

1
2
3
4

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	Digestibility and Physicochemical Characteristics of Blood Sausages Made with Korean Traditional Fermented Food
Running Title (within 10 words)	
Author	Su-Kyung Ku, Jake Kim, Yea-Ji 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 (https://orcid.org/0000-0002-9158-8254) Jake Kim (https://orcid.org/0000-0002-3016-7659) Yea-Ji Kim (https://orcid.org/0000-0003-0937-5100) Yun-Sang Choi (https://orcid.org/0000-0001-8060-6237)
Conflicts of interest List any present or potential conflicts of interest for all authors. (This field may be published.)	The authors declare no potential conflict of interest.
Acknowledgements State funding sources (grants, funding sources, equipment, and supplies). Include name and number of grant if available. (This field may be published.)	This work was supported by the Main Research Program(E0211200-04) of the Korea Food Research Institute (KFRI)funded by the Ministry of Science and ICT (Republic of Korea). This work was also partially supported by the High Value-added Food Technology Development Pro-gram (321053-5) of the Ministry of Agriculture, Food and Rural Affairs (Republic of Korea).
Author contributions (This field may be published.)	Conceptualization: Ku SK, Choi YS. Data curation: Ku SK, Choi YS. Formal analysis: Ku SK, Kim J, Kim YJ. Methodology: Ku SK, Kim J, Kim YJ. Validation: Choi YS. Investigation: Choi YS. Writing - original draft: Ku SK, Kim J, Kim YJ, Choi YS. Writing - review & editing: Ku SK, Kim J, Kim YJ, Choi YS. (This field must list all authors)
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.

5
6

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

7

8 **Physicochemical Characteristics and Protein Digestibility of Blood**

9 **Sausages Made with Korean Traditional Fermented Food**

11 **Abstract**

12 This study evaluated the quality characteristics of blood sausages where salt (NaCl) was
13 replaced with traditional Korean fermented foods. Five types of traditional fermented
14 materials were used as salt substitutes: *Doenjang* (T1), *Gochujang* (T2), *Cheonggukjang* (T3),
15 *Makjang* (T4), and Kimchi (T5). The salinity of each traditional fermented food material was
16 measured, and materials corresponding to a salt concentration of 1.5% were added. Blood
17 sausages containing these fermented foods had higher protein content and pH compared to the
18 control ($p<0.05$). The protein digestibility of blood sausages was improved by the using these
19 fermented foods ($p<0.05$). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
20 showed that the protein distribution in T3 had a higher amount of low-molecular-weight
21 proteins in the 25-50 kDa range compared to the other treatments. The hardness, gumminess,
22 and chewiness were the highest in the control and lowest in T3 ($p<0.05$). Springiness and
23 cohesiveness did not show significant differences among the treatments. The amino acid
24 composition showed no difference in total content ($p>0.05$), but Glu and Ser were higher in
25 the traditional fermented food-added sausage compared to the control ($p<0.05$). Apparent
26 viscosity and storage modulus (G'), of blood sausages with Korean traditional fermented
27 foods were lower than those of the control. Loss modulus (G'') and $\tan \delta$ tended to be lower in
28 all treatments compared to the control, except for T4. Therefore, traditional Korean fermented
29 foods are effective at tenderizing the physical properties and improving the protein
30 digestibility of blood sausages.

31 **Key words:** blood sausage, fermented food, protein digestibility, SDS-PAGE, texture profile
32 analysis

33 **Introduction**

34 Duck meat is a preferred protein source for consumers because of its excellent nutritional
35 value and flavor (Biswas et al., 2019; Ofori and Hsieh, 2014). With growth in the duck meat
36 industry, the production of byproducts during the slaughter process has also increased (Shim
37 et al., 2018). Maximizing the utilization of these duck by-products can reduce industrial
38 waste, efficiently utilizing the available proteins to minimize environmental pollution
39 (Jayathilakan et al., 2012; Wan et al., 2002). Blood is one of the first by-products produced
40 during the slaughter of livestock, consisting of approximately 17–18% protein and 75–82%
41 water, with hemoglobin accounting for approximately 70% of the total blood protein found in
42 red blood cells (Leoci 2014, Tarté 2011). Duck blood contains protein and iron, making it an
43 excellent source of protein for meeting the amino acid requirements of adults
44 (WHO/FAO/UNU 2007, Silva and Silvestre, 2003). Therefore, duck blood is a valuable
45 source of protein and iron. Additionally, blood plays a functional role in sausage production
46 by binding fats, pork, and various other ingredients, thereby maintaining the sausage shape
47 (Choi et al., 2009; Grasbeck et al., 1982). Currently, blood is widely utilized as a protein
48 source in various countries across Europe and Asia, and is used commercially in products,
49 such as blood sausages, black pudding, blood curd, and blood tofu (Lui, 2016). Blood
50 sausages are traditionally favored as meat products and consumed in various countries. The
51 ingredients used may vary depending on the production region (Stiebing, 1990; Dieza et al.,
52 2009).

53 Traditional Korean fermented foods are acidic foods that break large nutrient molecules into
54 smaller ones, promoting protein digestibility and enhancing flavor and texture.

55 Microorganisms involved in fermentation are beneficial and edible, producing hydrolytic
56 enzymes and other enzymes that break-down carbohydrates, lipids, and proteins (Park, 2012).

57 Fermentation is a biochemical reaction that converts complex organic substances into

58 relatively simpler ones, thereby enhancing the nutritional value of food and increasing the
59 absorption rate in the body. This process plays an important role in enhancing the functional
60 and nutritional characteristics of food (Hwang et al., 2017). Proteins or peptides with different
61 molecular weights exhibit different physicochemical properties (Sato et al., 1995).
62 Consequently, factors that influence protein interactions can also alter gel characteristics.
63 Traditional fermented foods contain proteolytic enzymes, which break down proteins into
64 low-molecular-weight amino acids (Ann, 2011). The application of fermented foods to blood
65 sausage could potentially interfere with the gelation of myofibrillar and blood proteins during
66 heating, resulting in lower gel strength. Lower gel strength may cause the product to
67 degradation more quickly during digestion, leading to higher protein digestibility. Therefore,
68 this study aimed to investigate how replacing salt with traditional fermented foods in the
69 production of blood sausages affects protein digestibility and physicochemical characteristics,
70 aiming to enhance the utilization of traditional fermented foods in meat products.

71
72

73 **Materials and Methods**

74 **Preparing and processing blood sausage using Korean traditional fermented food**
75 **materials**

76 Fresh pork ham, back fat, skin, and duck blood were purchased from a local market in
77 Jeonju, South Korea. Blood sausages containing traditional fermented food materials were
78 prepared by modifying the manufacturing methods described by Choi et al. (2009). The
79 control blood sausage was prepared by mixing 55% pork ham, 20% blood, 15% back fat, 5%
80 skin, 5% ice, and 1.5% salt. Five types of traditional fermented materials were used as salt
81 substitutes: *Doenjang* (T1), *Gochujang* (T2), *Cheonggukjang* (T3), *Makjang* (T4), and
82 Kimchi (T5). The salinity of each traditional fermented food material (T1: 7.7%, T2: 9.5%,
83 T3: 3.3%, T4: 6.8%, T5: 1.9%) was measured, and materials corresponding to a salt
84 concentration of 1.5% were added. Pork ham, back fat, and skin were chopped using a 6 mm
85 plate. Salt and traditional fermented materials (1.5% salinity and 0.01% nitrite) were added to
86 chopped pork ham, back fat, and skin. The mixture was then tumbled for 1 h and
87 subsequently heated for 30 min at 80 °C. The heated materials, along with blood, ice, and sub-
88 materials (0.8% sugar, 4.0% onion powder, 3.0% garlic powder, 0.7% ginger powder, 0.3%
89 black pepper, 9.7% monosodium glutamate, 0.7% carrageenan, and 2.0% isolated soy protein)
90 were combined in a silent cutter, mixed for 5 min, and stuffed into casings. Subsequently, the
91 sausages were heated for 30 min at 80 °C and then cooled to 4 °C.

92

93 **Proximate compositions**

94 The compositional properties of blood sausages containing traditional fermented food
95 materials were analyzed using the methods outlined by the AOAC (2000). The moisture
96 content was determined using a drying oven (AOAC 950.46B), protein content was measured
97 using the Kjeldahl method (AOAC 981.10), and fat content was assessed using the Soxhlet

98 method (AOAC 960.69). The ash content (AOAC 920.153) was quantified in a muffle
99 furnace.

100

101 **Amino acid profile**

102 To estimate the amino acid composition of the heat-induced mixed protein gel, a cooling gel
103 was prepared and analyzed using the method described by Fountoulakis and Lahm (1998).
104 Briefly, the amino acid composition of the hydrolyzed protein, processed under nitrogen using
105 6 M HCl, was analyzed using an L-8800 amino acid analyzer (Hitachi, Tokyo, Japan). The
106 hydrolysate was filtered using a 0.20 μm membrane filter and an ion-exchange resin column
107 (4.6 mm inside diameter \times 60 mm). The nutritional value, particularly the essential amino acid
108 index, of the gel was calculated according to the FAO/WHO/UNU (1985) guidelines.

109

110 **pH**

111 The pH was measured using a pH meter (Accumet Model AB15+; Fisher Scientific,
112 Hampton, NH, USA). For pH measurement, 5 g of the sample was homogenized with 20 mL
113 of distilled water at 8,000 rpm for 3 min.

114

115 **Color**

116 The color of the blood sausages was measured using a colorimeter (CR-210; Minolta, Osaka,
117 Japan). The CIE L* (lightness), CIE a* (redness), and CIE b* (yellowness) values were
118 measured thrice using illuminant C. The colorimeter was calibrated using a white plate ($L^* =$
119 $+97.83$, $a^* = -0.43$, $b^* = +1.98$).

120

121

122 **Protein digestibility**

123 *In vitro* digestion of blood sausages was performed as described by Lee et al. (2020). Blood
124 sausage sample (3 g) was mixed with distilled water (9 mL) and heated in a water bath at
125 80°C for 30 min. After cooling at room temperature (20°C), the mixture was then
126 homogenized at 13,000 rpm for 1 min. The homogenate (4 mL) was treated with 10 mL of
127 gastric digestive juice (pepsin = 182 units/mg protein and gastric lipase = 21 units/mg protein
128 dissolved in 0.15 M NaCl, pH = 1.8, adjusted with 0.1 M HCl) and digested at 37 °C for 2 h
129 in a shaking water bath. Duodenal (10 mL) and bile fluids (5 mL) were added to the material
130 from the gastric phase, and digestion was performed under identical conditions. The
131 compositions of the duodenal and bile fluids were as follows: duodenal fluid (trypsin = 34.5
132 units/mg protein, chymotrypsin = 0.4 units/mg protein, and pancreatic lipase = 2,000 units/mg
133 protein dissolved in distilled water, pH = 7.5, adjusted with 1 M NaOH) and bile fluid (4 mM
134 bile extract dissolved in distilled water, pH = 7.5, adjusted with 1 M NaOH). For the control,
135 the same amounts of distilled water and digestion solution were used instead of the sample
136 during digestion. The digesta after digestion was stored at -70 °C and stored until further
137 analysis and the protein content was determined by the Kjeldahl method (AOAC, 2000). The
138 digesta samples were fractionated based on size through filtration using centrifugal filters
139 (Amicon Ultra-15, Millipore, Billerica, MA, USA) with molecular weight cut-offs of 3 kDa.
140 The *in vitro* protein digestibility of the digesta after gastrointestinal digestion was calculated
141 using the following equation:

142 Protein digestibility (%) = (crude protein content of blood sausage before the digestion –
143 crude protein content of blood sausage after the digestion)/ crude protein content of blood
144 sausage before the digestion) *100%

145

146 **Texture profile analysis**

147 The textural properties were analyzed using a texture analyzer (TA-XT2i; Stable Micro
148 Systems, Surrey, UK) under the following conditions: maximum load = 2 kg, pre-test speed =
149 2.0 mm/s, post-test speed = 5.0 mm/s, force = $10 \times g$, distance = 8.0 mm, and head speed =
150 2.0 mm/s.

151

152 **Apparent viscosity**

153 The apparent viscosities of the samples were measured using a Brookfield viscometer (DV3T'
154 Brookfield, MA, USA). The emulsion temperature was maintained at 20 °C *via* distilled water
155 circulation using a refrigerated circulator bath (VB-07; UITECH, Suwon, Korea), and the
156 viscosity change was measured at 10 rpm for 30 s using an SC4-29 standard spindle.

157

158 **Dynamic viscosity**

159 The dynamic viscosities of blood sausages containing traditional fermented food materials
160 were measured using a Physica MCR 102 rheometer (Anton Paar Ltd., Graz, Austria). A flow
161 curve test was conducted using a parallel plate with a diameter of 25 mm and a gap of 1 mm.
162 The angular frequency ranged between 0.1–100 rad/s at a strain of 1%, with the temperature
163 maintained at 25 °C. Storage modulus (G'), loss modulus (G''), and loss factor ($\tan \delta = G''/G'$)
164 were recorded using RheoCompass v.1.19 software.

165

166 **SDS-PAGE**

167 Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed to
168 determine the distribution of proteins based on their molecular weight. The samples were
169 prepared at identical concentrations (1 mg/mL). The sample buffer was mixed with 20 μ g of
170 protein sample (Bio-Rad Lab, Inc., USA) at a ratio of 3:1 (protein to sample buffer). The

171 mixture was heated at 100 °C for 5 min, and subsequently cooled to 25 °C for 5 min. Protein
172 bands were separated using 12% Mini-PROTEIN® TGX™ Precast Protein Gels (Bio-Rad),
173 and Precision Plus Protein™ Dual Color Standards (Bio-Rad Lab) were used as standard
174 proteins. After electrophoresis, the gels were stained with Coomassie Brilliant Blue R-250 (Bio-
175 Rad).

176

177 **Statistical analysis**

178 The data were analyzed using SPSS Statistics 20 software (IBM Corp., Armonk, NY, USA).
179 One-way analysis of variance (ANOVA) with Duncan's range test was performed with the
180 significance level set at $p < 0.05$. Each experimental analysis was conducted twice with three
181 replicates.

182

183 **Results and Discussion**

184 **Proximate composition**

185 The proximate compositions of blood sausages containing traditional fermented food materials
186 are shown in Table 2. The moisture content was the highest in T3 (*Cheonggukjang*) at 61.50%
187 ($p < 0.05$), with no significant differences observed in other treatments. Arrese et al. (1991)
188 reported soybean protein exhibit a high water-holding capacity. Meanwhile the protein content
189 was higher in T3 compared to the other treatments. Therefore, it is thought that the high
190 moisture content in T3 was due to its higher protein content and water-holding capacity. The
191 protein content tended to be higher in sausages prepared with traditional fermented foods than
192 in the control. Lee (1973) reported that soybean, soybean paste, and red pepper paste contained
193 13.31–38.06% protein, and Kim et al. (1998) reported the presence of protein in Kimchi
194 ingredients. Additionally, Lee and Lyu (2008) found that the protein content increased with the
195 addition of *Cheonggukjang* powder to patties. The fat content was the lowest in T4 (*Magjang*)

196 at 11.93%, with no significant differences observed in other treatments (12.67–13.12%).
197 Sausages with added blood have a fat content of approximately 20% (Pereira, 2000; Santos,
198 2007; Santos et al., 2003).

199

200 **Amino acid profile**

201 The amino acid compositions of blood sausages made from traditional Korean fermented
202 foods are presented in Table 3. There was no significant difference in the total amino acid
203 content of blood sausages containing traditional fermented foods, but there was a difference in
204 the amino acid composition ($p < 0.05$). *Cheonggukjang* has been reported to generate amino
205 acids such as glutamic acid (Glu) and aspartic acid (Asp) through the breakdown of soybean
206 protein by *Bacillus spp.* during the fermentation process (Rozan et al., 2000). Glu and Asp
207 values were higher in T3 than in the control ($p < 0.05$), indicating the influence of
208 *Cheonggukjang* addition. Similarly, *Doenjang* and *Gochujang* have been reported to have
209 increased levels of Glu and Asp during fermentation, which is considered to have influenced
210 the blood sausage as well (Choi et al., 2000; Lee et al., 1980; Lee et al., 2012). *Kimchi* also has
211 relatively high levels of Glu in its amino acid composition (Woo et al., 2006). Therefore, adding
212 fermented foods could influence the amino acid composition of blood sausage due to the amino
213 acids contained in the fermented foods.

214

215 **pH and color**

216 The pH and color characteristics of the blood sausages containing traditional Korean
217 fermented foods are shown in Table 4. The pH showed no significant difference between the
218 control and the blood sausages with traditional fermented foods. Gašperlin (2014) reported that
219 the pH of blood sausages (Krvavica) in various regions ranged between 6.60–6.94, which is
220 slightly higher than the values observed in the present study. Lightness was the highest in T3

221 (*Cheonggukjang*) and lowest in the control ($p < 0.05$). Redness was prominent in T2 (*Gochujang*)
222 and T3, with no significant differences observed in other treatments. Yellowness was the
223 highest in T4 (*Makjang*) ($p < 0.05$), with no significant differences observed in other treatments.
224 The addition of blood to meat products results in a dark color because of the presence of
225 hemoglobin in the blood. Heat treatment of blood can affect the color because of the destruction
226 of heme, as reported by Oellingrath and Slinde (1985) and Schriket and Miller (1983). The
227 differences observed in the treatment groups in the present study can be attributed to the unique
228 color of traditional fermented foods.

229

230 ***In vitro* protein digestibility**

231 Table 4 shows the protein digestibility of blood sausages containing traditional Korean
232 fermented foods. The proteolysis of free amino acids and small peptides indicates their
233 absorption into the body, highlighting the importance of assessing the protein digestibility of
234 meat products to determine their health profile (Hsu et al., 1977). The protein digestibility of
235 sausages with traditional Korean fermented foods showed the highest in T3 (*Cheonggukjang*).
236 Protein digestibility increases with smaller particle size or higher low-molecular-weight
237 protein content (Sicard et al., 2018). SDS-Page suggests that T3 showed higher digestibility
238 because it contained more low-molecular-weight proteins. In addition, T3 exhibited lower gel
239 hardness and elasticity. During the gelation of proteins, a three-dimensional network of
240 polypeptides that can enclose water is formed. This process involves the stages of protein
241 denaturation and aggregation. Protein gelation is affected by various factors such as protein
242 concentration, temperature, pH, salt, or other additives (Opazi-Navarrete et al., 2018).
243 Changes in the form or structure of proteins affect the digestibility of meat proteins (Kaur et
244 al., 2014). These results suggest that fermented foods interfere with the gelation of
245 myofibrillar and blood proteins during the heating process, leading to rapid dissociation of the

246 product during digestion, thus increasing protein digestibility. Meanwhile, Gan et al. (2009)
247 reported that an improvement in gel strength leads to a decrease in digestibility, which is
248 consistent with the results of this study.

249

250 **Texture profile analysis**

251 Table 5 shows the texture analysis of blood sausages containing traditional fermented foods.
252 The hardness of the control was the highest at 2207.66 g, whereas the lowest value was observed
253 for T3 among the blood sausages with traditional Korean fermented foods ($p < 0.05$). Springiness
254 and cohesiveness did not show significant differences between the control and any of the
255 experimental samples, with values between 0.40–0.47 and 0.26–0.28, respectively. The
256 gumminess and chewiness of the control group were the highest, whereas those of the T3 group
257 were the lowest ($p < 0.05$). Although the texture index of blood sausages containing traditional
258 Korean fermented foods decreased, no significant difference was observed in cohesiveness,
259 indicating that the tightness of the bond between the components of the sausage was not
260 significantly affected. Koak et al. (2011) reported a reduction in breaking strength during a
261 study on the physical properties of sausages with the addition of a tenderizer (over-matured
262 fruits). Furthermore, several studies have shown tenderizing effects on physical properties, such
263 as reduction in hardness and shear force, by applying proteolytic enzymes to meat, which aligns
264 with the results of the present study (Choi et al., 1992; Han and Chin, 2004; Yang, 2006).

265

266 **Apparent viscosity**

267 The apparent viscosities of the blood sausages prepared using traditional fermented foods are
268 presented in Fig. 1. Blood sausages made with traditional fermented foods showed lower values
269 than the control; T3 (*Cheonggukjang*) and T1 (*Doenjang*) tended to have the lowest values.
270 Degradation of meat proteins increases the viscosity of meat emulsions because of an increase

271 in the number of degraded protein molecules (Richardson, 1977). However, in the present study,
272 the lower apparent viscosity of blood sausages prepared with traditional Korean fermented
273 foods compared to the control was attributed to the suppression of emulsification caused by the
274 application of these fermented foods.

275

276 **Dynamic viscosity**

277 The structural characteristics of blood sausages prepared from traditional Korean fermented
278 foods are shown in Fig. 2. Storage modulus (G'), loss modulus (G''), and $\tan \delta$ are indicators
279 used to explain the physical properties of viscoelastic materials. The storage modulus is a
280 value representing the elastic response of a material, indicating how elastically the material
281 reacts to external deformation forces. The higher the storage modulus, the stronger the
282 material's resistance to external deformation forces. The loss modulus represents the viscous
283 response of a material and indicates how viscously the material flows in response to external
284 strain. The higher the loss modulus, the more flexibly the material flows under external strain,
285 consuming more energy. $\tan \delta$ represents the relative relationship between the viscosity and
286 elasticity of a material. It is defined as the ratio of the loss modulus to the storage modulus
287 (Lee and Kim, 2017). Both storage modulus (G') and loss modulus (G'') exhibited an
288 increasing trend with increasing angular frequencies, indicating a similar trend across all
289 treatment groups. Over the entire frequency range, all the samples formed stable gel systems
290 without any crossover points. Storage modulus (G'), loss modulus (G''), and $\tan \delta$ were the
291 lowest in T2 (Gochujang), followed by T3, T5, and T1, with T4 and the control showing
292 similar values. The G' decreased for all treatments except 4 compared to the control,
293 indicating that the elasticity of the blood sausage gels decreased in the treatments added with
294 fermented products. Therefore, it is judged that the addition of fermented foods inhibited
295 emulsification and gel formation. $\tan \delta$, representing the relative ratio of storage modulus to

296 loss modulus, was consistently less than 1 for all treatments, indicating elastic properties
297 rather than viscous properties (Liu et al., 2019).

298

299 **SDS-PAGE**

300 The SDS-PAGE results for blood sausages prepared with traditional Korean fermented foods
301 are shown in Fig. 3. The protein degradation patterns of both the control and the groups treated
302 with traditional fermented foods showed a similar trend. Low-molecular-weight bands at
303 approximately 60 kDa and 10–15 kDa were observed for all samples. However, unlike the other
304 treatment groups, T3 exhibited bands in the 30–60 kDa range. The protein content of
305 *Cheonggukjang* is the highest among the added fermented foods (18.8%, Table 1). The
306 *Cheonggukjang* has been reported to have high proteolytic enzyme activity (Kwon et al., 2004),
307 and has also been found that higher crude protein content is associated with increased protease
308 activity (Baek et al. 2014). It is judged that the interaction between the proteins in the blood
309 sausage and the proteolytic enzymes in *Cheonggukjang* resulted in the presence of fractions in
310 the 30-60 kDa range, which were not observed in other treatments. While protein-degrading
311 enzymes derived from fruits exhibit high activity around 50 °C, the enzymes from traditional
312 fermentation agents have relatively high activity at higher temperatures, a finding corroborated
313 by the results of the present study (Yoo et al., 2013).

314

315 **Conclusion**

316 In this study, we evaluated the effects of applying traditional Korean fermented foods to blood
317 sausages on their protein digestibility and physicochemical properties. Blood sausages made
318 with traditional fermented foods exhibited improved digestibility and decreased gel hardness
319 and elasticity. Among them, the *Cheonggukjang* treatment exhibited more extensive
320 degradation into small molecular peptides and the highest protein digestibility. This is thought

321 to be the result of the inhibition of emulsification and gel formation of blood sausages due to
322 fermented foods. Therefore, the application of *Cheonggukjang* to blood sausage is expected to
323 contribute to the utilization of blood by-products and to have a positive effect on digestion and
324 absorption by degrading them into low molecular amino acids.

325

326 **Reference**

327 AOAC. 2000. Official Methods of Analysis (16th ed.). Washington, DC, USA: Association of
328 Official Analytical Chemist

329 Arrese EL, Sorgentini DA, Wagner JR, Anon MC. 1991. Electrophoretic, solubility and
330 functional properties of commercial soy protein isolates. *J Agric Food Chem* 39:
331 1029-1032.

332 Baek JE, Choi YH, Song J, Yun HT, Choi HS, Park SY. 2014. Physicochemical properties of
333 Chemggukjang with fermentation period for a variety of soybean cultivars. *Korean J*
334 *Food & Nutr* 27: 742-750.

335 Biswas S, Banerjee R, Bhattacharyya D, Patra G, Das A, Das S. 2019. Technological
336 investigation into duck meat and its products - a potential alternative to chicken.
337 *World's Poult Sci J* 75:609-620.

338 Choi C, Son GM, Cho YJ, Chun SS, Lim SI, Seok YR. 1992. Purification and characteristics
339 of bromelain from Korean pineapple. *J Korean Agric Chem Soc* 35: 23-29.

340 Choi JY, Lee TS, Noh BS. 2000. Quality characteristics of the kochujang prepared with
341 mixture of meju and koji during fermentation. *Korean J Food Sci Technol* 32:125-
342 131.

343 Choi YS, Choi JH, Han DJ, Kim HY, Lee MA, Kim HW, Kim CJ. 2009. Physicochemical
344 and sensory characterization of Korean blood sausage with added rice bran fiber.
345 *Food Sci Anim Resour* 29:260-268.

346 Dieza AM., Björkroth J, Jaimea I, Rovira J. 2009. Microbial, sensory and volatile changes
347 during the anaerobic cold storage of morcilla de Burgos previously inoculated with
348 *Weissella viridescens* and *Leuconostoc mesenteroides*. Int J Food Microbiol 131:
349 168-177.

350 Fountoulakis M, Lahm HW. 1998. Hydrolysis and amino acid composition analysis of
351 proteins. J Chromatogr A, 826:109-134.

352 Gan CY, Cheng LH, Azahari B, Easa AM. 2009. In-vitro digestibility and amino acid
353 composition of soy protein isolate cross-linked with microbial transglutaminase
354 followed by heating with ribose. Int J Food Sci Nutr 60:99-108.

355 Gašperlin L, Skvarča M, Žlender B, Lušnic M, Polak T. 2014. Quality assessment of
356 Slovenian Krvavica, a traditional blood sausage: sensory evaluation. J Food Process
357 Pres 38:97-105.

358 Grasbeck R, Majuri J, Kouvonen I, Thohunen R. 1982. Spectral and other studies on the
359 intestinal heme receptor of the pig. Protides Biol Fluids 29:467-470.

360 Han SK, Chin KB. 2004. Study on meat tenderness of a protease extracted from domestic
361 pear. Korean J Food Sci Ani Resour.24: 326-328.

362 Hsu HW, Vavak DL, Satterlee L, Miller GA. 1977. A multienzyme technique for estimating
363 protein digestibility. J food Sci 42:1269-1273.

364 Hwang YJ, Chae IS, Lee YK. 2017. Anti-inflammatory effects of fermented *Laminaria*
365 *japonica* and *Hizikia fusiforme* water extracts with probiotics in LPS-stimulated
366 RAW 264.7 macrophage cell line. J East Asian Soc Diet Life 27:1-8.

367 Jayathilakan K, Sultana K, Radhakrishna K, Bawa AS. 2012. Utilization of byproducts and
368 waste materials from meat, poultry and fish processing industries: a review. J Food
369 Technol 49:278-293.

370 Kaur L, Maudens E, Haisman DR, Boland MJ, Singh H. 2014. Microstructure and protein
371 digestibility of beef: The effect of cooking conditions as used in stews and curries.
372 LWT-Food Sci Technol 55:612-620.

373 Kim HJ, Lee JJ, Cheigh MJ, Choi SY. 1998. Amylase, Pretease, Peroxidase and Ascorbic
374 Acid Oxidase Activity of Kimchi Ingredients. Korean J Food Sci Technol 30:1333-
375 1338.

376 Kwon HY, Kim YS, Kwon GS, Kwon CS, Sohn HY. 2004. Isolation of immuno-stimulating
377 strain *Bacillus pumilus* JB-1 from Chungkook-jang and fermentational
378 characteristics of JB-1. Korean J Microbiol Biotechnol. 32: 291-296.

379 Koak JH, Baik MY, Kim BY. 2011. Rheological studies of the sausage added the over-
380 matured fruits tenderizer. Food Eng Prog 15:116-121.

381 Lee CH. 1973. Studies on the amino acid composition of Korean fermented soybean Meju
382 products and evaluation of the protein quality. Korean J Food Sci Technol 5:210-
383 214.

384 Lee KS, Chung DH. (1973). Effects of *Bacillus natto* on the soybean paste. Korean J Food Sci
385 and Technol 5:163-168.

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

388 Lee SY, Kim YN. 2017. Food rheology. Soohaksa. pp105-108.

389 Lee SY, Park NY, Kim JY, Choi HS. 2012. Quality characteristics of rice-doenjang during
390 fermentation by differently shaped meju and adding starter. The Korean J Food Nutr.
391 25:505-512.

392 Lee TS, Cho HO, Ryoou MK. 1980. Approach to the taste components of kochujang(part I),
393 Content of amino acids and other nitrogen compounds. Korean J Nutr 13:43-50

394 Lee YM, Lyu ES. 2008. Physico-chemical and sensory characteristics of *Chungkukjang*
395 powder added hamburger patty. Korean J food cook sci 24:742-747.

396 Leoci R. 2014. Animal by-products (ABPs): origins, uses, and European regulations.
397 Universitas Studiorum; Mantova, Italy: Chapter 4

398 Lui DC. 2016. Better utilization of by-product from the meat industry. Available from:
399 <http://www.agnet.org/library.php?func=view&id=20110706135001>/Accessed April
400 15, 2024.

401 Liu X, Ji L, Zhang T, Xue Y, Xue C. 2019. Effects of pre-emulsification by three food-grade
402 emulsifiers on the properties of emulsified surimi sausage. J Food Eng 247:30-37.

403 Oellingrath IM, Slinde E. 1985. Color, pigment and iron content of meat loaves with blood,
404 blood emulsion, or mechanically deboned meat added. J Food Sci 50:1551-1555.

405 Ofori JA, Hsieh YHP. 2014. Issues related to the use of blood in food and animal feed. Food
406 Sci Nutr 54:687-97.

407 Opazi-Navarrete M, Altenburg MD, Boom RM, Janssen AE. 2018. The effect of gel
408 microstructure on simulated gastric digestion of protein gels. Food Biophys, 13:124-
409 138.

410 Park KY. 2012. Increased health functionality of fermented foods. Food Industry and
411 Nutrition. 17(1): 1-8.

412 Pereira AD. 2000. Effect of blood treated with carbon monoxide on the colour and chemical
413 characteristics of mortadella. 117 f. Thesis (PhD in Food Science and Technology),
414 Federal University of Viçosa, Minas Gerais, Brazil.

415 Richardson T. 1977. Funcuionality changes in proteins following action of enzymes. In: RE
416 Feeney, JR Whitaker. Editors. Food Proteins 7:185-243.

417 Rozan P, Kuo YH, Lambein F. 2000. Free amino acids present in commercially available
418 seedlings sold for human consumption. A potential hazard for consumers. *J Agric*
419 *Food Chem* 48:716-723.

420 Santos REV. 2007. Physical, chemical, microbiological evaluation of mortadella formulated
421 with mixtures of pig blood and concentrate protein whey. 113f. Thesis (PhD in Food
422 Science and Technology), Federal University of Viçosa, Minas Gerais, Brazil.

423 Santos EM, Gonzáles-Fernández C, Jaime I, Rovira J. 2003. Physicochemical and sensory
424 characterization of Morcilla de Burgos, a traditional Spanish blood sausage. *Meat Sci*
425 65:893-898.

426 Sato KI, Sato A, Aoto M, Fukami Y. 1995. C-Src phosphorylates epidermal growth factor
427 receptor on tyrosine 845. *Biochem Biophys Res Commun* 215:1078-1087.

428 Sicard J, Mirade PS, Portanguen S, Clerjon S, Kondjoyan A. 2018. Simulation of the gastric
429 digestion of proteins of meat bolus using a reaction–diffusion model. *Food*
430 *Funct* 9:6455–6469.

431 Shim JY, Kim TK, Kim YB, Jeon KH, Ahn KI, Paik HD, Choi YS. 2018. The ratios of pre-
432 emulsified duck skin for optimized processing of restructured ham. *Korean J Food*
433 *Sci Anim Resour* 38:162-171.

434 Silva VDM, Silvestre MPC. 2003. Functional properties of bovine blood plasma intended for
435 use as a functional ingredient in human food. *LWT - Food Sci Technol* 36:709-718.

436 Stiebing A. 1990. Blood sausage technology. *Fleischwirtschaft*. 70: 424-428.

437 Tarté R. 2011. Meat protein ingredients In: Phillips G. O., Williams P. A., editors. *Handbook*
438 *of food proteins*. Woodhead Publishing; Cambridge, UK. pp 56–91.

439 Wan Y, Ghosh R, Cui Z. 2002. High-resolution plasma protein fractionation using
440 ultrafiltration. *Desalination*, 144:301-306.

- 441 WHO/FAO/UNU Expert Consultation. 2007. Protein and amino acid requirements in human
442 nutrition. World Health Organ Tech Rep Ser 935:1-265.
- 443 Woo SM, Jeong YJ, Whang K. 2006. Effect of germinated brown rice extract powder on free
444 amino acid content, antioxidant and nitrite scavenging ability of the Korean cabbage
445 kimchi. Korean J Food Preser 13(5):548-554.
- 446 Yang CY. 2006. Physicochemical properties of chicken jerky with pear, pineapple and kiwi
447 extracts. Korean J Culinary Res 12:237-250.
- 448 Yoo SA, Seo SH, Hyun SY, Son HS. 2013. Characteristics of crude protease from fruits and
449 traditional Korean fermentation starters. J Korean Soc Food Sci Nutr 42:1461-1466.

ACCEPTED

450 Table 1. Proximate compositions of Korean traditional fermented food

	T1	T2	T3	T4	T5
Moisture	50.77±0.19 ^b	39.67±0.26 ^c	51.14±0.44 ^b	51.02±0.25 ^b	87.82±0.27 ^a
Protein	11.08±0.75 ^b	4.95±0.01 ^d	18.81±0.37 ^a	9.57±0.16 ^c	2.01±0.00 ^e
Fat	0.26±0.00 ^c	0.30±0.15 ^c	8.63±0.41 ^a	2.31±0.76 ^b	0.36±0.06 ^c
Ash	12.04±0.08 ^a	7.40±0.09 ^b	4.76±0.31 ^d	6.68±0.23 ^c	2.10±0.11 ^e

451 ¹T1: *Doenjang*, T2: *Kochujang*, T3: *Cheonggukjang*, T4: *Magjang*, T5: *Kimchi*

452 ^{a-c} Different letters within a row are significantly different (p<0.05).

453 Table 2. Proximate compositions of blood sausages made with Korean traditional fermented
 454 food

	Control ¹	T1	T2	T3	T4	T5
Moisture	60.87±0.18 ^b	60.53±0.39 ^b	60.55±0.09 ^b	61.50±0.24 ^a	60.82±0.22 ^b	60.93±0.22 ^b
Protein	17.23±0.10 ^c	18.09±0.13 ^{ab}	17.62±0.24 ^{abc}	17.44±0.35 ^{bc}	18.17±0.57 ^{ab}	18.26±0.04 ^a
Fat	13.12±0.00	12.79±0.38	12.88±0.26	12.67±0.37	11.93±0.30	12.88±0.18
Ash	1.28±0.02	1.32±0.05	1.31±0.06	1.32±0.18	1.37±0.10	1.27±0.17

455 ¹Control: Salt, T1: *Doenjang*, T2: *Kochujang*, T3: *Cheonggukjang*, T4: *Magjang*, T5: *Kimchi*
 456 ^{a-c} Different letters within a row are significantly different (p<0.05).

Table 3. Amino acid profile of blood sausages made with Korean traditional fermented food (Unit: mg/100 g)

	Control ¹	T1	T2	T3	T4	T5
Essential amino acid (EAA)						
His	674.79±2.85	660.91±4.17	669.85±28.65	683.74±10.60	685.96±3.38	680.40±6.23
Ile	618.84±16.91 ^{ab}	610.65±17.77 ^{ab}	594.58±33.09 ^{ab}	660.42±32.99 ^a	562.74±28.45 ^b	621.99±25.73 ^{ab}
Leu	1,371.23±33.81	1,383.53±11.50	1,389.9±59.65	1,450.14±38.86	1,417.50±12.17	1,421.08±1.57
Lys	1,429.13±11.11 ^b	1,421.03±12.84 ^b	1,429.34±71.32 ^b	1,487.19±25.20 ^{ab}	1,444.55±21.56 ^b	1,529.98±2.29 ^a
Met	255.64±57.15 ^b	335.36±8.57 ^{ab}	334.25±41.73 ^{ab}	319.61±40.26 ^{ab}	358.49±15.91 ^a	355.71±4.83 ^a
Phe	770.58±14.18	761.17±6.51	769.71±38.67	791.46±14.93	766.49±7.53	779.37±1.55
Tyr	346.34±25.34 ^c	397.76±0.02 ^b	390.31±25.18 ^{bc}	413.96±15.45 ^{ab}	448.01±15.85 ^a	432.13±17.88 ^{ab}
Thr	735.46±5.43	737.67±6.09	748.97±39.00	775.28±7.59	769.85±7.85	769.28±12.80
Val	913.74±9.33 ^a	880.40±17.94 ^{ab}	858.94±43.04 ^{ab}	894.03±26.92 ^a	795.67±42.58 ^b	868.98±42.45 ^{ab}
Sum of EAA	7,115.75±165.24	7,188.48±68.24	7,185.25±380.32	7,475.82±132.28	6,613.42±152.20	7,458.93±36.57
Non-essential amino acid						
Ala	1,058.22±2.05	1,059.69±4.64	1,111.37±64.27	1,059.50±10.61	1,078.18±16.38	1,085.12±12.95
Arg	1,097.71±2.29	1,120.42±1.84	1,148.35±36.11	1,127.29±18.02	1,129.73±31.47	1,141.98±1.78

Asp	1,633.15±20.62	1,623.67±9.24	1,659.17±58.91	1,691.13±22.29	1,684.31±2.08	1,686.49±16.69
Glu	2,981.45±11.88 ^b	3,011.36±12.46 ^{ab}	3,038.26±37.34 ^{ab}	3,068.60±44.40 ^a	3,0771.47±29.74 ^a	3,072.99±25.45 ^a
Pro	520.63±30.46	531.00±2.13	624.78±150.41	527.45±9.02	547.55±66.30	592.70±26.65
Gly	946.76±27.16	1,016.91±27.36	1,112.22±339.55	860.98±6.96	916.85±50.97	959.32±27.27
Ser	689.66±4.19 ^c	717.89±5.76 ^b	740.98±5.35 ^{ab}	732.09±8.51 ^{ab}	761.42±0.29 ^a	738.86±25.27 ^{ab}
Sum of total AA	8,927.59±33.65	9,080.93±20.04	9,435.13±488.75	8,623.49±94.35	8,745.74±158.23	9,277.44±136.06

457 ¹Control: Salt, T1: *Doenjang*, T2: *Kochujang*, T3: *Cheonggukjang*, T4: *Magjang*, T5: *Kimchi*

458 ^{a,b} Different letters within a row are significantly different (p<0.05)

459 Table 4. pH color and digestibility of blood sausages made with Korean traditional fermented
 460 food

	Control ¹	T1	T2	T3	T4	T5	
pH	6.29±0.01	6.30±0.02	6.31±0.01	6.29±0.02	6.31±0.02	6.32±0.02	
L	35.27±0.75 ^d	36.58±0.22 ^{ab}	35.82±0.51 ^{cd}	37.06±0.57 ^a	36.33±0.36 ^{bc}	36.31±0.54 ^{bc}	
Color	a	16.13±0.92 ^c	15.78±0.91 ^c	17.56±0.85 ^{ab}	17.76±0.36 ^a	16.42±0.76 ^c	16.65±0.73 ^{bc}
	b	10.17±0.75 ^b	10.31±0.30 ^{ab}	10.53±0.43 ^{ab}	10.72±0.31 ^{ab}	10.87±0.50 ^a	10.53±0.42 ^{ab}
Digestibility (%)	96.31±0.01 ^c	96.66±0.02 ^b	96.79±0.04 ^{ab}	96.86±0.14 ^a	96.81±0.11 ^{ab}	96.83±0.01 ^{ab}	

461 ¹Control: Salt, T1: *Doenjang*, T2: *Kochujang*, T3: *Cheonggukjang*, T4: *Magjang*, T5: *Kimchi*

462 ^{a-d} Different letter within a row are significantly different (p<0.05)

463 Table 5. pH color and digestibility of blood sausages made with Korean traditional fermented food

	Control ¹	T1	T2	T3	T4	T5
Hardness (Kg)	2.21±0.45 ^a	2.03±0.28 ^{ab}	2.16±0.34 ^a	1.85±0.18 ^b	2.03±0.30 ^{ab}	1.91±0.22 ^b
Springiness	0.47±0.08	0.43±0.12	0.40±0.09	0.40±0.08	0.42±0.09	0.41±0.09
Cohesiveness	0.28±0.03	0.26±0.03	0.27±0.03	0.28±0.02	0.27±0.03	0.27±0.02
Gumminess (g)	619.88±114.06 ^a	527.58±100.21 ^b	577.87±116.99 ^{ab}	508.83±51.20 ^b	545.37±124.88 ^{ab}	508.99±76.51 ^b
Chewiness (g)	298.36±109.40 ^a	239.02±125.44 ^{ab}	237.53±83.69 ^{ab}	205.62±43.13 ^b	234.69±92.06 ^{ab}	209.92±75.03 ^b

464 ¹Control: Salt, T1: *Doenjang*, T2: *Kochujang*, T3: *Cheonggukjang*, T4: *Magjang*, T5: *Kimchi*

465 ^{a,b} Different letters within a row are significantly different (p< 0.05)

Figure Legends

Fig. 1. Apparent viscosity of blood sausages made with Korean traditional fermented food

Control: Salt; T1: *Doenjang*; T2: *Kochujang*; T3: *Cheonggukjang*; T4: *Magjang*; T5: *Kimchi*

^{a-d}Values with different letters are significantly different at the 5% level

Fig. 2. Dynamic viscosity of blood sausages made with Korean traditional fermented food

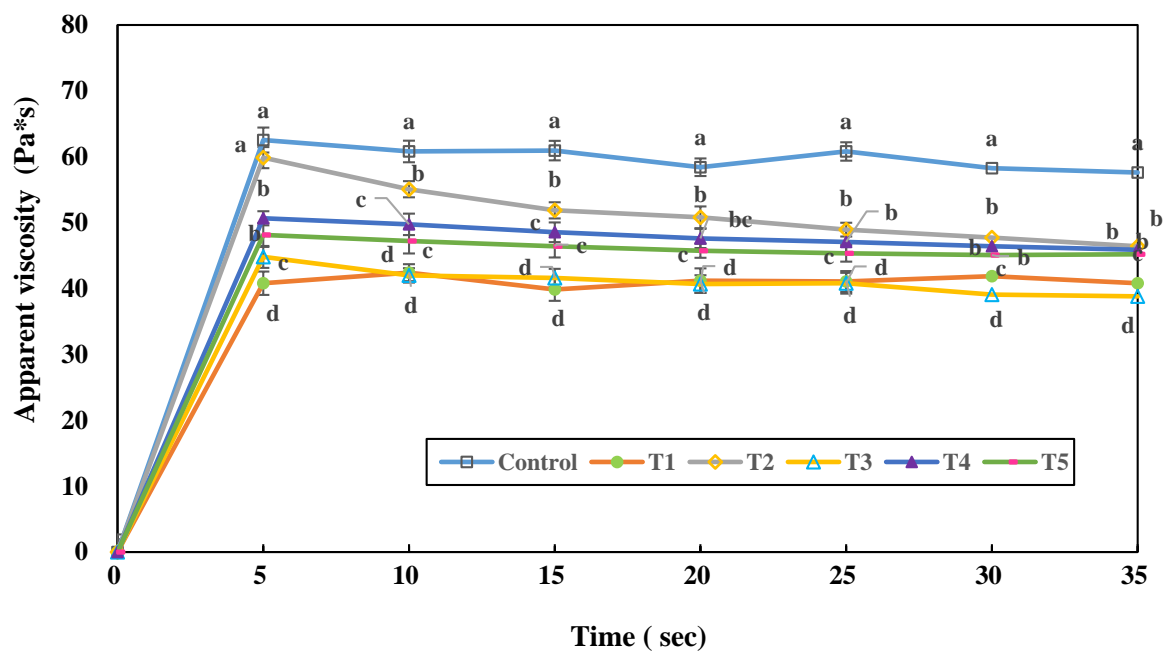
Control: Salt; T1: *Doenjang*; T2: *Kochujang*; T3: *Cheonggukjang*; T4: *Magjang*; T5: *Kimchi*

^{a,b}Values with different letters are significantly different at the 5% level

Fig. 3. SDS-page of blood sausages made with Korean traditional fermented food

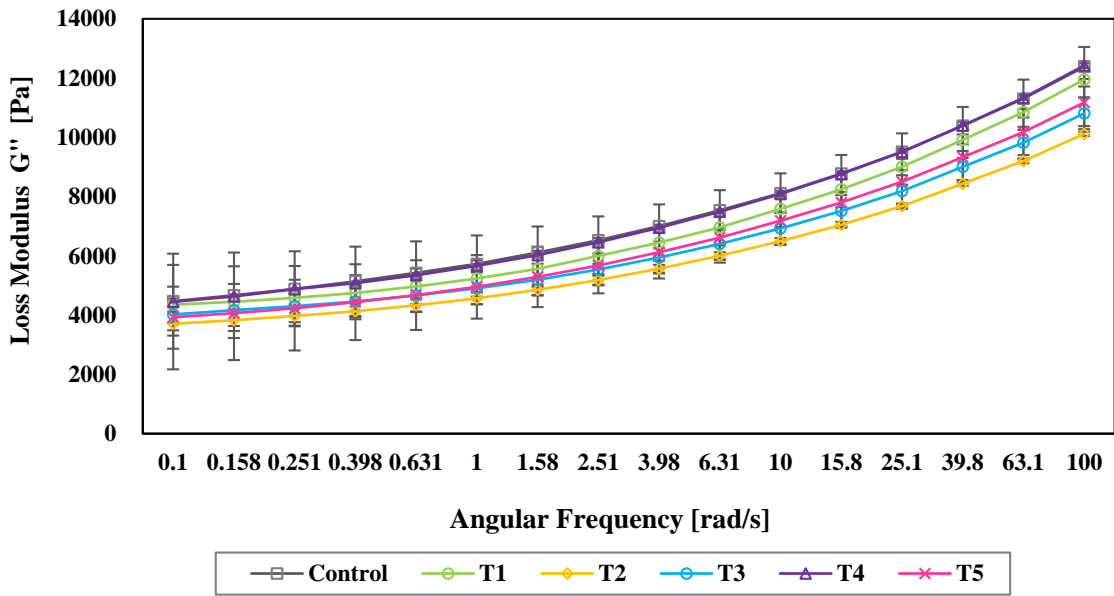
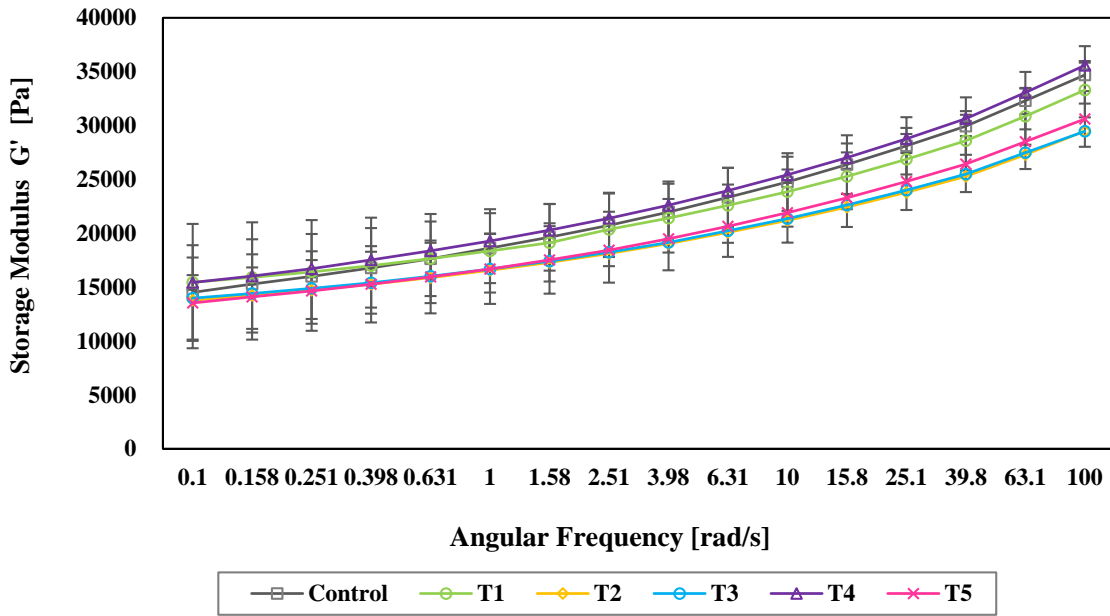
Control: Salt; T1: *Doenjang*; T2: *Kochujang*; T3: *Cheonggukjang*; T4: *Magjang*; T5: *Kimchi*

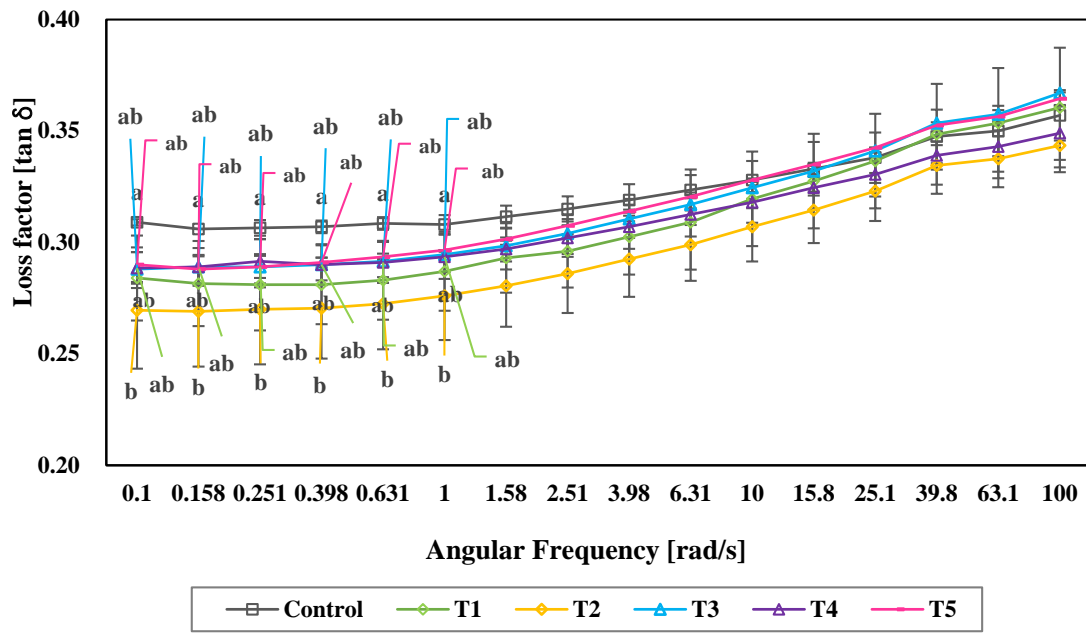
Fig. 1



ACCEPTED

Fig. 2





ACCEPTED

Fig. 3

