SL were measured at a time from the bottom of the first SL with an Olympus BX40 system microscope at a 1000× magnification. A mean of 50 SL per sample was recorded for statistical analysis.

Warner-Bratzler shear force determinations

The frozen beef samples were thawed at 4°C for 24 h, and then about 200 g was removed from each sample and broiled (American Meat Science Association, 2015) in a broiling oven (Mielé model H217; Mielé & Cie, Gütersloh, Germany) at 260°C (preset) to an internal temperature of 70°C and cooled down to room temperature (18°C) (American Meat Science Association, 2015). Six cores from each cooked sample were removed parallel to the fiber orientation, using a hollow metal probe with 8 cm length and 1.27 cm diameter. A Warner-Bratzler shear device attached to the Universal Instron apparatus (Model 4301; Instron Ltd, Buckinghamshire, UK; crosshead speed 200 mm/min) was used to shear the cores perpendicular to the muscle fiber orientation (Honikel, 1998). An average of 6 single peak force values per sample was taken for statistical analysis.

Myofibril fragment length measurements

The lengths of the myofibril fragments of the injected and noninjected muscles were measured using an Olympus BX41 system microscope and video image analysis (VIA; Soft Imaging System, Olympus, Japan). The myofibrils were extracted using the method of Culler et al. (1978) as modified by Heinze and Brüggemann (1994). Sample slices were cut from frozen muscle samples using a knife, and any visible fat and connective tissue were removed. The sample was then finely minced with scissors, and 3 g was weighed into a 50 mL Bühler glass. Subsequently, 30 mL of MFL extraction buffer (0.02 M potassium phosphate buffer containing 100 mM KCl, 1 mM $MgCl_2$, 1 mM EDTA, and 1 mM NaN_3) was added at 4°C. The sample was allowed to thaw for 60 s and homogenized for exactly 30 s in a Bühler HO4 homogenizer at 20 000 rpm while chilled in ice water. The blade was turned around in order to fragment the myofibrils rather than to cut them. The samples were subsequently transferred into centrifuge tubes and centrifuged at 4°C at 3000 rpm for 15 min. The pellet was washed once with 30 mL MFL extraction buffer and centrifuged at 3000 rpm. The supernatant was then discarded, and the pellet was suspended in 10 mL MFL extraction buffer. The suspension was then filtered under vacuum through a 1000 μm polyethylene strainer, and an additional 5 mL MFL buffer was used

to facilitate the passage of the myofibrils through the strainer. The samples were transferred on to a slide and covered with a slip. The excess water on the slide was dried with a paper towel. The MFL measurements were determined at a magnification of 400×. A mean of 100 MFL (μm) per sample was recorded for statistical analysis.

Collagen solubility

The collagen solubility of the injected and noninjected samples was determined following the procedure of Bergman and Loxley (1963), Hill (1966), and Weber (1973). One gram of freeze-dried meat samples was pulverized and added to 12 mL of a 1% NaCl solution. The samples were heated in a shaking water bath for 60 min at 78°C. Then, they were allowed to cool for 15 min and centrifuged at 6000 × g for 10 min. The supernatant was hydrolyzed by adding 30 mL of 6 N HCl and heated for 16 h at 110°C. A 0.5 g portion of active carbon was added, stirred, and the homogenate was filtered into a 100-mL volumetric flask. The flasks were filled to the mark with distilled water. Hydroxyproline was colorimetrically determined by neutralizing the acid in the samples with 10% KOH, then oxidizing the hydroxyproline with Chloramine-T for 20 min. Ehrlich's reagent was then added, and the samples were placed in a water bath for 15 min at 60°C. The absorbance of the pink color was measured at 558 nm in a 1-cm^3 cuvette. All determinations were performed in triplicate. Collagen solubility was calculated by expressing hydroxyproline in the filtrate as a percentage of the total hydroxyproline content (filtrate plus residue). Collagen content was further quantified using hydroxyproline nitrogen relative to total protein nitrogen.

Quantification of meat degradation

To determine the fiber detachment, fiber breaks, and percentage fiber separation score, blocks of approximately 7 mm × 4 mm were cut from the frozen muscles and fixed on a Cryotome disk. A Shandon Cryotome E (Thermo Fisher Scientific, Pittsburgh, USA) was used to obtain sections of 15-μm thickness by cutting parallel to the orientation of the muscle fibers, and they were then mounted on a microscope slide. Two sections from each muscle sample were stained with Amaranth (Sigma A 1016-100G), after which the stained sections were observed under a microscope (Olympus BX41 system) at a magnification of 100× (Olympus, Tokyo, Japan). The entire muscle fiber areas and the fiber detachments (% white to red area) in a field of 0.57 mm^2 were measured using the AnalySIS Life Science software package (Soft Imaging Systems GmbH, Münster, Germany). The fiber breaks were scored by the analyst on a scale of 1 to 5 (Taylor and Frylinck, 2003).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis

The changes in the myofibrillar proteins in the LTL were also measured by means of SDS-PAGE. Myofibrillar proteins were extracted from 200 mg of the LTL samples that were frozen in liquid nitrogen and stored at −80°C. Each sample was homogenized in 1 mL TES buffer and selectively extracted to isolate myofibrillar proteins, following the method described by Jia et al. (2006). Protein concentrations were determined with the RC-DC protein assay kit (Bio-Rad, USA) at 750 nm in a Universal Micro Plate Rader (Bio-Tek Elx800) with bovine serum albumin as standard (Moloto el at., 2015). Twelve percent gel of SDS-PAGE was used to separate the protein bands using the Ettan DALTsix large format vertical system (GE HealthCare Bio-Sciences), after which the Coomassie brilliant blue G250 stain was used to stain the protein bands in PAGE gels (Gallagher, 2012). A Chemi-Doc™ MP imaging system (Bio-Rad Hercules, CA, USA) was used to image and process the gels.

Statistical analyses

The data were analyzed using the linear mixed model (LMM) procedure in SPSS 11.5 for Windows (2003; SPSS version 11.5, SPSS Inc., Chicago, IL, USA), with injection treatment (control vs injected) as fixed effect and carcass and carcass side as random effects. For the determination of WBSF, the test date was also included as random effect. Nonsignificant terms were removed from the model. LMM were used to determine the relationships between WBSF and predictors. The models are as follows:

Model 1. WBSF = fixed effect (injection treatment) + covariates (SL + MFL + fiber breaks + fiber detachment + collagen solubility) + random effects (carcass + carcass side); and

Model 2. WBSF = covariates (SL + MFL + fiber breaks + fiber detachment + collagen solubility) + random effects (injection treatment + carcass + carcass side).

In model 2, nonsignificant variables were excluded from the model using the forward stepwise selection. The fiber breaks scores were transformed to ranks

before running the analysis. Variance components were estimated using restricted maximum likelihood method. The data were expressed as mean values ± standard error of the mean.

Results and Discussion

Meat quality characteristics and collagen solubility

In general, exogenous protease enzymes degrade muscle protein structures, causing significant structural modifications in meat (Bekhit, 2017). This enzymatic process enhances the light-scattering properties, leading to increased instrumental color values (Hughes et al., 2018). However, injection with the protease extract of Gubbain (*S. dubium*) seeds did not influence ($P > .05$) the meat color parameters, except for lightness (L^*) values (Table 1). These contradictions can be attributed to the color of the aqueous extract, which is relatively dark yellow. The meat injected with the protease extract showed higher L^* values than the control samples. These findings were somewhat similar to the observations in the preliminary study conducted on *longissimus* muscles from Sudanese beef cattle (Biraima and Webb, 2018), which found that meat injected with 10% aqueous protease extract of *S. dubium* did not show significant differences in the values of L^*, a^*, and chroma compared with noninjected samples. These authors also reported that the injected muscles had higher ($P < .05$) b^* and hue-angle values than muscles that were not injected. The color of the aqueous extract may also explain the observed increase in muscle yellowness (b^*) due to injection with protease extract of *S. dubium* seeds. The meat samples treated with the protease extract showed longer ($P < .05$) SL, shorter ($P < .001$) MFL, and lower ($P < .001$) WBSF values (Table 1). The SL of injected muscles with the protease extract of *S. dubium* increased by only 1.05% but was statistically significant compared to control samples. However, Cruz et al. (2020) reported that the increase in SL of chicken breasts treated with 5% crude enzymatic ginger extract was not statistically significant, indicating that the enzymatic treatment had a minimal effect on SL. The length of the myofibril fragments can give a good indication of the amount of myofibrillar protein degradation (Li et al., 2014). Shorter myofibril fragments typically suggest greater proteolytic activity, which accelerates myofibrillar protein degradation, leading to improved tenderness in meat (Došler et al., 2007; Frylinck et al., 2009). The observed effect of the protease extract injection on MFL was probably because the fragment length became shorter due to proteolysis by the exogenous proteolytic enzyme of the *S. dubium* seeds (Figure 1). In this study, the injection with the protease extract lowered the WBSF values by nearly

Table 1. Instrumental color, sarcomere length (μm), myofibril fragment length (μm), and Warner-Bratzler shear force (N) of beef *m. longissimus thoracis et lumborum* injected with *Solanum dubium* protease extract.

	Injection Treatment			
Item	Control	Injected	SEM	*P* Value
Lightness (L^*)	32.36	33.36	0.24	.038
Redness (a^*)	11.34	11.38	0.20	.891
Yellowness (b^*)	11.95	12.45	0.21	.074
Chroma	16.48	16.88	0.27	.238
Hue-angle	46.50	47.54	0.37	.074
SL (μm)	1.90	1.92	0.01	.027
MFL (μm)	33.09	23.65	1.52	.000
WBSF (N)	50.27	19.13	1.61	.000

MFL, myofibril fragment length; SEM, standard error of the mean; SL, sarcomere length; WBSF, Warner-Bratzler shear force.

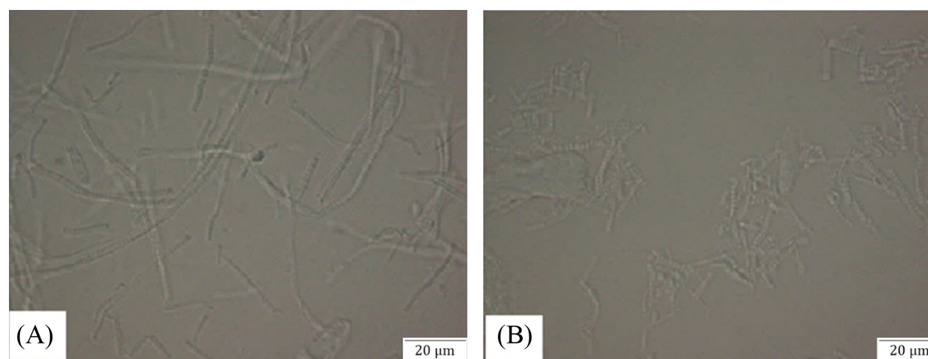

Figure 1. Myofibril fragment lengths (μm) of beef *m. longissimus thoracis et lumborum* samples subjected to different injection treatments (injected vs control). A. Control sample (noninjected), and B. injected sample with protease extract of *Solanum dubium* seeds.

62% relative to the control samples. In support of this, Biraima and Webb (2018) reported that beef muscles injected with the protease extract of *S. dubium* seeds had significantly lower WBSF values (2.12 kg) than noninjected muscles (6.00 kg) and produced more tender meat by almost 65% relative to the noninjected meat. The shorter MFL, with an increase in the myofibrillar fragmentation of treated LTL with the protease extract (Figure 1B), may explain the observed improvement in tenderness, since the shorter length of the myofibril fragments contributes to more tender meat (Frylinck et al., 2009).

Collagen is one of the major protein components of animal connective tissues and is the main factor in determining the tenderness and texture of meat (Weston et al., 2002; Torrescano et al., 2003). The muscles injected with the aqueous protease extract of *S. dubium* seeds showed a higher ($P < .001$) percentage of collagen solubility than the noninjected samples (Figure 2). This great hydrolysis of collagen protein was probably due to the proteolytic activity of the *S. dubium* seed protease extract, which facilitates the breakdown of collagen fibers, thereby improving meat tenderness. The hydrolysis of collagen weakens the structural integrity of connective tissues, reducing their resistance to shear force and resulting in tougher meat (Roy and Bruce, 2023). This may explain the lower WBSF values observed in the injected muscle samples. A meta-analysis by Li et al. (2021) reported a negative correlation between collagen solubility and WBSF values in beef, suggesting that higher collagen solubility is associated with improved tenderness. In contrast, Chriki et al. (2013) found that collagen characteristics have a low impact on beef tenderness in a cut with a low amount of connective tissue such as LTL. However, the intensive degradation of connective tissue in the injected muscle samples may have resulted in a significant tenderization effect despite the general assumption that collagen plays a minor role in low-connective tissue cuts.

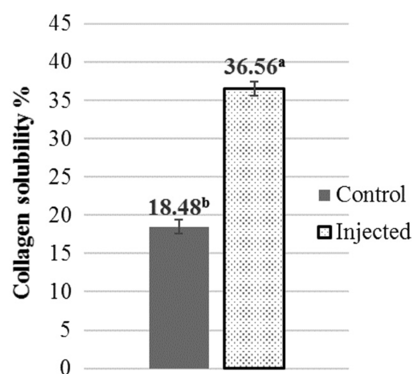

Figure 2. Effects of *Solanum dubium* protease extract injections on the collagen solubility (%) of beef *m. longissimus thoracis et lumborum* samples. The error bars represent the SEM. [a,b]Bar means with different superscripts differ significantly at $P < .05$. SEM, standard error of the means.

Quantification meat degradation

The muscle fibers from the muscles injected with the protease extract of *S. dubium* seeds exhibited more fractures and breaks (Figure 3C and D) than those from the control samples (Figure 3A and B). Fiber breaks and detachment (% white to red area) were quantified and found to be higher ($P < .001$) for injected muscles than for noninjected ones (Table 2). The proteolytic action observed in muscle fiber fractures, breaks, and detachment can indeed be attributed to the protease extract from *S. dubium* seeds. These findings suggest that the exogenous protease enzyme of *S. dubium* seeds actively hydrolyzes myofibrillar proteins, leading to a higher degree of structural disruption. This enzymatic degradation contributes to lower WBSF values, as muscle fiber fractures, breaks, and detachment are key indicators of proteolytic action that enhance meat tenderness (Taylor and Frylinck, 2003; Veiseth-Kent et al., 2010).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis

The results of the SDS-PAGE pattern indicated pronounced proteolytic changes between the injected and noninjected muscles (Figure 4A). The myofibrillar proteins extracted from the meat injected with the protease extract of *S. dubium* seeds had a lower number of protein bands of high molecular weights (50–230 kDa) (Figure 4A and B) than the control samples (Figure 4A and C). However, the number of protein bands of low molecular weights (<50 kDa) increased in the injected meat (Figure 4A and B) compared with the control samples (Figure 4A and C). The increase in low molecular weight protein bands (<50 kDa) observed in the injected muscles is a direct result of the proteolytic action of the *S. dubium* protease extract, demonstrating extensive protein degradation. This enzymatic breakdown leads to the fragmentation of myofibrillar proteins, causing structural disruption and producing smaller peptides, ultimately enhancing meat tenderness. Liu et al. (2011) reported that meat injected with the protease enzyme of kiwifruit juice showed a significant loss of higher molecular weight fractions with the presence of many new lower molecular weight bands

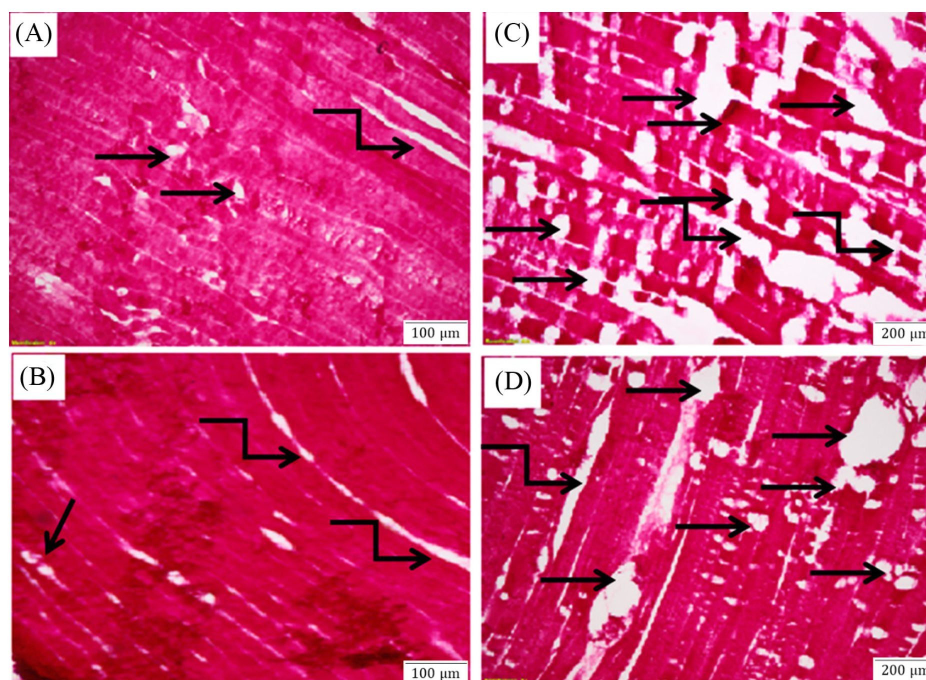

Figure 3. A. and B. Longitudinal sections of beef *m. longissimus thoracis et lumborum* of control samples (noninjected), and C. and D. samples injected with protease extract of *Solanum dubium* seeds. Fractured muscle fibers are indicated by straight arrows, whereas breaks are indicated by elbow arrows.

Table 2. Rank means (mean) for the fiber breaks score and means for fiber detachment of beef *m. longissimus thoracis et lumborum* samples affected by injection treatment (injection with *Solanum dubium* protease extract vs control).

	Injection Treatment			
Item	Control	Injected	SEM	*P* Value
Fiber breaks score (1–5)	7.17 (1.58)	17.83 (4.5)	1.02	.000
Fiber detachment: % white area	16.92	25.92	1.07	.000

SEM, standard error of the mean.

underneath because of the breakdown of the myosin-heavy chain, thus improving the meat's tenderness.

Regression coefficients

Two LMM were used to analyze the relationships between WBSF and its predictors (SL, MFL, fiber breaks, fiber detachment and collagen solubility). Model 1 examined which predictors had a significant impact on WBSF variation, while model 2 excluded nonsignificant predictors through forward stepwise selection to develop the optimal regression model for predicting WBSF. As shown by the results of model 1, injection treatment, SL, MFL, and collagen solubility were significant variables associated with the prediction of WBSF (Table 3). However, the fiber breaks and detachment showed no significant regression coefficients on WBSF ($P > .05$; Table 3). Model 2 was the best regression model built for predicting the WBSF (Table 3). Model 2 included the presence of SL, MFL, and collagen solubility ($R^2 = 0.95$, $P < .001$). As expected, injection with protease extract *S. dubium* seeds had a significant effect on the WBSF of the beef LTL muscles. Similarly, Stolowski et al. (2006) reported that tenderness increases as SL increase. In contrast, Taylor and Frylinck (2003) assessed the quantification of SL and myofibrillar structure degradation in different beef breeds and stated that meat tenderness is related to muscle fiber fractures, breaks, and detachment but not to the lengths of a sarcomere. The strong relationships among MFL, collagen solubility, and WBSF were expected since, in this study, the exogenous protease enzyme from the seeds of *S. dubium* hydrolyzed the myofibrillar and collagen proteins greatly, resulting in more tender meat. Good relationships between myofibrillar fragmentation and tenderness have been reported (Strydom et al., 2000; Muchenje et al., 2008). In general, shorter MFL or a higher myofibril fragmentation index are usually related to a higher degree of proteolysis and a decreased shear force (Došler et al., 2007; Frylinck et al., 2009). Strydom et al. (2005) reported that MFL was a good predictor of improved tenderness during prolonged aging. The current study indicated that MFL was a

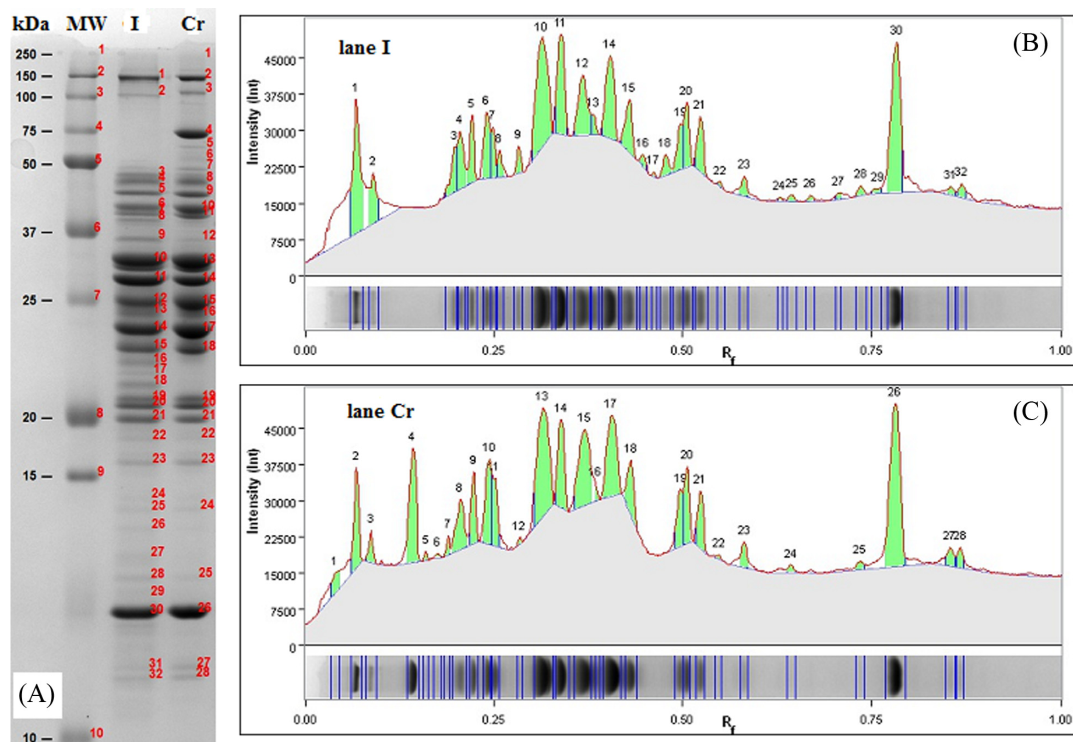

Figure 4. A. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis pattern of myofibrillar proteins isolated from beef *m. longissimus thoracis et lumborum* subjected to injection treatment (injected vs control). Lane MW marker; lane I, injected muscle sample with protease extract of *Solanum dubium* seeds; and lane Cr, control muscle sample (noninjected). B. The expression of the bands from the injected muscle sample (lane I). C. The expression of the bands from the control muscle sample (lane Cr). MW, molecular weight.

Table 3. Summary of the effects of fixed-term (injection treatment) and covariates (histologic characteristics and collagen solubility) on Warner-Bratzler shear force.

Model Term	Coefficient	SEM	*P* Value
Model 1			
Intercept	−72.28	38.46	.078
Treatment = control	−29.46	8.50	.003
Treatment = injected	0		
SL (μm)	86.84	20.91	.001
MFL (μm)	1.31	0.36	.002
Fiber breaks	0.52	0.27	.076
Fiber detachment (%)	−0.14	0.25	.563
Collagen solubility (%)	−3.07	0.35	.000
Model 2			
Intercept	−110.90	39.42	.013
SL (μm)	98.00	20.02	.000
MFL (μm)	1.29	0.35	.001
Collagen solubility (%)	−2.84	0.34	.000

MFL, myofibril fragment length; SEM, standard error of mean; SL, sarcomere length.

good predictor of the differences in tenderness. Furthermore, the relationship between meat pH and texture has been widely investigated (Jankowiak et al., 2021). pH is believed to influence protein solubility and myofibrillar structural integrity, which are important factors in meat tenderness (Feng et al., 2020). However, pH was not measured in this study and should be considered in future research to better understand how enzymatic treatment interacts with meat pH and texture. This remains a limitation of the current study, but our findings on myofibrillar fragmentation and collagen hydrolysis still support the effectiveness of *S. dubium* protease in improving meat texture.

Conclusions

The present study confirmed that the protease present in the seeds of *S. dubium* was a powerful meat tenderizer and resulted in a lower shear force without a negative influence on meat color. The results for MFL, quantification of myofibrillar structure degradation, and SDS-PAGE reflected the strong proteolytic activity of the protease on myofibrillar proteins. The protease extract of *S. dubium* seeds exhibited extensive hydrolysis of myofibrillar and connective tissue (collagen) proteins. This study showed that meat tenderness is strongly associated with collagen solubility, SL, and

MFL. The protease enzyme from the seeds of *S. dubium* is a promising source of an exogenous proteolytic enzyme that could have a wide application in the meat industry. Further research is required to identify the specific proteins affected by the protease extract of *S. dubium* seeds and the tenderizing effect of purified Dubiumin enzyme. The current results could pave the way for a promising new meat tenderizer to improve the tenderness of meat.

Conflict of Interest

The authors declare no conflict of interest.

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Authors Contributions

Ahmed Biraima: Conceptualization; Methodology; Data curation; Formal analysis; Writing—original draft. Edward Webb: Conceptualization; Methodology; Writing—review & editing; Supervision; Funding acquisition

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