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

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Abstract This study aimed to determine the effect of enzyme, 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 enzyme and guar gum; T3, pressure-cooking; T4, proteolytic enzyme and pressure-cooking; T5, proteolytic enzyme, 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 was the lowest. 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 treatments. 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, B₂, and B₁₂, 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 highly effective emulsifier for processing owing to its unique taste and technical function (Fischer, 1982; Hammer, 1982; Han et al., 2018).

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

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

Guar gum has a strong water-holding capacity and is used as a binder and lubricant for manufacturing sausage and stuffed meat products (Bakhsh et al., 2021). The addition of guar gum can contribute to quality improvement by stabilizing enzymatic treatment and improving the water holding capacity. Moreover, it has been reported that the interaction of proteins and polysaccharides improves the stability of enzyme (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 enzyme and guar gum), T3 (pressure cooking), T4 (proteolytic enzyme and pressure cooking), and T5 (proteolytic enzyme, 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 (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°C for 15 h. Subsequently, the first cooking was performed to stop the enzymatic reaction. The control, T1 and T2, were cooked at 80°C for 30 min using a water bath (JSR JSSB-30T, JS Research, Gongju, Korea), and the pressure treatments (T3, T4, and T5) were cooked at 110°C using an autoclave (Jeio Tech AC-13, JeioTech, 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. Maschinenfabrik KG, Witten, Germany) and then stuffed into the cellulose casing. After stuffing, the samples were cooked at 80°C for 30 min in a water bath (JSR JSSB-30T).

pH

The pH values were determined using a pH meter (Accumet Model AB15+, Fisher Scientific, Hampton, NH, USA) after homogenizing (8,000 rpm, 3 min) the sample (5 g) with distilled water (20 mL).

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-pickling salt.

Color

The color of liver sausage was measured using a colorimeter (CR-210, Minolta, Osaka, Japan). The values of CIE L* (lightness), CIE a* (redness), and CIE b* (yellowness) were measured (illuminant C). The colorimeter was calibrated with a white plate (L* = +97.83, a* = -0.43, b* = +1.98).

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 glass 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 fat and water separated by heating the glass tube at 75°C for 30 min, followed by cooling for 30 min (Choi et al., 2015).

Digestibility

The *in vitro* digestion of liver sausages was analyzed using the method of 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°C 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°C, 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°C 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-XT2i, Stable Micro Systems, Godalming, 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 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 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, 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°C for 5 min in a water bath and cooled at 25°C for 5 min. Then, 15 μL of each sample was injected into the well of 12% Mini-PROTEIN® TGXTM Precast Gels (Bio-Rad Laboratories, Hercules, CA, 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 Laboratories).

Statistical analysis

SPSS Statistics 20 software (IBM Corp., Armonk, NY, 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 enzyme 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

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 ^c | 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 enzyme; T2, proteolytic enzyme and guar gum; T3, pressure-cooking; T4, proteolytic enzyme and pressure cooking; T5, proteolytic enzyme, guar gum, and pressure cooking.

^{a-c} Values with different letters in the same row are significantly different ($p < 0.05$).

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 enzyme 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 enzyme (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

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 enzyme; T2, proteolytic enzyme and guar gum; T3, pressure-cooking; T4, proteolytic enzyme and pressure cooking; T5, proteolytic enzyme, guar gum, and pressure cooking.

^{a-c} Values with different letters in the same row are significantly different ($p < 0.05$).

pressurization treatment increased the moisture retention.

Apparent viscosity

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

Hardness

Sausage hardness indicates the degree of ripening due to the denaturation and gelation of meat proteins and loss of moisture (Gimeno et al., 2001). Enzyme and pressure processing can affect the hardness of spreadable liver sausages. The enzyme and pressure treatments led to lower hardness than the control, and T4 had the lowest hardness at 20,911.3 N/m² (Fig. 2). Pressure treatment induces a change in the muscle microstructure, myofibrillar contractions, fragmentation, and gelation

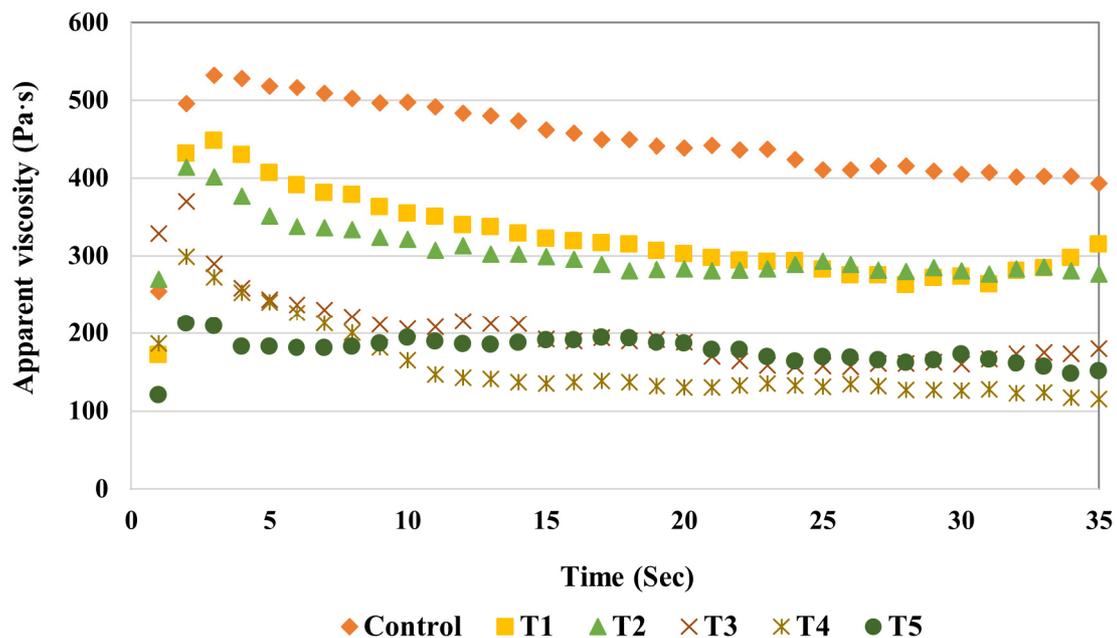


Fig. 1. Apparent viscosity of spreadable liver sausages after pressure and proteolytic enzyme treatment. Control, without proteolytic enzyme; T1, proteolytic enzyme; T2, proteolytic enzyme and guar gum; T3, pressure-cooking; T4, proteolytic enzyme and pressure cooking; T5, proteolytic enzyme, guar gum,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

of myofibril structural proteins that damage myofibers (Chen et al., 2014; Morton et al., 2017). Plant 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/m²; level 2 is food that can be eaten with gums and has a hardness of 50,000–22,000 N/m²; and level 3 is food that can be consumed with the tongue and has a hardness lower than 20,000 N/m² 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 enzyme 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 enzyme 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 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

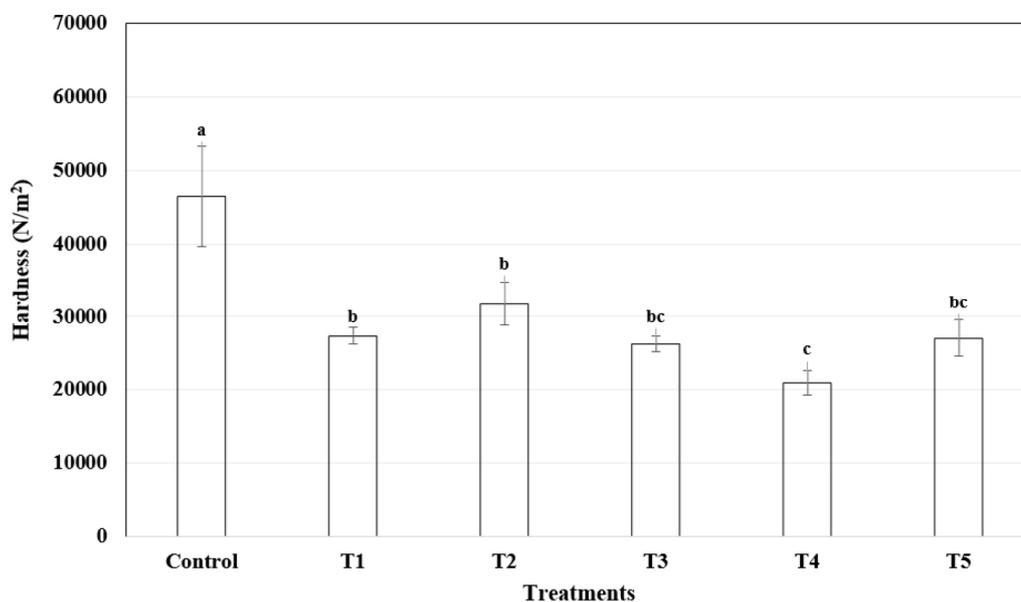


Fig. 2. Hardness of spreadable liver sausages after pressure and proteolytic enzyme treatment. Control, without proteolytic enzyme; T1, proteolytic enzyme; T2, proteolytic enzyme and guar gum; T3, pressure-cooking; T4, proteolytic enzyme and pressure-cooking; T5, proteolytic enzyme, guar gum, and pressure cooking. ^{a-c} Different letters above the bars indicate that the results are significantly different ($p < 0.05$).

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.

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

The SDS-PAGE results of the spreadable liver sausages with enzyme and pressure processing are shown in Fig. 4. The

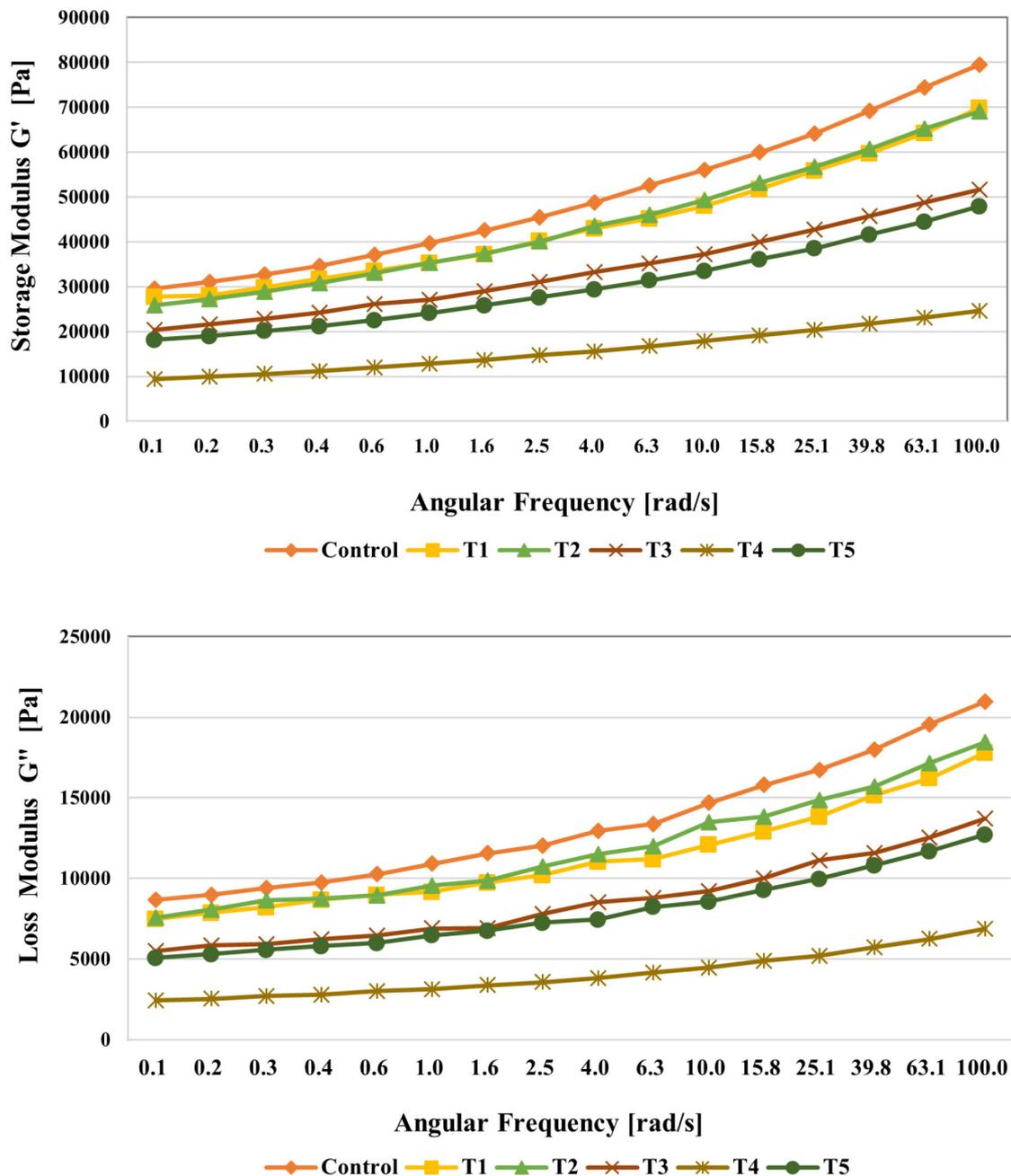


Fig. 3. Viscoelasticity of spreadable liver sausages after pressure and proteolytic enzyme treatment. Control, without proteolytic enzyme; T1, proteolytic enzyme; T2, proteolytic enzyme and guar gum; T3, pressure-cooking; T4, proteolytic enzyme and pressure-cooking; T5, proteolytic enzyme, guar gum, and pressure-cooking.

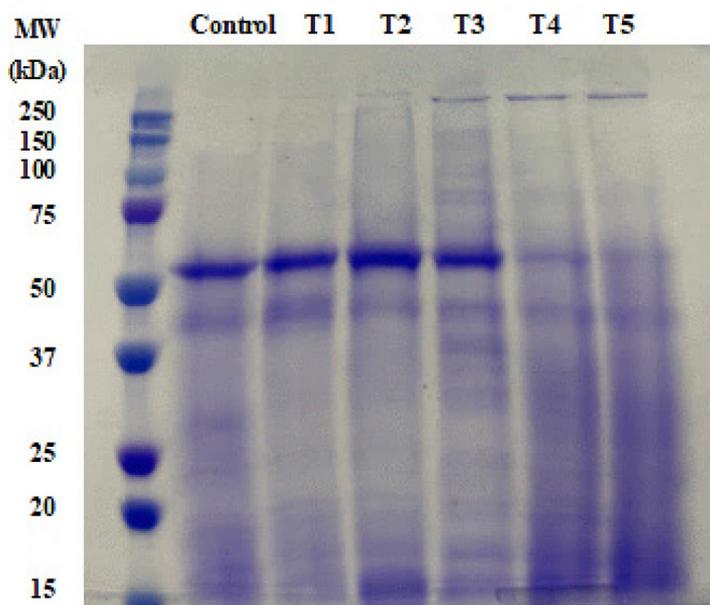


Fig. 4. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) patterns of spreadable liver sausages after pressure and proteolytic enzyme treatment. Control, without proteolytic enzyme; T1, proteolytic enzyme; T2, proteolytic enzyme and guar gum; T3, pressure-cooking; T4, proteolytic enzyme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

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 (Koochmaraie 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 (Chen et al., 2018; Pazos et al., 2014). 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°C–70°C, the quaternary structure of the protein is reversibly changed by unfolding, the disulfide bond is broken in the range of 70°C–80°C, and protein polymerization occurs at 90°C–100°C (Davis and Williams, 1998). Therefore, it was concluded that a polymer band formed because of protein polymerization because pressure treatment was conducted at 110°C.

Conclusion

In this study, combined enzyme and pressure processing was conducted to produce spreadable liver sausage with improved digestibility, and the effect of different treatments was evaluated. The enzyme and pressure treatments had higher pH and lower emulsion stability, viscosity, and hardness than the control. Treatments also decreased the viscoelasticity. As for digestibility, the enzyme and pressurized combination treatments led to higher digestibility than those in the control. Therefore, the results of this study suggest that enzyme and pressure are effective at tenderizing the physical properties of spreadable liver sausage, improving digestibility, and allowing their use to produce age-friendly foods.

Conflicts of Interest

The authors declare no potential conflicts of interest.

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Author Contributions

Conceptualization: Ku SK, Kim BK, Choi YS. Data curation: Ku SK, Kim BK, 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 BK, Choi YS. Writing - original draft: Ku SK, Kim BK, Choi YS. Writing - review & editing: Ku SK, Kim J, Kim SM, Yong HI, Kim BK, Choi YS.

Ethics Approval

This article does not require IRB/IACUC approval because there are no human and animal participants.

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