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TITLE PAGE
- Food Science of Animal Resources -
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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
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Special remarks – if authors have additional information to inform the editorial office	
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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.

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

14

15 **Abstract**

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

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

38 **Introduction**

39 The aging society significantly impacts the global food industry because sensory
40 perception and food preferences change with age (Zizza et al., 2007). Kim (2018)
41 reported that elderly people had problems with insufficient dietary intake and
42 malnutrition due to chewing difficulties. The food types that older people with
43 difficulties in chewing and swallowing can eat are limited. Accordingly, it has been
44 reported that the ratio of protein and lipid energy intake is lower in foods that are
45 difficult to chew than in foods that are easy to chew (Park et al., 2013). Many studies
46 have cited protein as an important nutrient for the elderly and reported that protein
47 intake could improve the rapid loss of muscle mass associated with aging (Morais et al.,
48 2006; Wolfe et al., 2008). Therefore, the adequate intake of easily digestible protein is
49 important for elderly individuals with muscle weakness, mastication, and dysphagia
50 (Gagaoua et al., 2021).

51 Additionally, a study on the exploration of the snacking behavior of the elderly for
52 the development of processed meat products showed that meat sticks and Chinese beef
53 jerky were difficult to consume because of their hard texture. However, prosciutto and
54 liver pâté were recognized as foods that could be eaten in special cases (Mena et al.,
55 2020). Spreadable meat products such as liver pâté and liver sausages have a high
56 nutritional value and density.

57 Nutritionally, the liver contains approximately 20% protein and is an excellent source
58 of many mineral substances, vitamins A, D, B₂, and B₁₂, and folic acid (Jayathilakan et
59 al., 2012). Therefore, liver products could be an excellent alternative to fresh meat
60 because they can provide high value-added nutrients in small amounts to the elderly
61 with dysphagia (Delgado-Pando et al., 2011). In addition, as an edible by-product, the
62 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 (Dransficee 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

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

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

93

94 **Materials and Methods**

95 *Spreadable liver sausage preparation and processing*

96 Spreadable liver sausages were prepared by referring to the methods of Choi et al.
97 (2019). Lean pork, back fat, duck liver, and duck skin were purchased from a local
98 market (Jeonju, Korea). Spreadable liver sausages were manufactured through
99 treatments involving the addition of a proteolytic enzyme and guar gum, and pressure-
100 cooking, as shown in Table 1 and as follows: Control (without proteolytic enzyme), T1
101 (proteolytic enzyme), T2 (proteolytic enzymes and guar gum), T3 (pressure cooking),
102 T4 (proteolytic enzyme and pressure cooking), and T5 (proteolytic enzymes, guar gum,
103 and pressure cooking). Spreadable liver sausage was prepared using the following
104 method. After each raw meat (lean pork, back fat, duck liver, and duck skin) was ground
105 through a \varnothing 6 mm plate using a meat chopper (SMC-22A, SL company, Incheon,
106 Korea), nitrite-pickling salt (NPS; salt/nitrite = 99.4:0.6) and plant protease (complex
107 seasoned food containing bromelain, tender enzyme S1, ES food, Gunpo, Korea) were
108 added at 4 °C for 15 h. Subsequently, the first cooking was performed to stop the
109 enzymatic reaction. The control, T1 and T2, were cooked at 80 °C for 30 min using a
110 water bath (JSR JSSB-30T, Gongju, Korea), and the pressure treatments (T3, T4, and
111 T5) were cooked at 110 °C using an autoclave (Jeio tech AC-13, Daejeon, Korea) at a
112 pressure of 0.06 MPa for 10 min. After adding ingredients to the cooked pork, back fat,

113 duck skin, and duck liver, they were mixed for 2 min in a silent cutter (Hermann
114 Scharfen GmbH & Co., Witten, Germany) and then stuffed into the cellulose casing.
115 After stuffing, the samples were cooked at 80 °C for 30 min in a water bath (JSR JSSB-
116 30T, Gongju, Korea).

117 *pH*

118 The pH was determined by mixing 5 g of sample with 20 mL of distilled water at
119 8,000 rpm (Ultra-Turrax, T25, Janke & Kunkel, Staufen, Germany) after
120 homogenizing the liver sausage for 3 min using a pH meter (Accumet Model AB15+,
121 Fisher Scientific, NH, USA).

122 *Color*

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

128 *Emulsion stability*

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

135 *Digestibility*

136 The *in vitro* digestion of liver sausages was carried out as described by as Lee et al.
137 (2020). The homogenate (4 mL) was treated with 10 mL of gastric digestive juice

138 (pepsin 182 unit/mg protein and gastric lipase 21 unit/mg protein dissolved in 0.15 M
139 NaCl, pH 1.8 with 0.1 M HCl) and digested at 37 °C for 2 h in a shaking water bath.
140 Duodenal fluid (10 mL) and bile fluid (5 mL) were added to the product of the gastric
141 phase, and digestion was performed under the same conditions as in the gastric phase.
142 The compositions of duodenal and bile fluids were as follows: duodenal fluid (trypsin
143 34.5 unit/mg protein, chymotrypsin 0.4 unit/mg protein, and pancreatic lipase 2,000
144 unit/mg protein dissolved in distilled water, pH 7.5 adjusted with 1 M NaOH), and bile
145 fluid (4 mM bile extract dissolved in distilled water, pH 7.5 adjusted with 1 M NaOH).
146 For the control, the same amount of distilled water and digestion solution were added
147 instead of the sample used during digestion. The digesta was stored at -70 °C, and the
148 protein content was determined using the Kjeldahl method (AOAC, 2000).

149 *Proximate composition*

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

153 *Apparent viscosity*

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

158 *Texture profile analysis*

159 The textural properties were analyzed using a texture analyzer (TA-XT2i, Stable
160 Micro Systems, Surrey, UK). The sample was placed in a container with a diameter of
161 40 mm and height of 20 mm, a probe (circular, 20 mm in diameter at the bottom) was
162 mounted, and compression was measured. Analytic conditions were determined by

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

165 *Viscoelasticity*

166 For the viscoelastic properties, the shear strain (1%) corresponding to the linear
167 viscosity range (LVR) was fixed, and a frequency sweep test was performed to measure
168 the storage modulus (G) and loss modulus (G'') according to the angular frequency
169 (0.1–100 rad/s).

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

171 The protein concentration was measured using the Bradford method (Kruger, 2009). A
172 sample aliquot of 50 μ L and 200 μ L of Bradford reagent (Sigma-Aldrich, St. Louis,
173 MO, USA) were mixed, and absorbance was measured at 595 nm using a
174 spectrophotometer (Optizen 2120 UV plus, Mecasys Co. Ltd., Daejeon, Korea). The
175 standard curve was calculated using bovine serum albumin obtained from Sigma-
176 Aldrich, and distilled water was used as the blank. The sample buffer was mixed with
177 20 μ g of the protein sample, and the protein to sample buffer was 3:1. The mixture was
178 heated at 100 °C for 5 min in a water bath and cooled at 25 °C for 5 min. Then, 15 μ L
179 of each sample was injected into the well of 12% Mini-PROTEIN® TGXTM Precast
180 Gels (Bio-Rad Lab, Inc., USA), and the Precision Plus Protein TM dual-color standard
181 presented standard molecular weight bands on the gel. After separation, the gel was
182 stained with Coomassie Brilliant Blue R250 (Bio-Rad Lab, Inc., USA).

183 *Statistical analysis*

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

188 **Results and Discussion**

189 *pH, color, emulsion stability, and digestibility*

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

206 The emulsion stability of the spreadable liver sausage was in the range of 12.21–
207 13.99%, with no statistical differences among different treatments, but it showed
208 relatively lower values during enzyme and pressure treatment compared to that in the
209 control (Table 2). When manufacturing ground meat products, the emulsification
210 capacity of meat proteins affects the degree of meat tenderness. This is because of the
211 correlation between the concentration of water-soluble proteins released into emulsion
212 and meat tenderness (Aminlari et al., 2009).

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

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

226 *Proximate composition*

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

240 *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/m² (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

266 (Maiti et al., 2008). In addition, the combined treatment with enzyme and pressure
267 improved the digestibility owing to the partial degradation of muscle protein (Ma et al.,
268 2019), consistent with the results of this study. The texture of the liver sausages
269 prepared in this study was analyzed according to the texture analysis method specified
270 in Korean Industrial Standard (KS) for aging-friendly food. The Korea Industrial
271 Standards and the Ministry of Food and Drug Safety have defined “age-friendly food”
272 and prepared specifications and standards. Korean industrial standards are classified
273 into three stages based on their physical properties. Level 1 is food that can be ingested
274 with teeth and has a hardness of 500,000–50,000 N/m²; level 2 is food that can be eaten
275 with gums and has a hardness of 50,000–22,000 N/m²; and level 3 is food that can be
276 consumed with the tongue and has a hardness lower than 20,000 N/m² and a viscosity of
277 1,500 mPa/s or higher (Korean Industrial Standards, 2017). As a result of this study, the
278 liver sausages treated with enzymes and pressure processing can be considered to be
279 products equivalent to level 2 age-friendly food.

280 *Viscoelasticity*

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

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

297 *SDS-PAGE*

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

312

313 **Conclusions**

314 In this study, combined enzyme and pressure processing was conducted to produce
315 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

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483 **Tables**

484 **Table 1.** Formulation of spreadable liver sausages by pressure and proteolytic enzyme
 485 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

486 NPS, nitrite-picked salt.

487 **Table 2.** pH, color, emulsion stability, and digestibility of spreadable liver sausages after
 488 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
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}
Color						
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

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

492 CIE L^{*}, lightness; CIE a^{*}, redness; and CIE b^{*}, yellowness.

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

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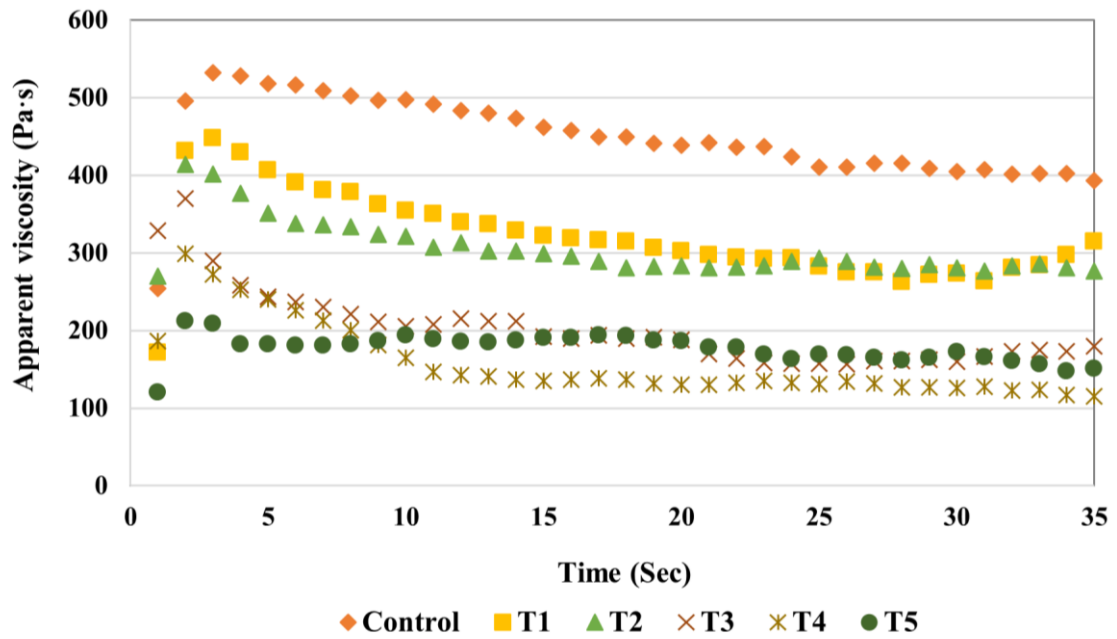
496 **Table 3.** Proximate composition (%) of spreadable liver sausages by pressure and
 497 proteolytic enzyme treatment.

	Control¹	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

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

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

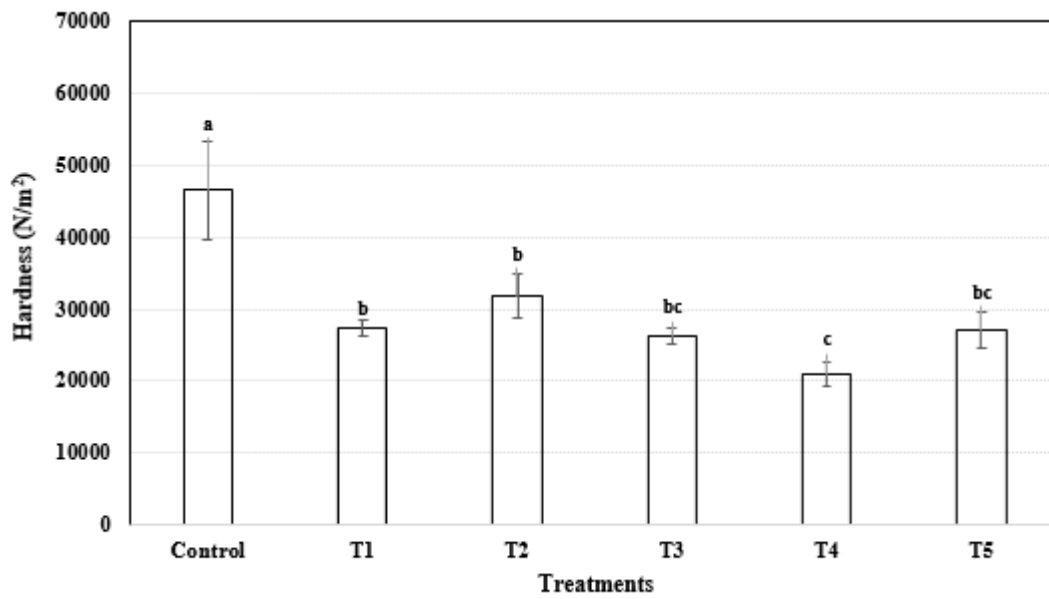
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505 **Fig. 1.** Apparent viscosity of spreadable liver sausages after pressure and proteolytic
 506 enzyme treatment. Control, without proteolytic enzyme; T1, proteolytic enzymes; T2,
 507 proteolytic enzymes and guar gum; T3, pressure-cooking; T4,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

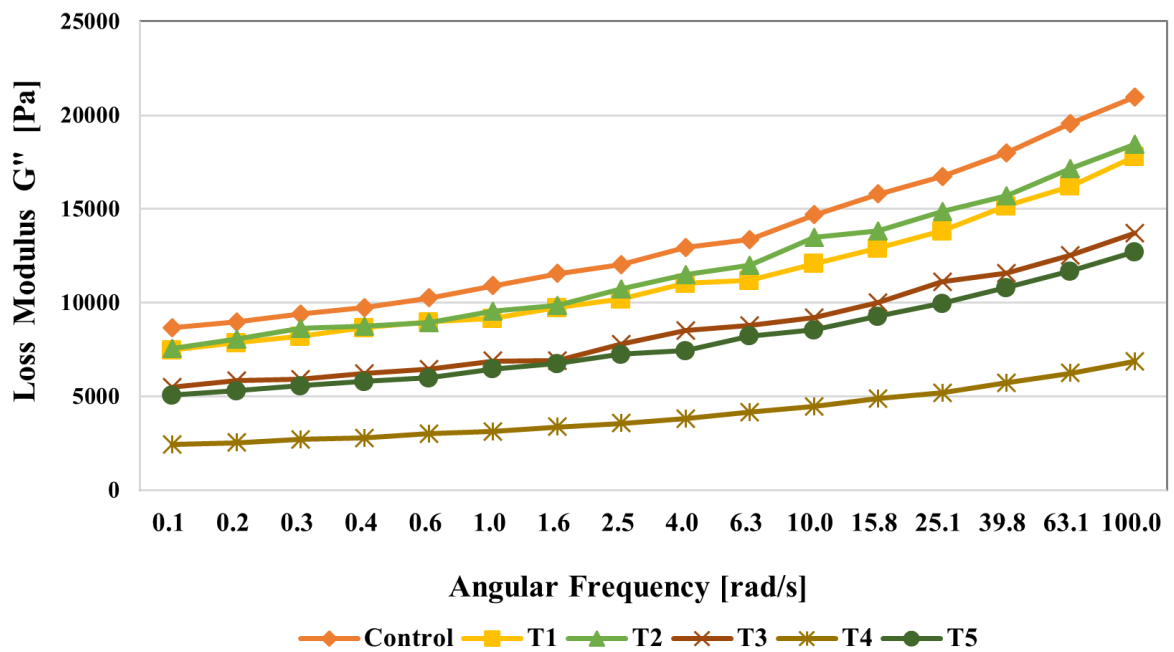
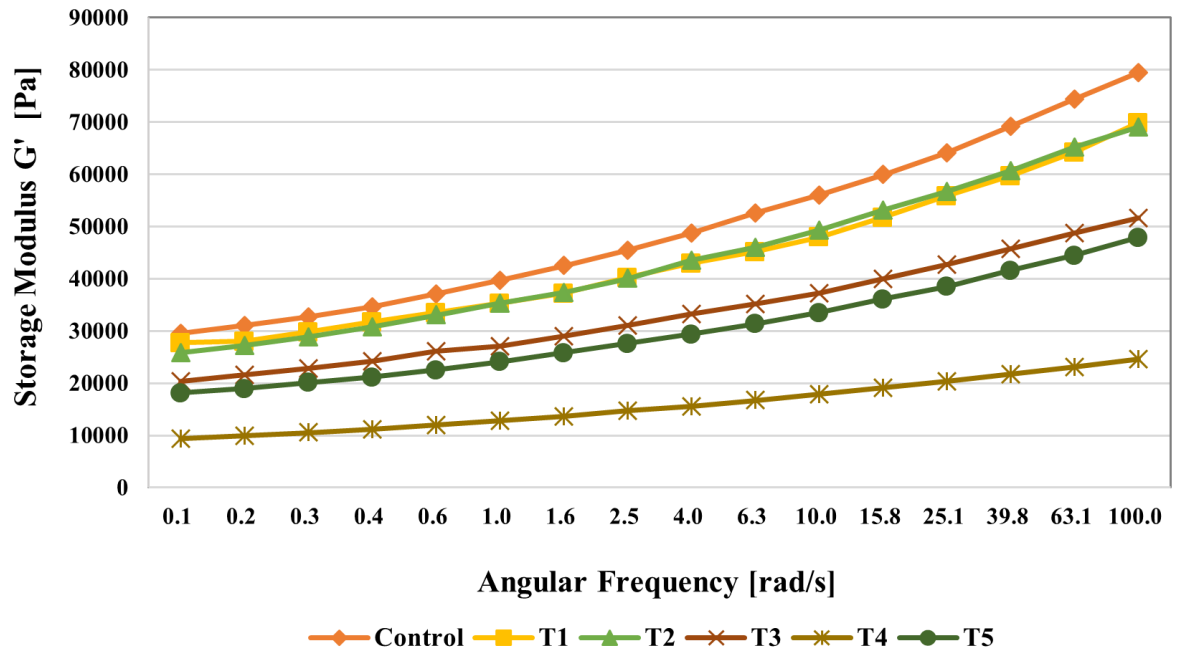
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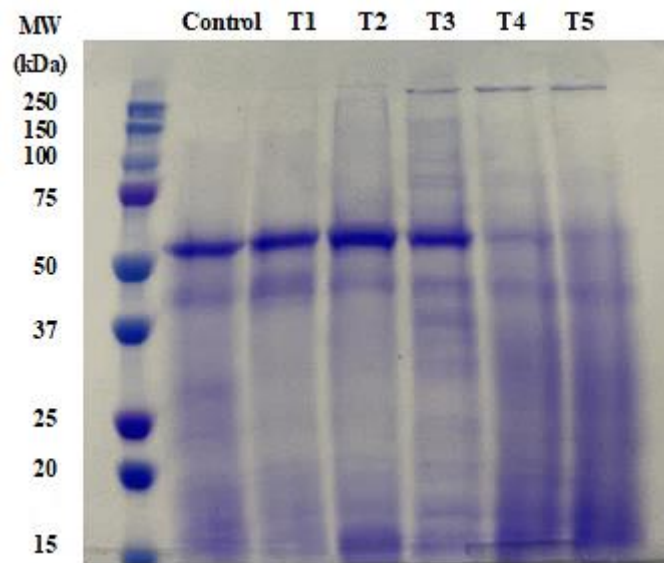
511 **Fig. 2.** Hardness of spreadable liver sausages after pressure and proteolytic enzyme
 512 treatment.

513 Control, without proteolytic enzyme; T1, proteolytic enzymes; T2, proteolytic enzymes
 514 and guar gum; T3, pressure-cooking; T4, proteolytic enzymes and pressure-cooking; T5,
 515 proteolytic enzymes, guar gum, and pressure cooking. ^{a-c} Different letters above the bars
 516 indicate that the results are significantly different ($P < 0.05$).

518



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.



525

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

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