TITLE PAGE

- Food Science of Animal Resources -

Upload this completed form to website with submission

ARTICLE INFORMATION	Fill in information in each box below
Article Type	Research article
Article Title	Combined Effects of Pressure cooking and Enzyme Treatment to Enhance The Digestibility and Physicochemical Properties of Spreadable Liver Sausage
Running Title (within 10 words)	Combined Pressure and Enzyme Properties of Liver Sausage
Author	Su-Kyung Ku, Jake Kim, Se-Myung Kim, Hae In Yong, Bum-Geun Kim, Yun-Sang Choi
Affiliation	Research Group of Food Processing, Korea Food Research Institute, Wanju 55365, Republic of Korea
Special remarks – if authors have additional information to inform the editorial office	
ORCID (All authors must have ORCID) https://orcid.org	Su-Kyung Ku (orcid.org/0000-0002-9158-8254) Jake Kim (orcid.org/0000-0002-3016-7659) Se-Myung Kim (orcid.org/ 0000-0003-2250-7243) Hae In Yong (orcid.org/ 0000-0003-0970-4496) Bum-Geun Kim (orcid.org/0000-0001-9452-9391) Yun-Sang Choi (orcid.org/0000-0001-8060-6237)
Conflicts of interest List any present or potential conflict s of interest for all authors. (This field may be published.)	The authors declare no potential conflict of interest. All authors declare that unknown financial and personal relationships with other people or organizations that could inappropriately influence their work do not exist.
Acknowledgements State funding sources (grants, funding sources, equipment, and supplies). Include name and number of grant if available. (This field may be published.)	This research was supported by the Main Research Program [E0211100-02] of the Korea Food Research Institute (KFRI) funded by the Ministry of Science and ICT (Korea).
Author contributions (This field may be published.)	Conceptualization: Ku SK, Kim BG, Choi YS. Data curation: Ku SK, Kim BG, Choi YS. Formal analysis: Ku SK, Kim J, Kim SM. Methodology: Ku SK, Kim J, Yong HI. Software: Ku SK, Kim J, Yong HI. Validation: Ku SK, Kim J, Yong HI. Investigation: Kim BG, Choi YS. Writing - original draft: Ku SK, Kim BG, Choi YS. Writing - review & editing: Ku SK, Kim J, Kim SM, Yong HI, Kim BG. Choi YS.
Ethics approval (IRB/IACUC) (This field may be published.)	This article does not require IRB/IACUC approval because there are no human and animal participants.

CORRESPONDING AUTHOR CONTACT INFORMATION

For the <u>corresponding</u> author (responsible for correspondence, proofreading, and reprints)	**Fill in information in each box below**
First name, middle initial, last name	Yun-Sang Choi
Email address – this is where your proofs will be sent	kcys0517@kfri.re.kr
Secondary Email address	
Postal address	Research Group of Food Processing, Korea Food Research Institute, Wanju 55365, Korea
Cell phone number	

Office phone number	82-63-219-9387
Fax number	82-63-219-9076

Co-CORRESPONDING AUTHOR CONTACT INFORMATION

For the corresponding author (responsible for correspondence, proofreading, and reprints)	**Fill in information in each box below**
First name, middle initial, last name	Bum-Geun Kim
Email address – this is where your proofs will be sent	bkkim@kfri.re.kr
Secondary Email address	
Postal address	Research Group of Food Processing, Korea Food Research Institute, Wanju 55365, Korea
Cell phone number	
Office phone number	82-63-219-9335
Fax number	82-63-219-9076

Combined Effects of Pressure cooking and Enzyme Treatment to Enhance the Digestibility and Physicochemical Properties of Spreadable Liver Sausage

Abstract

This study aimed to determine the effect of enzymes, guar gum, and pressure processing on the digestibility and physicochemical properties of age-friendly liver sausages. Liver sausages were manufactured by adding proteolytic enzyme (Bromelain) and guar gum, and pressure-cooking (0.06 MPa), with the following treatments: Control, without proteolytic enzyme; T1, proteolytic enzyme; T2, proteolytic enzymes and guar gum; T3, pressure-cooking; T4, proteolytic enzyme and pressure-cooking; T5, proteolytic enzymes, guar gum, and pressure-cooking. The pH was high in the enzyme- and pressure-processed groups. The pressure-processed groups had lower apparent viscosity than other cooking groups, and it decreased during enzyme treatment. Hardness was lower in the enzyme- and pressure-processed groups than in the control, and the T4 led to the lowest hardness. Digestibility was the highest in T4 at 82.58%, and there was no significant difference with that in T5. The general cooking group with enzyme and guar gum also showed higher digestibility than the control (77.50%). As a result of the sodium dodecyl sulfate-polyacrylamide gel electrophoresis, the enzyme- and pressure-treated groups (T4, T5) were degraded more into low-molecular-weight peptides (≤37 kDa) than the control and other treatment groups. Viscoelasticity showed similar trends for viscous and elastic moduli. Similarly, combined pressure processing and enzymatic treatment decreased viscoelasticity, while guar gum increased elasticity but decreased viscosity. Therefore, the tenderized physical properties and improved digestibility by enzyme and pressurization treatment could be used to produce age-friendly spreadable liver sausages.

Keywords: liver sausage, enzyme, pressure, digestibility, hardness

Introduction

The aging society significantly impacts the global food industry because sensory perception and food preferences change with age (Zizza et al., 2007). Kim (2018) reported that elderly people had problems with insufficient dietary intake and malnutrition due to chewing difficulties. The food types that older people with difficulties in chewing and swallowing can eat are limited. Accordingly, it has been reported that the ratio of protein and lipid energy intake is lower in foods that are difficult to chew than in foods that are easy to chew (Park et al., 2013). Many studies have cited protein as an important nutrient for the elderly and reported that protein intake could improve the rapid loss of muscle mass associated with aging (Morais et al., 2006; Wolfe et al., 2008). Therefore, the adequate intake of easily digestible protein is important for elderly individuals with muscle weakness, mastication, and dysphagia (Gagaoua et al., 2021).

Additionally, a study on the exploration of the snacking behavior of the elderly for the development of processed meat products showed that meat sticks and Chinese beef jerky were difficult to consume because of their hard texture. However, prosciutto and liver pâté were recognized as foods that could be eaten in special cases (Mena et al., 2020). Spreadable meat products such as liver pâté and liver sausages have a high nutritional value and density.

Nutritionally, the liver contains approximately 20% protein and is an excellent source of many mineral substances, vitamins A, D, B_2, and $B_{12,}$ and folic acid (Jayathilakan et al., 2012). Therefore, liver products could be an excellent alternative to fresh meat because they can provide high value-added nutrients in small amounts to the elderly with dysphagia (Delgado-Pando et al., 2011). In addition, as an edible by-product, the liver is an important raw material with potential for high-quality development and a

63 highly effective emulsifier for processing owing to its unique taste and technical
64 function (Fisher, 1982; Han et al., 2018; Hammer, 1982).

65 According to the mechanism of action, generally used meat tenderization methods
66 can be classified into electrical, mechanical, chemical, and enzymatic treatments
67 (Dransfiee et al., 1981; Elkahalifa et al., 1990; Macfarlane, 1985; Zhang et al., 2021).
68 Pressure treatment disaggregates actin and myosin filaments, the major constituents of
69 myofibrils, and promotes tenderizing by inducing changes in protein molecular
70 interactions and noncovalent bonds (Bouton et al., 1977). Therefore, pressure can affect
71 the structure of myofibrillar proteins. Results depend on protein susceptibility, pressure
72 and temperature, and the degree of pressure treatment (Sun and Holley, 2010). It has
73 also been reported that high-pressure treatment promotes the activation of proteolytic
74 enzymes in the muscle (Homma et al., 1994). Proteolytic enzyme treatment is a widely
75 used method for meat tenderization. Bromelain is a proteolytic enzyme extracted from
76 plants and has been widely used as a meat tenderizer (Naveena et al., 2004). Gerelt et al.
77 (2000) reported that proteolytic enzymes promote the fragmentation of myofibrils and
78 weaken the connective tissue structure in the muscles.

79 Manufacturing methods significantly influence the digestibility of meat proteins (Lie
80 et al., 2017). Xue et al. (2020) reported that structural changes through autoclaving
81 affect the digestion of meat. It has been reported that proteolysis due to enzymatic
82 tenderizing weakens the protein structure and can increase digestibility by increasing
83 protein accessibility to digestive proteases (Zhao et al., 2019).

84 Guar gum has a strong water-holding capacity and is used as a binder and lubricant
85 for manufacturing sausage and stuffed meat products (Bakhsh et al., 2021). The addition
86 of guar gum can contribute to quality improvement by stabilizing enzymatic treatment
87 and improving the water holding capacity. Moreover, it has been reported that the

interaction of proteins and polysaccharides improves the stability of enzymes (Jadhav and Singhal, 2013).

Therefore, this study aimed to produce age-friendly spreadable liver sausages with improved digestibility by applying enzyme, guar gum, pressure processing, and analyzing the physicochemical properties of the produced sausages.

Materials and Methods

Spreadable liver sausage preparation and processing

Spreadable liver sausages were prepared by referring to the methods of Choi et al. (2019). Lean pork, back fat, duck liver, and duck skin were purchased from a local market (Jeonju, Korea). Spreadable liver sausages were manufactured through treatments involving the addition of a proteolytic enzyme and guar gum, and pressure-cooking, as shown in Table 1 and as follows: Control (without proteolytic enzyme), T1 (proteolytic enzyme), T2 (proteolytic enzymes and guar gum), T3 (pressure cooking), T4 (proteolytic enzyme and pressure cooking), and T5 (proteolytic enzymes, guar gum, and pressure cooking). Spreadable liver sausage was prepared using the following method. After each raw meat (lean pork, back fat, duck liver, and duck skin) was ground through a Ø 6 mm plate using a meat chopper (SMC-22A, SL company, Incheon, Korea), nitrite-pickling salt (NPS; salt/nitrite = 99.4:0.6) and plant protease (complex seasoned food containing bromelain, tender enzyme S1, ES food, Gunpo, Korea) were added at 4 ℃ for 15 h. Subsequently, the first cooking was performed to stop the enzymatic reaction. The control, T1 and T2, were cooked at 80 ℃ for 30 min using a water bath (JSR JSSB-30T, Gongju, Korea), and the pressure treatments (T3, T4, and T5) were cooked at 110 ℃ using an autoclave (Jeio tech AC-13, Daejeon, Korea) at a pressure of 0.06 MPa for 10 min. After adding ingredients to the cooked pork, back fat,

duck skin, and duck liver, they were mixed for 2 min in a silent cutter (Hermann Scharfen GmbH & Co., Witten, Germany) and then stuffed into the cellulose casing. After stuffing, the samples were cooked at 80 ℃ for 30 min in a water bath (JSR JSSB-30T, Gongju, Korea).

pH

The pH was determined by mixing 5 g of sample with 20 mL of distilled water at 8,000 rpm (Ultra-Turrax, T25, Janken & Kunkel, Staufen, Germany) after homogenizing the liver sausage for 3 min using a pH meter (Accumet Model AB15+, Fisher Scientific, NH, USA).

Color

The color was measured using a chromameter (CR-210, Minolta, Osaka, Japan) at the center of the cut liver sausage. The values of CIE L^* (lightness), CIE a^* (redness), and CIE b^* (yellowness) were measured thrice (illuminant C). A standard white plate with an "L" value of 97.83, "a" value of –0.43, and "b" value of +1.98 was used as the background.

Emulsion stability

The emulsion stability of the liver sausage was measured according to the method described by Ensor et al. (1987). After two layers of wire mesh (4×4 cm) were placed on the prepared centrifuge tube, 20 g of the emulsion was filled, and the inlet was sealed with aluminum foil. The emulsion stability was evaluated by measuring the amount of free fat and water by heating the centrifuge tube at 75 ℃ for 30 min, followed by cooling for 30 min (Choi et al., 2015).

Digestibility

The *in vitro* digestion of liver sausages was carried out as described by as Lee et al. (2020). The homogenate (4 mL) was treated with 10 mL of gastric digestive juice

(pepsin 182 unit/mg protein and gastric lipase 21 unit/mg protein dissolved in 0.15 M NaCl, pH 1.8 with 0.1 M HCl) and digested at 37 ℃ for 2 h in a shaking water bath. Duodenal fluid (10 mL) and bile fluid (5 mL) were added to the product of the gastric phase, and digestion was performed under the same conditions as in the gastric phase. The compositions of duodenal and bile fluids were as follows: duodenal fluid (trypsin 34.5 unit/mg protein, chymotrypsin 0.4 unit/mg protein, and pancreatic lipase 2,000 unit/mg protein dissolved in distilled water, pH 7.5 adjusted with 1 M NaOH), and bile fluid (4 mM bile extract dissolved in distilled water, pH 7.5 adjusted with 1 M NaOH). For the control, the same amount of distilled water and digestion solution were added instead of the sample used during digestion. The digesta was stored at –70 ℃, and the protein content was determined using the Kjeldahl method (AOAC, 2000).

Proximate composition

Moisture, crude protein, and crude fat contents were determined using a drying oven, the Kjeldahl method, and Soxhlet method (AOAC, 2000), respectively. Ash content was determined using a muffle furnace (AOAC, 2000).

Apparent viscosity

The apparent viscosities of the liver sausage were measured using a rheometer (DV3THB; Brookfield Engineering Laboratories, Middleborough, MA, USA) at 35 ℃ for 10 s. The apparent viscosity was assessed at a constant shear rate of 50/s for 30 s. The maximum apparent viscosity is presented in mPa/s.

Texture profile analysis

The textural properties were analyzed using a texture analyzer (TA-XT2*i*, Stable Micro Systems, Surrey, UK). The sample was placed in a container with a diameter of 40 mm and height of 20 mm, a probe (circular, 20 mm in diameter at the bottom) was mounted, and compression was measured. Analytic conditions were determined by

setting the pre-test speed to 10.0 mm/s, test speed to 10.0 mm/s, post-test speed to 10.0 mm/s, distance to 10.0 mm, and trigger distance to 10.0 mm.

Viscoelasticity

For the viscoelastic properties, the shear strain (1%) corresponding to the linear viscosity range (LVR) was fixed, and a frequency sweep test was performed to measure the storage modulus (G) and loss modulus (G″) according to the angular frequency (0.1–100 rad/s).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

The protein concentration was measured using the Bradford method (Kruger, 2009). A sample aliquot of 50 µL and 200 µL of Bradford reagent (Sigma-Aldrich, St. Louis, MO, USA) were mixed, and absorbance was measured at 595 nm using a spectrophotometer (Optizen 2120 UV plus, Mecasys Co. Ltd., Daejeon, Korea). The standard curve was calculated using bovine serum albumin obtained from Sigma-Aldrich, and distilled water was used as the blank. The sample buffer was mixed with 20 µg of the protein sample, and the protein to sample buffer was 3:1. The mixture was heated at 100 ℃ for 5 min in a water bath and cooled at 25 ℃ for 5 min. Then, 15 µL of each sample was injected into the well of 12% Mini-PROTEIN® TGXTM Precast Gels (Bio-Rad Lab, Inc., USA), and the Precision Plus Protein TM dual-color standard presented standard molecular weight bands on the gel. After separation, the gel was stained with Coomassie Brilliant Blue R250 (Bio-Rad Lab, Inc., USA).

Statistical analysis

SPSS Statistics 20 software (SPSS Inc., Chicago, IL, USA) was used to analyze the data statistically. One-way analysis of variance (ANOVA) with Duncan's range test was performed ($P < 0.05$). Each experimental analysis was performed twice for all three replicates.

Results and Discussion

pH, color, emulsion stability, and digestibility

The pH and color of the spreadable liver sausages with enzymes and pressure processing are shown in Table 2. The pH is affected by enzymatic and pressure processing. Additionally, the pH was higher after enzyme- and pressure-processing than that in the control. The combination of enzyme and pressure treatment in T4 was the highest at 6.25, which was not significantly different from that of T2 and T5. The higher pH values in pressure-processing may be attributed to fast cooking rates, which can lead to higher loss of free acidic groups. It has been that free hydrogen sulfide begins to form when cooked at a high temperature above 80°C, which increases with increasing temperature (Lawrie, 1998). The lightness was the highest in T4 and lowest in the control. The pressure-treated group showed a higher redness than the general heat treatments, while yellowness showed the opposite trend. Myoglobin is one of the most incomplete proteins with respect to pH and temperature (Faustman and Cassens, 1990). It has been reported that color change can be caused by protein denaturation and the emulsification of water and protein by pressure (Jung et al., 2003). Therefore, the difference in color owing to pressure processing was likely caused by the denaturation of myoglobin.

The emulsion stability of the spreadable liver sausage was in the range of 12.21–13.99%, with no statistical differences among different treatments, but it showed relatively lower values during enzyme and pressure treatment compared to that in the control (Table 2). When manufacturing ground meat products, the emulsification capacity of meat proteins affects the degree of meat tenderness. This is because of the correlation between the concentration of water-soluble proteins released into emulsion and meat tenderness (Aminlari et al., 2009).

The *in vitro* digestibility of the spreadable liver sausages upon enzyme and pressure processing is shown in Table 2. A chemical method used to analyze meat tenderization was used to determine the solubility and effectiveness of connective tissues and protein

digestion (Mahendrakar et al., 1989). The enzyme and pressurized combination treatment (T4) showed the highest digestion at 82.58%, and there was no significant difference compared to that in T5. The general heat treatments with enzyme and guar gum also showed higher digestibility than the control (77.50%). Steam cooking positively affects the overall muscle protein digestion (Rakotondramavo et al., 2019). Xue et al. (2020) reported that high-pressure treatment improved the digestibility of gel-based meat products. By measuring the digestibility of bovine muscle according to the heating time, it was found that the digestibility decreased as the cooking time increased (Santé-Lhoutellier et al., 2008). Therefore, heating under vapor pressure shortened the heating time and improved the digestibility due to steam and pressure in this study.

Proximate composition

The proximate components of spreadable liver sausages with enzymes and pressure processing are listed in Table 3. The moisture content did not significantly differ between the control and general heat treatments. However, the pressure processing groups (T3–T5) showed a higher moisture content than the control ($P < 0.05$). Pawar et al. (2000) reported that the moisture content and cooking time showed an inverse relationship. It was determined that the yield decreased as the cooking time increased. In addition, water retention increases upon treatment with plant proteolytic enzymes (Aminlari et al., 2009). The protein content did not significantly differ, at 17.30–18.76%. The fat content was higher in the pressure treatment group than in the control and general heat treatment groups, similar to the moisture content. Ash content was higher in the general heat treatments than in the control and pressure treatments. The study results also indicate that the enzyme and pressurization treatment increased the moisture retention.

Apparent viscosity

241 The apparent viscosity of the spreadable liver sausage batters with enzymes and
242 pressure processing is shown in Fig 1. Enzyme and pressure processing affected the
243 viscosity of liver sausages. Additionally, all batters showed a decrease in apparent
244 viscosity with rotation time and thixotropic behavior. The apparent viscosity of the
245 pressure-treated group was lower than that of the heat-treated group, and the viscosity
246 decreased during enzymatic treatment. In addition, the guar gum-treated group showed
247 a relatively high viscosity in both the general and pressure heating treatments. It was
248 reported that when guar gum is dispersed in water, the galactose side chains of the
249 molecules interact with water molecules, causing intermolecular chain entanglement in
250 aqueous solutions, thereby increasing the viscosity (Zhang et al., 2005), which is
251 consistent with the results of this study. Emulsions with a high apparent viscosity are
252 correlated with high emulsion stability, which affects the quality characteristics of meat
253 products (Zayas, 1997). There was a clear difference in apparent viscosity among
254 treatments in this study. However, it was judged that the effect of particle size and
255 distribution degree was greater than that of emulsion stability when there was no
256 significant difference in emulsion stability.

257 *Hardness*

258 Sausage hardness indicates the degree of ripening due to the denaturation and
259 gelation of meat proteins and loss of moisture (Gimeno et al., 2001). Enzyme and
260 pressure processing can affect the hardness of spreadable liver sausages. The enzyme
261 and pressure treatments led to lower hardness than the control, and T4 had the lowest
262 hardness at 20,911.3 N/m^2 (Fig. 2). Pressure treatment induces a change in the muscle
263 microstructure, myofibrillar contractions, fragmentation, and gelation of myofibril
264 structural proteins that damage myofibers (Morton et al., 2017; Chen et al., 2014). Plant
265 proteases affect meat tenderization through microstructural and biochemical changes

(Maiti et al., 2008). In addition, the combined treatment with enzyme and pressure improved the digestibility owing to the partial degradation of muscle protein (Ma et al., 2019), consistent with the results of this study. The texture of the liver sausages prepared in this study was analyzed according to the texture analysis method specified in Korean Industrial Standard (KS) for aging-friendly food. The Korea Industrial Standards and the Ministry of Food and Drug Safety have defined “age-friendly food” and prepared specifications and standards. Korean industrial standards are classified into three stages based on their physical properties. Level 1 is food that can be ingested with teeth and has a hardness of 500,000–50,000 N/m^2; level 2 is food that can be eaten with gums and has a hardness of 50,000–22,000 N/m^2; and level 3 is food that can be consumed with the tongue and has a hardness lower than 20,000 N/m^2 and a viscosity of 1,500 mPa/s or higher (Korean Industrial Standards, 2017). As a result of this study, the liver sausages treated with enzymes and pressure processing can be considered to be products equivalent to level 2 age-friendly food.

Viscoelasticity

The viscoelastic properties of liver paste products are essential, as they provide fundamental insights into the structural organization of the product (Steen et al., 2014). The storage modulus showed an increasing trend as the angular frequency increased in all the treatments. The storage modulus according to the treatments was the highest in the control and lowest in T4. The high-pressure treatment led to a lower value than the general heat treatment, and it was found that the enzymatic treatment decreased the elasticity. In contrast, the guar gum-treated groups (T2 and T5) treated with enzymes and showed lower values than the control and T1 but higher than that of T4, suggesting that guar gum increases the elasticity (Fig. 3). The results of the loss modulus (G″) of liver sausages with improved digestibility upon applying enzyme and pressure

processing are shown in Fig. 4. The loss modulus (G″), which indicates the viscosity, showed a similar tendency to the elastic modulus. It was found that the enzymatic treatment had a greater effect on viscosity reduction than the heating method. The addition of guar gum did not show a significant difference during general heating treatments, but it was found that pressure treatment reduced the G″ value and decreased the viscosity.

SDS-PAGE

The SDS-PAGE results of the spreadable liver sausages with enzymes and pressure processing are shown in Fig. 4. The combined enzyme and pressurized treatments (T4 and T5) led to more degraded, low-molecular-weight peptides of 37 kDa or less than those in the control and other treatments. A major determinant of softening is the degree of proteolysis of key target proteins in muscle fibers (Koohmaraie and Geesink, 2006). The three-dimensional structure of a protein can be broken even by pressure (Son, 1997). Myofibrillar proteins are sensitive to autoclaving, which has been confirmed in many studies (Pazos et al., 2014; Chen et al., 2017). In addition, a large protein band of 259 kDa appeared in T3, T4, and T5, which were pressurized. In general, when the change in protein occurs at 55–70 ℃, the quaternary structure of the protein is reversibly changed by unfolding, the disulfide bond is broken in the range of 70–80 ℃, and protein polymerization occurs at 90–100 ℃ (Davis and Williams, 1998). Therefore, it was concluded that a polymer band formed because of protein polymerization because pressure treatment was conducted at 110 ℃.

Conclusions

In this study, combined enzyme and pressure processing was conducted to produce spreadable liver sausage with improved digestibility, and the effect of different

316 treatments was evaluated. The enzyme and pressure treatments had higher pH and lower
317 emulsion stability, viscosity, and hardness than the control. Treatments also decreased
318 the viscoelasticity. As for digestibility, the enzyme and pressurized combination
319 treatments led to higher digestibility than those in the control. Therefore, the results of
320 this study suggest that enzyme and pressure are effective at tenderizing the physical
321 properties of spreadable liver sausage, improving digestibility, and allowing their use to
322 produce age-friendly foods.
323
324 **References**

325 Aminlari M, Shekarforoush SS, Gheisari HR, Golestan L. 2009. Effect of actinidin on
326 the protein solubility, water holding capacity, texture, electrophoretic pattern of
327 beef, and on the quality attributes of a sausage product. J Food Sci 74(3): C221-
328 C226.
329 AOAC 2000. Official Methods of Analysis. 17th ed. Association of Official Analytical
330 Chemists, Washington, DC, USA.
331 Bakhsh A, Lee SJ, Lee EY, Hwang YH, Joo ST. 2021. evaluation of rheological and
332 sensory characteristics of plant-based meat analog with comparison to beef and
333 pork. Food Sci Anim Resour 41(6):983-996.
334 Bouton PE, Ford AL, Harris PV, Macfarlane JJ, O'Shea JM. 1977. Pressure-heat
335 treatment of postrigor muscle: Effects on tenderness. J Food Sci 42(1): 132-135.
336 Buckow R, Sikes A, Tume R. 2013. Effect of high pressure on physicochemical
337 properties of meat. Crit Rev Food Sci Nutr 53(7): 770-786.
338 Chen X, Li PJ, Nishiumi T, Takumi H, Suzuki A, Chen CG. 2014. Effects of high-
339 pressure processing on the cooking loss and gel strength of chicken breast
340 actomyosin containing sodium alginate. Food Bioproc Tech 7(12): 3608–3617.
341 Chen X, Tume RK, Xiong Y, Xu X, Zhou G, Chen C, Nishiumi T. 2018. Structural
342 modification of myofibrillar proteins by high-pressure processing for functionally

343 improved, value-added, and healthy muscle gelled foods. Crit Rev Food Sci Nutr
344 58(17): 2981-3003.
345 Choi YS, Jeong TJ, Hwang KE, Kim HW, Kim CJ, Sung JM, Kim YB. 2015. Effects of
346 Emulsion Mapping in Different Parts of Pork and Beef. Korean J Food Sci
347 Technol 31(3): 241-247.
348 Choi YS, Kim YB, Jeon KH, Ku SK, Kim EM, Kim MJ. 2019. Liver sausages
349 comprising vegetables powder and its preparation method. Patent 1020170151549.
350 Colmenero FJ. 2002. Muscle protein gelation by combined use of high
351 pressure/temperature. Trends Food Sci Technol 13(1): 22–30
352 Davis PJ, Williams SC. 1998. Protein modification by thermal processing. Allergy 53:
353 102-105.
354 Delgado-Pando G, Cofrades S, Rodríguez-Salas L, Jiménez-Colmenero F. 2011. A
355 healthier oil combination and konjac gel as functional ingredients in low-fat pork
356 liver pâté. Meat Sci 88(2): 241-248.
357 Dransfiled E, Etherrington D. 1981. Enzymes and food processing. Elsvier Applied
358 Science Pub., London and New York. p 177
359 Elkahalifa E.A. and Marriott NG. 1990. Comparison of the effects of Achromobacter
360 iophagus and splennic pulp on collagen of restructured beef. J Muscle Foods 1:
361 115-128.
362 Ensor SA, Mandigo RW, Calkins CR, Quint LN. 1987. Comparative evaluation of whey
363 protein concentrate, soy protein isolate and calcium-reduced nonfat dry milk as
364 binders in an emulsion-type sausage. J Food Sci 52(5): 1155-1158.
365 Faustman C, Cassens R. 1990. The biochemical basis for discoloration in fresh meat: a
366 review. J Muscle Foods 1:217–243.
367 Fischer A. 1982. Leberwurst. Emulgiereigenschafen von Leber in Abhäangigkeit von
368 Zustand und Vorbehandlung. Fleischerei 33: 371.

Gagaoua M, Dib AL, Lakhdara N, Lamri M, Botineştean C, Lorenzo JM. 2021. Artificial meat tenderization using plant cysteine proteases. Curr Opin Food Sci 38: 177-188.

Gerel B, Ikeuchi Y, Suzuki A. 2000. Meat tenderization by proteolytic enzymes after osmotic dehydration. Meat Sci 56(3):311-318.

Gimeno O, Astiasaran I, Bello J. 2001. Calcium ascorbate as a potential partial substitute for NaCl in dry fermented sausages: effect on colour, texture and hygienic quality at different concentrations. Meat Sci 57: 23–29

Hammer GF. 1982. Zur Leberwursttechnologie: Verarbetungseignung von Schweinefettegewbe und Leber, Temperaturführung. Fleischwirtschaf 60(2): 190.

Han YJ, Kim TK, Hwang KE, Choi JH, Jeon KH, Kim YB, Choi YS. 2018. Quality characteristics of pork liver sausage containing natural vegetable. Korean J Food Cook Sci 34(3): 237-246.

Homma N, Ikeuchi Y, Suzuki A. 1994. Effects of high pressure treatment on the proteolytic enzymes in meat. Meat Sci 38(2):219-228.

Iwasaki T, Noshiroya K, Saitoh N, Okano K, Yamamoto K. 2006. Studies of the effect of hydrostatic pressure pretreatment on thermal gelation of chicken myofibrils and pork meat patty. Food Chem 95(3):474.

Jadhav SB, Singhal RS. 2013. Screening of polysaccharides for preparation of alpha-amylase conjugate to enhance stability and storage life. Carbohydr Polym 92(2):1724–1729.

Jayathilakan K, Sultana K, Radhakrishna K, Bawa AS. 2012. Utilization of byproducts and waste materials from meat, poultry and fish processing industries: a review. J Food Sci Technol 49(3): 278–293.

Jung S, Ghoul M, de Lamballerie-Anton M. 2003. Influence of high pressure on the color and microbial quality of beef meat. LWT-Food Sci Technol 36(6): 625-631.

Jung SH. 2010. Sausage recipe. Korea Meat Industries Association. Seoul. Korea. pp 171-172.

Kang CK, Rice EE. 1970. Degradation of various meat fractions by tenderizing enzymes. J Food Sci 35: 563-565.

Kim SH. 2018. Effects of nutrient intake on oral health and chewing difficulty by age group. Journal of Korea Academia-Industral Cooperation Society. 19(2): 202-209.

Koohmaraie M, Geesink GH. 2006. Contribution of postmortem muscle biochemistry to the delivery of consistent meat quality with particular focus on the calpain system. Meat Sci 74: 34-43.

Kruger NJ. 2009. The Bradford method for protein quantitation. In J. M. Walker (Ed.), The protein protocols handbook. Springer protocols handbooks (pp 17–24). Totowa, NJ, USA: Humana Press.

Lawrie RA, Ledward DA. 1998. The storage and preservation of meat II. Moisture control. Lawrie's Meat Science. Woodhead Publishing, Cambridge, UK, 191-194.

Lee S, Jo K, Lee HJ, Jo C, Yong HI, Choi YS, Jung S. 2020. Increased protein digestibility of beef with aging in an infant in vitro digestion model. Meat Sci 169: 108210.

Li L, Liu Y, Zou X, He J, Xu X, Zhou G, Li C. 2017. In vitro protein digestibility of pork products is affected by the method of processing. Food Res Int 92: 88-94.

Ma Y, Yuan Y, Bi X, Zhang L, Xing Y, Che Z. 2019. Tenderization of yak meat by the combination of papain and high-pressure processing treatments. Food Bioproc Technol 12(4): 681-693.

Macfarlane JJ. 1985. High pressure technology and meat quality. In developments in meat science (3). Elsevier Applied Science Pub., London and New York. p 155.

Mahendrakar NS, Dani NP, Ramesh BS, Amla BL. 1989. Studies on the influence of age of sheep and post-mortem carcass conditioning treatments on muscular collagen content and its thermal ability. J Food Sci Technol 26 (2): 102-105.

Maiti AK, Ahlawat SS, Sharma DP, Khanna N. 2008. Application of natural tenderizers in meat-a review. Agric Rev 29: 226–230.

Mena B, Hutchings S, Ashman H, Warner RD. 2020. Exploring meal and snacking behaviour of older adults in Australia and China. Foods 9(4): 426.

Morais JA, Chevalier S, Gougeon R. 2006. Protein turnover and requirements in the healthy and frail elderly. J Nutr Health Aging. 10: 272-283.

Morton JD, Pearson RG, Lee HY, Smithson S, Mason SL, Bickerstaffe R. 2017. High pressure processing improves the tenderness and quality of hot-boned beef. Meat Sci 133: 69–74.

Mudgil D, Barak S, Khatkar BS. 2014. Guar gum: processing, properties and food applications—a review. J Food Sci Technol 51(3): 409-418.

Naveena BM, Mendiratta SK, Anjaneyulu ASR. 2004. Tenderization of Buffalo Meat Using Plant Proteases from *Cucumis Trigonus Roxb* (Kachri) and *Zingiber Officinale Roscoe* (Ginger Rhizome). Meat Sci 68(3): 363-369.

Park JE, An HJ, Jung SU, Lee YA, Kim CI, Jang YA. 2013. Characteristics of the dietary intake of Korean elderly by chewing ability using data from the Korea National Health and Nutrition Examination Survey 2007-2010. J Nutr Health 46: 287-295.

Pawar VD, Khan FA, Agarkar BS. 2000. Quality of chevon patties as influenced by different methods of cooking. J Food Sci Technol 37(5): 545-548.

Pazos M, Méndez L, Gallardo JM, Aubourg SP. 2014. Selective-targeted effect of high-pressure processing on proteins related to quality: a proteomics evidence in Atlantic mackerel (Scomber scombrus). Food Bioproc Technol 7(8): 2342-2353.

Rakotondramavo A, Rabesona H, Brou C, Lamballerie MD, Pottier L. 2019. Ham processing: effects of tumbling, cooking andhigh-pressure on proteins. Eur Food Res Technol 245: 273–284.

Santé-Lhoutellier, V., Astruc, T., Marinova, P., Greve, E., & Gatellier, P. (2008). Effect of meat cooking on physicochemical state and in vitro digestibility of myofibrillar proteins. J Agric Food Chem 56(4): 1488-1494.

Sikes AL, Tobin AB, Tume RK. 2009. Use of high pressure to reduce cook loss and improve texture of low-salt beef sausage batters. Innov Food Sci Emerg Technol 10(4): 405–412.

Son KH. 1997. Use of high pressure to food industry. Proceed. Korean Soc Food Sci of Ani Res Conference, Seoul, Korea. pp 105-118.

Steen L, Fraeye I, De Mey E, Goemaere O, Paelinck H, Foubert I. 2014. Effect of salt and liver/fat ratio on viscoelastic properties of liver paste and its intermediates. Food Bioproc Technol 7: 496-505.

Sun XD, Holley RA. 2010. High hydrostatic pressure effects on the texture of meat and meat products. J Food Sci 75(1): R17-R23.

Sun XD, Holley RA. 2011. Factors influencing gel formation by myofibrillar proteins in muscle foods. Compr Rev Food Sci Food Saf 10(1): 33–51.

Wolfe RR., Miller SL, Miller KB. 2008. Optimal protein intake in the elderly. Clin Nutr 27(5): 675-684.

Xue S, Wang C, Kim YHB, Bian G, Han M, Xu X, Zhou G. 2020. Application of high-pressure treatment improves the in vitro protein digestibility of gel-based meat product. Food Chem 306: 125602.

Zayas JF. 1997. Emulsifying properties of proteins In: Zayas J. F., editor. Functionality of proteins in food. Springer-Verlag Berlin Heigedlbeg; Berlin, Germany. pp 134-227.

Zhang LM, Zhou JF, Hui PS. 2005. A comparative study on viscosity behavior of water-soluble chemically modified guar gum derivatives with different functional lateral groups. J Sci Food Agric 85(15): 2638-2644.

Zhang Y, Li X, Zhang D, Ren C, Bai Y, Ijaz M, Wang X, Zhao Y. 2021. Acetylation of sarcoplasmic and myofibrillar proteins were associated with ovine meat quality attributes at early postmortem. Food Sci Anim Resour 41(4):650-663.

Zhao D, Xu Y, Gu T, Wang H, Yin Y, Sheng B, Zhou G. 2019. Peptidomic investigation of the interplay between enzymatic tenderization and the

digestibility of beef semimembranosus proteins. J Agric Food Chem 68(4):1136-1146.

Zizza CA, Tayie FA, Lino M. 2007. Benefits of snacking in older Americans. J Am Diet Assoc 107(5): 800-806.

Tables

Table 1. Formulation of spreadable liver sausages by pressure and proteolytic enzyme treatment (unit, %).

Ingredients	Control	T1	T2	T3	T4	T5
Pork ham	45	45	45	45	45	45
Pork back fat	20	20	20	20	20	20
Duck skin	15	15	15	15	15	15
Duck liver	20	20	20	20	20	20
Total	100	100	100	100	100	100
NPS (salt/nitrite=99.4:0.6)	2.0	2.0	2.0	2.0	2.0	2.0
Isolated soy protein	1.9	1.9	1.9	1.9	1.9	1.9
Onion powder	2.4	2.4	2.4	2.4	2.4	2.4
Pepper	0.2	0.2	0.2	0.2	0.2	0.2
Ginger powder	0.2	0.2	0.2	0.2	0.2	0.2
Rosemary	0.05	0.05	0.05	0.05	0.05	0.05
Guar gum	-	-	0.25	-	-	0.25
Protease			0.5	0.5	0.5	0.5

NPS, nitrite-picked salt.

Table 2. pH, color, emulsion stability, and digestibility of spreadable liver sausages after pressure and proteolytic enzyme treatment.

		Control[1]	T1	T2	T3	T4	T5
pH		6.17±0.02^{c}	6.20±0.00^{b}	6.23±0.00^{a}	6.19±0.00^{b}	6.25±0.00^{a}	6.24±0.00^{a}
Color	CIE L*	54.42±0.12^{d}	54.95±0.54^{c}	55.52±0.17^{b}	55.58±0.36^{b}	56.31±0.44^{a}	54.65±0.48^{cd}
	CIE a*	7.41±0.09^{c}	7.73±0.13^{b}	7.35±0.07^{c}	7.96±0.05^{a}	7.81±0.14^{b}	7.94±0.12^{a}
	CIE b*	11.37±0.32^{b}	11.20±0.24^{b}	12.37±0.13^{a}	10.98±0.22^{c}	10.36±0.11^{e}	10.65±0.08^{d}
Emulsion stability (%)		13.99±1.29	13.59±1.23	13.24±1.89	12.96±0.28	12.95±0.23	12.21±0.78
Digestibility (%)		77.50±1.39^{c}	80.07±0.08^{b}	80.08±0.12^{b}	80.15±0.28^{b}	82.58±0.41^{a}	81.54±0.45^{a}

[1] Control, without proteolytic enzyme; T1, proteolytic enzymes; T2, proteolytic enzymes and guar gum; T3, pressure-cooking; T4, proteolytic enzymes and pressure cooking; T5, proteolytic enzymes, guar gum, and pressure cooking.
CIE L*, lightness; CIE a*, redness; and CIE b*, yellowness.
$^{a–e}$ Values with different letters in the same row are significantly different ($P < 0.05$).

Table 3. Proximate composition (%) of spreadable liver sausages by pressure and proteolytic enzyme treatment.

	Control[1]	T1	T2	T3	T4	T5
Moisture	49.46±1.84^{b}	49.16±2.69^{b}	48.52±1.47^{b}	53.54±0.58^{a}	54.38±0.09^{a}	53.67±1.15^{a}
Protein	18.76±0.63	18.14±0.21	17.30±1.09	17.62±0.66	17.77±0.33	17.98±0.96
Fat	22.95±0.37^{abc}	22.25±0.83^{bc}	21.51±0.09^{c}	24.66±1.48^{a}	24.09±0.33^{a}	23.42±0.65^{ab}
Ash	1.99±0.01^{b}	2.28±0.14^{a}	2.04±0.08^{ab}	1.84±0.02^{b}	1.93±0.06^{b}	1.93±0.22^{b}

[1] Control, without proteolytic enzyme; T1, proteolytic enzymes; T2, proteolytic enzymes and guar gum; T3, pressure-cooking; T4, proteolytic enzymes and pressure cooking; T5, proteolytic enzymes, guar gum, and pressure cooking.

[a–c] Values with different letters in the same row are significantly different ($P < 0.05$).

Figures

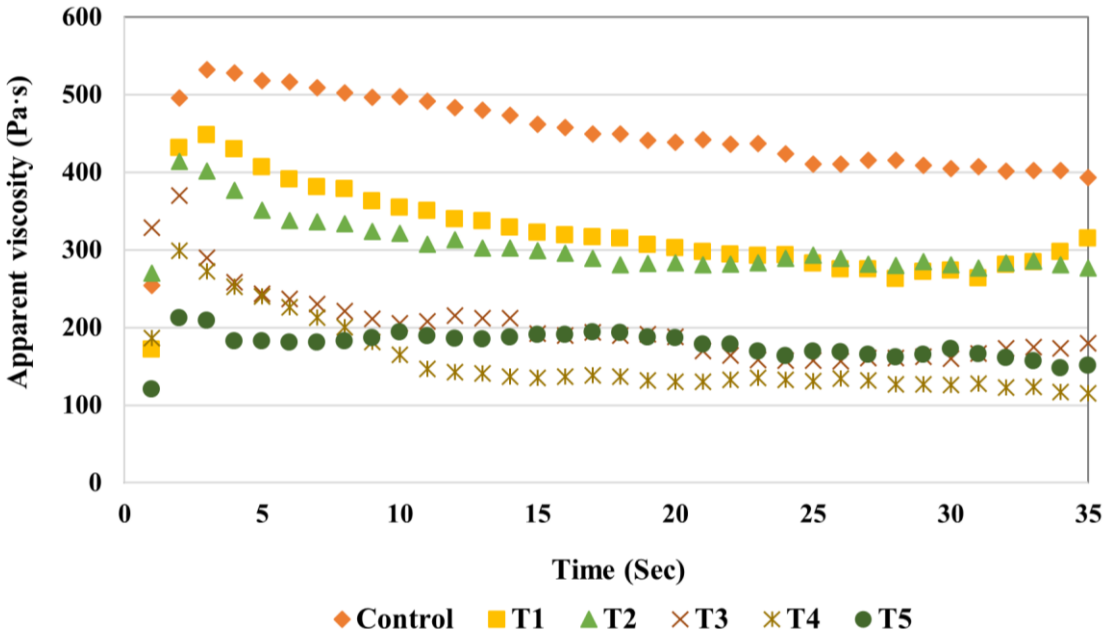


Fig. 1. Apparent viscosity of spreadable liver sausages after pressure and proteolytic enzyme treatment. Control, without proteolytic enzyme; T1, proteolytic enzymes; T2, proteolytic enzymes and guar gum; T3, pressure-cooking; T4, proteolytic enzymes and pressure cooking; T5, proteolytic enzymes, guar gum, and pressure cooking.

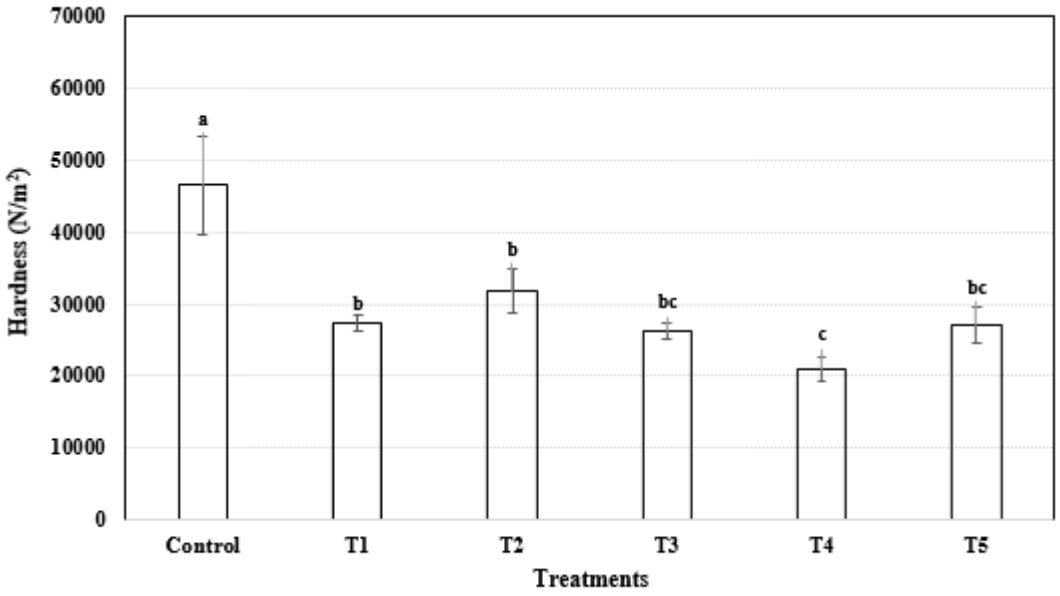
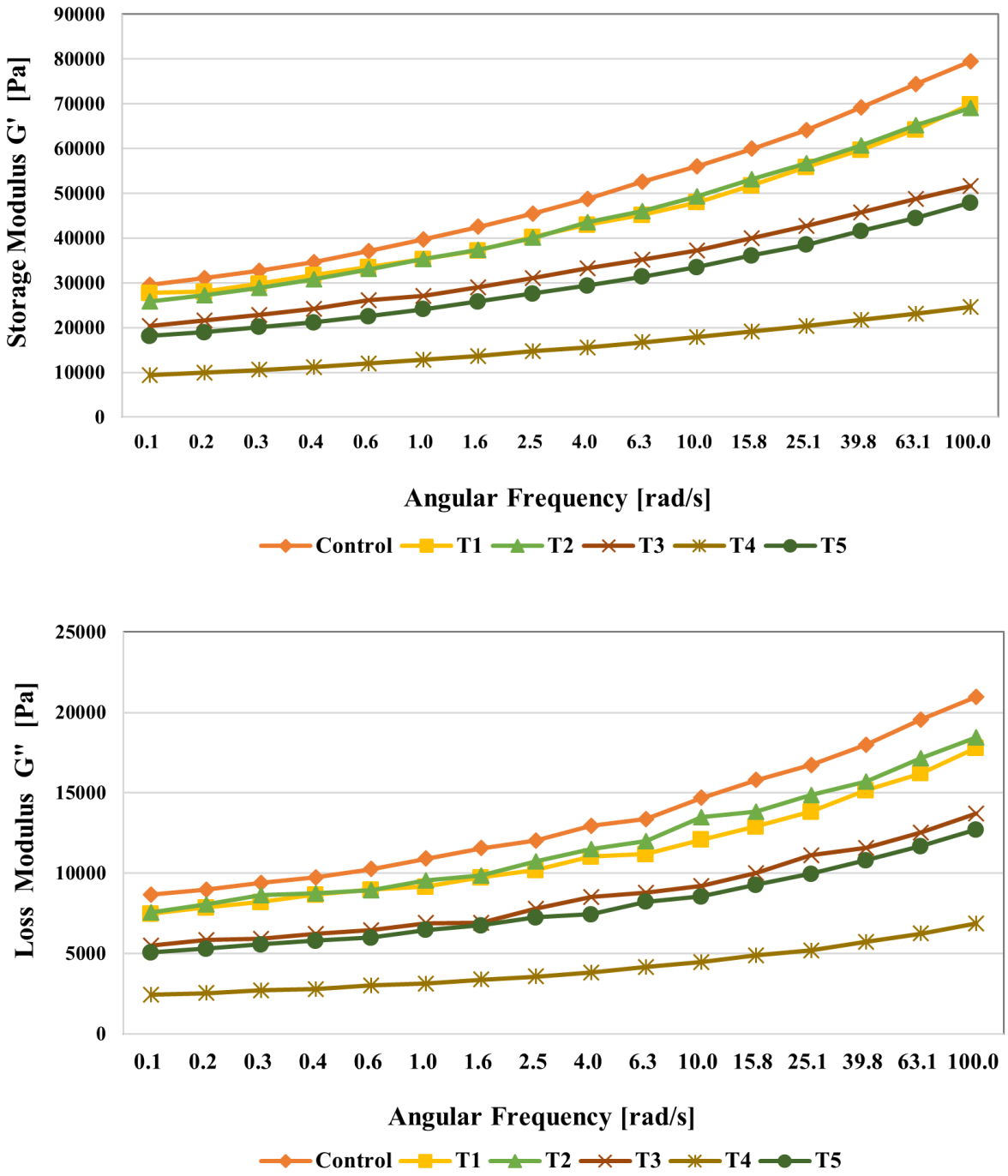


Fig. 2. Hardness of spreadable liver sausages after pressure and proteolytic enzyme treatment.

Control, without proteolytic enzyme; T1, proteolytic enzymes; T2, proteolytic enzymes and guar gum; T3, pressure-cooking; T4, proteolytic enzymes and pressure-cooking; T5, proteolytic enzymes, guar gum, and pressure cooking. [a–c] Different letters above the bars indicate that the results are significantly different ($P < 0.05$).



519
520 **Fig. 3**. Viscoelasticity of spreadable liver sausages after pressure and proteolytic
521 enzyme treatment. Control, without proteolytic enzyme; T1, proteolytic enzymes; T2,
522 proteolytic enzymes and guar gum; T3, pressure-cooking; T4, proteolytic enzymes and
523 pressure-cooking; T5, proteolytic enzymes, guar gum, and pressure-cooking.

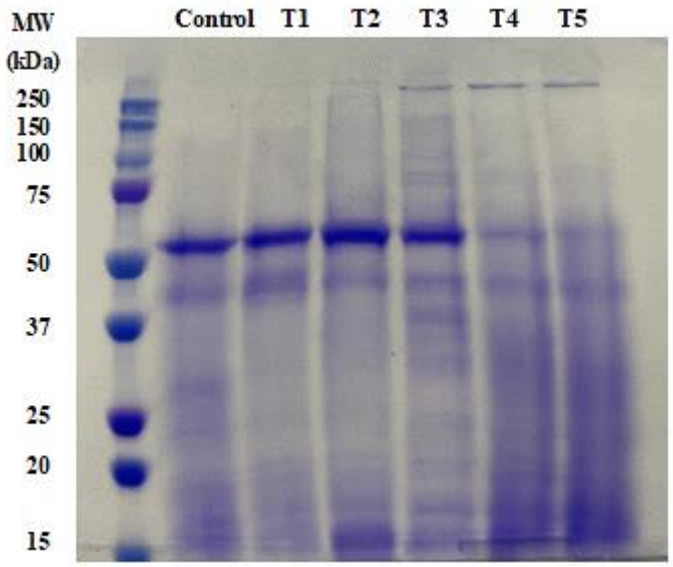


Fig. 4. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) patterns of spreadable liver sausages after pressure and proteolytic enzyme treatment. Control, without proteolytic enzyme; T1, proteolytic enzymes; T2, proteolytic enzymes and guar gum; T3, pressure-cooking; T4, proteolytic enzymes and pressure-cooking; T5, proteolytic enzymes, guar gum, and pressure-cooking.