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Article Title	Influences of red pepper seed powder on the physicochemical properties of a meat emulsion model system
Running Title (within 10 words)	Effects of by-product from red pepper powder on meat emulsion
Author	Jeong-A Lee[†], Gye-Woong Kim[†], Hack-Youn Kim*, Juhui Choe*
Affiliation	Department of Animal Resources Science, Kongju National University, Yesan, Chungnam, 32439, Korea
Special remarks – if authors have additional information to inform the editorial office	*These authors contributed equally to this work as joint corresponding author. [†]These authors contributed equally to this work as joint first author.
ORCID (All authors must have ORCID) **https://orcid.org**	Jeong-A Lee (0000-0003-3019-8321) Gye-Woong Kim (0000-0001-7325-9898) Hack-Youn Kim (000-0001-5303-4595) Juhui Choe (0000-0003-4585-0327)
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9 **CORRESPONDING AUTHOR CONTACT INFORMATION**

For the <u>corresponding</u> author (responsible for correspondence, proofreading, and reprints)	Fill in information in each box below
First name, middle initial, last name	**Hack-Youn Kim**
Email address – this is where your proofs will be sent	kimhy@kongju.ac.kr
Secondary Email address	
Postal address	Department of Animal Resources Science, Kongju National University, Yesan, Chungnam, 32439, Korea
Cell phone number	+82-10-8727-2928
Office phone number	+82-41-330-1241
Fax number	
First name, middle initial, last name	**Juhui Choe**
Email address – this is where your proofs will be sent	cjh1641@gmail.com
Secondary Email address	
Postal address	Department of Animal Resources Science, Kongju National University, Yesan, Chungnam, 32439, Korea
Cell phone number	+82-10-5125-9984
Office phone number	+82-41-330-1165
Fax number	

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11

12   **Influences of red pepper seed powder on the physicochemical properties of**

13   **a meat emulsion model system**

14

15   **Abstract**

16   Red pepper seed (RPS) is commonly removed during the production of red pepper powder,

17   which is contains large amounts of dietary fibers and is abundant in nutrients, readily available.

18   In this study, we determined the effects of adding RPS powder on the physicochemical

19   properties of emulsified meat products. Meat emulsion samples were prepared with pork hind

20   leg meat (60%) and back fat (20%), iced water (20%), various additives, and RPS powder at

21   different concentrations [0 (control), 1, 2, 3, and 4%]. For the physicochemical properties,

22   moisture and fat content, pH value, color, emulsion stability, cooking yield, appearance

23   viscosity, and textural properties were examined. Addition of RPS induced significantly higher

24   values in moisture content, pH, cooking yield, and a* values of the meat emulsion samples,

25   regardless of the amount added. However, lower values were obtained for emulsion stability,

26   cooking yield, and viscosity in samples with RPS powder at 3 or 4% among all groups. In

27   general, addition of RPS powder at 1 and 2% led to the greatest values in viscosity of the meat

28   emulsion samples. Higher values ($p<0.05$) in hardness and springiness were observed in

29   samples with RPS powder at 4% and 3%, respectively. For gumminess, chewiness, and

30   cohesiveness, the addition of RPS powder at 1, 2, and 3% induced the highest values ($p<0.05$)

3

31   in the meat emulsion samples. These results showed that addition of RPS powder at optimum

32   levels (2%) could be utilized to improve quality properties of emulsified meat products as a

33   non-meat ingredient.

34

37

Introduction

Red pepper (*Capsicum annuum* L.) is one of the most commonly consumed spices in Asian cuisine. In Korea, the red pepper is mostly consumed as a dried powder and the consumption per capita of which reached 3.7 kg in 2015 (KREI, 2019). In general, the seed of the red pepper is mostly removed prior to drying as a high seed content causes a deterioration in redness and in the flavor of the red pepper powder (Ku et al., 2008). However, the red pepper seed (RPS) contains 40-65% dietary fiber (Lee and Kim, 2016) and has both antioxidant and antimutagenic activity (Sim and Han, 2007).

Dietary fiber is used in processed meat products, attributing both health benefits for the consumer and desirable qualities for the products including water holding/binding capacity, oil holding ability, and textural or sensory properties (Rafiq et al., 2018). In particular, viscosity is an important parameter in emulsified meat products due to its close relationship with water holding or retention capacity as measured by internal friction or resistance to flow (Bourne, 2002). Ding et al. (2018) observed that the addition of 1% chia seed powder exhibited enhanced the water holding capacity, presented lower purge and centrifugation loss, and had similar textural properties by panel compared to samples without. In addition, Kaur et al. (2015) found that chicken nuggets containing pomegranate seed powder exhibited desirable sensory properties but with no change in emulsion stability or moisture content. In another study, the addition of pomelo peel to chicken emulsion-type sausages led to remarkable increases in

5

57 viscosity, emulsion stability, and moisture content in comparison to those without

58 (Srithongkerd et al., 2018). As mentioned above, many authors tried to incorporate with dietary

59 fiber obtained from agricultural by-products into meat products for enhancement in

60 physicochemical properties (Choe et al., 2017; Selani et al., 2016). While many of these studies

61 examined the effects of RPS powder on restructured or emulsified meat products for

62 improvement of physiochemical properties, to our knowledge, rheological characteristics

63 including emulsion stability and/or viscosity were not determined (Kim et al., 2013; Lee et al.,

64 2016a; Lee et al., 2016b). Hence, the aim of this study was to determine the effects of RPS

65 powder on the physicochemical properties of a meat emulsion model, including moisture and

66 fat contents, pH values, instrumental color, cooking yield, emulsion stability, viscosity, and

67 textural properties.

68

69   **Materials and methods**

70 **Preparation of RPS powder and meat emulsion samples**

71   The RPS powder was purchased from a local market (Chungnam, Korea), sifted through a

72 35-mesh sieve, and stored at −20°C until use.

73   To prepare the meat emulsion sample,   the hind leg meat and back fat from pork were

74 first purchased from a local butcher (Hongju meat Co., Chungnam, Korea). All subcutaneous

75 and intramuscular fat and visible connective tissues were removed from the pork muscle; these

76 were then cut and ground using an 8-mm plate grinder (PA-82, Mainca, Barcelona, Spain).

77 Next, the ground meat (60%) was mixed with back fat (20%), iced water (20%), nitrite

78 pickling salt (1.2%; nitrite content: 6,000 ppm), phosphate (0.3%), sugar (1%), mixed spice

79 (0.6%; Bockworst, Raps GmbH & Co., Krombacher, Germany), and RPS powder at different

80 concentrations [0 (control), 1, 2, 3, and 4%] in a silent cutter (K-30, Talsa Co., Valencia, Spain).

81 During the meat batter preparation, the temperature of the mixture was maintained below 10 ℃.

82 The meat batter was then divided into two parts. One portion was used for measurement of pH

83 values, color, cooking yield, emulsion stability, and viscosity, whereas the other portion was

84 stuffed into a collagen casing (#240, NIPPI Inc., Tokyo, Japan; approximately 25-mm diameter)

85 using a stuffer (EM-12, Manica, Barcelona, Spain) and cooked using a water bath (JSWB-30T,

86 JSR, Korea) at a of temperature 80 ℃ ± 1 ℃ until the internal temperature of the samples

87 reached 75 ℃. The samples were subsequently cooled in iced water for 30 min, vacuum packed,

88 and stored at 4 ℃ until moisture and fat content, pH, color, and textural properties could be

89 analyzed. All processing was performed in triplicate.

90

91 **Measurement of moisture and fat contents**

92 Moisture and fat contents of the cooked samples were determined using the method

93 described by AOAC (2012). Moisture content (AOAC method 950.46B) was determined by

94 measuring weight loss after 12 h of drying at 105 °C in a drying oven (SW-90D, Sang Woo

95 Scientific Co., Bucheon, Korea). Fat content (AOAC method 960.69) was determined using

96 the Soxhlet method in a solvent extraction system (Soxtec $^{®}$ Avanti 2050 Auto System, Foss

97 Tecator AB, Höganas, Sweden).

98

99 **Determination of pH values and instrumental color**

100 The pH values for a mixture of sausage samples and distilled water (1:4) were determined

101 using a pH-meter (Model 340, Mettler-Toledo GmbH Analytical, Schwerzenbach,

102 Switzerland). The cooking yield in each treatment was determined by weighing meat batters

103 before and after cooking and expressed in percentage.

104 The color of the samples was examined using a colorimeter (CR-10, Minolta, Japan;

105 illuminant C, calibrated with a white standard plate $L^* = 97.83$, $a^* = -0.43$, and $b^* = +1.98$),

106 which had a measuring area with a diameter of 8-mm and an illumination area of 50 mm

107 diameter. Color values (CIE L^*, a^*, and b^*) were measured on the surface of samples with

108 results taken in triplicate for each sample.

109

110 **Determination of emulsion stability**

111 The meat batter samples from each group were analyzed for emulsion stability using the

112 method described by Choe et al. (2013). The 15-mesh sieve (with 50-mm diameter) was placed

113 in the middle of a graduated glass tube. Approximately 25 g of the meat batter was weighed

114   and placed on the sieve in glass tubes and covered. Triplicate samples from each group were

115   cooked at $75 \pm 1°C$ for 30 min, followed by cooling to approximately $4 \pm 1°C$ in order to

116   facilitate fat and water layer separation. The fluid water and fat, which separated well in the

117   bottom of the graduated glass tube, were measured in milliliters and calculated as a percentage

118   of the original weight of the batter.

119

120   **Determination of cooking yield**

121   The cooking yield of each group was determined by weighing meat batters before and after

122   cooking and expressing this as a percentage.

123

124   **Determination of apparent viscosity of the meat emulsion samples**

125   The flow behavior and time dependence of the batters were investigated at 20 ± 1 °C using

126   a parallel plate rotational viscometer (HAKKE Viscotester 500 R®, Thermo Electron

127   Corporation, Karlsruhe, Germany). The batter allowed to equilibrate for 5 min at room

128   temperature (23±3°C) and was tested using a standard cylinder sensor (SV-2). Time

129   dependency of the batter viscosity was determined by measuring the apparent viscosity under

130   a constant shear rate of 10/s for 60 s.

131

132   **Texture profile analysis (TPA)**

9

133   Cooked samples, cut into sections with a height of 25 mm and φ 16 mm diameter, were used

134   in this study. A cylinder probe (φ 20 mm diameter) set attached to a Texture Analyzer (TA-

135   XT2*i*, Stable Micro System Ltd., Surrey, UK) was used to examine the textural properties for

136   each sample. The test conditions used in the study were as follows: stroke, 2 kg; test speed, 2.0

137   mm/s; and distance, 8 mm. The texture profile analysis (TPA) parameters, thus hardness (N),

138   springiness, cohesiveness, gumminess (N), and chewiness (N) were collected.

139

140   **Statistical analysis**

141   All experiments were repeated three times. Statistical analysis was performed by one-way

142   analysis of variance (ANOVA), and significant differences ($p<0.05$) were detected by

143   Duncan's multiple range test using SAS software (SAS, Release 9.3 for window, SAS Institute

144   Inc., USA). The values are expressed as means ± standard deviation (SD).

145

146   # Results and discussion

147   **Moisture and fat content**

148   The addition of RPS powder to the meat emulsion significantly influenced the moisture

149   and fat contents (Fig. 1.). The amount of RPS powder added had no effect on the moisture

150   contents ($p > 0.05$), similar to the results of Lee and Kim (2016) for restructured meat products

151   with RPS powder. Addition of RPS powder at levels higher than 3% led to a significant

152 decrease in fat contents of samples compared to that of the control. Moreover, samples with 4%

153 RPS powder showed the lowest ($p < 0.05$; 12.2% reduction) fat contents among all the groups.

154 This result may be linked to fat binding ability of RPS powder based on result of fat exudation

155 which showed the highest ($p < 0.05$) by addition of 4% RPS powder in emulsion samples (Fig.

156 2). Previous study observed moisture content was increased and fat content was decreased

157 (weight percentage basis) in sausage samples as increasing addition level of oat bran which is

158 soluble fiber (Chang and Carpenter, 1997). In hence, we speculated that PRS powder probably

159 contains large amounts of soluble fiber.

160

161 **pH and color**

162   The pH was affected both by the amount of RPS powder added and by heat treatment

163 (Table 1). The higher ($p<0.05$) pH of samples with RPS powder were most probably caused

164 by the pH value (pH 6.25) of the RPS powder alone. Interestingly, in this study, samples with

165 1% RPS powder showed the highest pH values among the samples containing RPS powder.

166 After cooking, the pH had increased in all groups. This phenomenon may be due to the

167 protonation of imidazole groups which could be exposed as a result of protein denaturation at

168 the temperatures used for cooking (Tanford, 1968). Xiong et al. (1999) reported a different

169 observation whereby meat batter with a higher pH than the isoelectric point (pI) rarely showed

170 a pH increase after heat treatment.

171   The color of meat and meat products has a large influence on consumers purchasing

172   decisions, and is therefore an important parameter (Choe et al., 2017). In this study, it was

173   found that the addition of RPS powder and the amount added resulted in a change of color (L*,

174   a*, and b*) (Table 1). In general, in all samples, by increasing the amount of RPS added, L*

175   values decreased and a* and b* values increased (p<0.05), regardless of cooking. Similar

176   results have been observed in sausages where significant decreases of L* values were related

177   to an increase in apple and orange fiber concentration (García, 2007). In this study, the increase

178   in a* and b* values was probably caused by the intrinsic color of the RPS powder (L*, 53.50;

179   a*, 24.46; b*, 22.10). Based on these results, incorporation of RPS powder into meat products

180   can lead to a positive change in a* values, whilst to a negative change in b* values.

181

182   **Emulsion stability and cooking yield**

183   Emulsion stability is critically affected by the properties of proteins, water and fat retention

184   or absorption capacity of non-meat ingredients in emulsified products (Haque et al., 2016;

185   Zorba and Kurt, 2006). In addition, the pH values of samples can influence emulsification and

186   the functional characteristics of emulsion; pH values further away from the pI possess greater

187   emulsifying stability (Cheftel et al., 1985; Zorba and Kurt, 2006). In this study, regarding non-

188   meat ingredients, water and fat exudation were dependent on the amount of RPS powder added

189   (Fig. 2). Among the groups, significantly lower values of water and fat exudation were

190 observed in emulsified samples containing 2% RPS powder. Additionally, the addition of 3%

191 RPS powder showed decreased (p<0.05) in fat exudation. The addition of RPS powder at

192 higher levels (4%) induced an increase in water and fat exudation. These results indicate that

193 the addition RPS powder at optimum levels (2 or 3%) forms a matrix which may be effective

194 at holding or absorbing water and fat during protein gelation. The emulsion stability of samples

195 is closely related with cooking yield loss. According to Surh et al. (2006), emulsified samples

196 with high stability showed low levels of water and fat exudation during cooking, hence

197 presenting highly sought after properties. In this study, the cooking yield of samples was

198 affected by the addition of RPS powder and the amount added (Table 1). The lowest ($p<0.05$)

199 cooking yield was observed in samples without RPS powder while significant differences in

200 yields were not observed among the groups with RPS powder at amounts up to 3%, with 3%

201 RPS powder exhibiting the highest cooking yield. A similar trend to that of the emulsion

202 stability analysis was observed in that the higher level (4%) of RPS powder added led to a

203 deterioration in the water holding capacity of the samples and therefore a lower cooking yield.

204

205

206 **Apparent viscosity of the meat emulsion samples**

207 　　The apparent viscosity of the meat emulsion samples, which ranged from 47.5 to 128.29

208 Pa•s, decreased with increasing rotation time (Fig. 3). The samples without RPS powder had

209 the lowest values ($p < 0.05$) up until 30 s while, thereafter, samples with RPS powder added at

210 0 and 4% had the lowest values ($p < 0.05$) of apparent viscosity. In general, the addition of

211 RPS powder at 1, 2, and 3% led to the similar values ($p > 0.05$) in apparent viscosity of the

212 meat emulsion samples. In general, the addition of dietary fiber brought about an increase in

213 viscosity in bologna samples regarding many fibers possess high fat absorption ability and

214 WHC (Claus and Hunt, 1991; Saricoban et al., 2010). However, in this study, the addition of

215 RPS powder at 4% induced decline in viscosity compared to samples with RPS powder at 1, 2,

216 3%, which was probably affected by the poor fat absorption ability (based on fat content) as

217 adding large amounts of RPS powder. Furthermore, according to Dhingra et al (2012),

218 incorporation of fiber with highly branched or short chain polymer can lead to low viscosity

219 depending on addition level, although having high WHC. Like emulsion stability, viscosity of

220 meat emulsion can change depending on the water-holding capacity of non-meat ingredients

221 including dietary fiber. Choe et al. (2013) showed a negative correlation between viscosity and

222 cooking loss (R^2=0.8305) in meat batter samples with wheat fiber and pork skin. Similarly, in

223 this study, viscosity of the emulsion samples exhibited positive correlation with cooking yield

224 [R^2=0.8291; Fig. 4(A)].

225

226 **TPA**

227   As shown in Table 2, textural properties (hardness, springiness, gumminess, chewiness,

228 and cohesiveness) were partially affected by addition of RPS powder and by the amount added.

229 Textural properties are important parameters for consumer preference and are closely related

230 to the viscosity of emulsion samples. A previous study reported that high-viscosity meat batter

231 exhibited greater textural properties, except for springiness (Choe et al., 2013). In this study,

232 higher values ($p < 0.05$) in hardness and springiness were observed in samples with RPS

233 powder at 4 and 3%, respectively. This result partially agreed with the finding of several

234 authors who reported that hardness is generally increased as decreasing in fat content in meat

235 emulsion consisting of same meat protein content (Jiménez Colmenero et al., 1995; Lee et al.,

236 1987). For gumminess, chewiness, and cohesiveness, the addition of RPS powder at 1, 2, and

237 3% showed the highest values ($p < 0.05$). Furthermore, positive correlations between viscosity

238 and hardness [R^2=0.7444; Fig 4(B)] and viscosity and cohesiveness [R^2=0.7118; Fig 4(C)]

239 were observed in the emulsion samples. The relatively lower correlation coefficients herein

240 were presented than that between viscosity and cooking yield.

241

242 **Conclusion**

243   Based on the results of this study, addition of the optimum amount of RPS powder (2%)

244 to emulsified meat products would lead to an enhancement in the quality of characteristics

245 associated with these products. In particular, addition of 1% RPS powder would have a potent

246 effect on the color of meat products due to its bright red color and acceptable increase in

247 yellowness.

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310

311 **Figure legends**

312 **Fig. 1. Moisture and fat contents of emulsion model sausages with red pepper seed**

313 **powder.** [a-c] Means within different letters in each parameter indicate differences between

314 treatments ($p<0.05$). Bar (SD) was determined using three independent experiments each

315 assayed in triplicates.

316

317 **Fig. 2. Emulsion stability of emulsion model sausages with red pepper seed powder.** [a-c]

318 Means within different letters in each parameter indicate differences between treatments

319 ($p<0.05$). Bar (SD) was determined using three independent experiments each assayed in

320 triplicates.

321

322 **Fig. 3. Apparent viscosity of meat emulsion with red pepper seed powder.**

323

324 **Fig. 4. Relationship between viscosity and each parameter [(A) cooking yield (B)**

325 **hardness (C) cohesiveness] of meat emulsion with red pepper seed powder.**

326

Table 1. pH and color of uncooked and cooked emulsion model sausages with red pepper seed powder

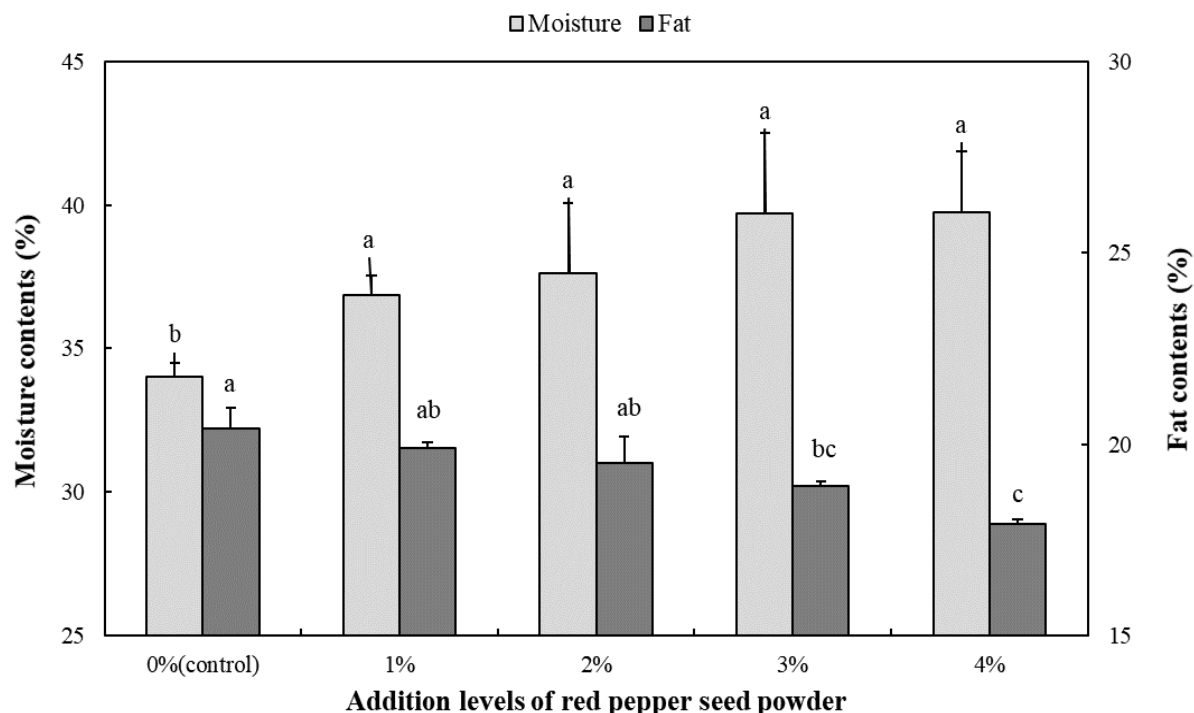| Traits			Addition levels of red pepper seed powder (%)				
			0 (control)	1	2	3	4
pH		Uncooked	6.13 ± 0.01^d	6.20 ± 0.02^a	6.17 ± 0.01^b	6.14 ± 0.01^c	6.15 ± 0.01^c
		Cooked	6.24 ± 0.01^d	6.32 ± 0.01^a	6.29 ± 0.01^b	6.26 ± 0.01^c	6.26 ± 0.01^c
Cooking yield			84.85 ± 0.33^c	87.53 ± 0.42^a	87.67 ± 0.60^a	87.55 ± 0.44^a	86.78 ± 0.32^b
Color	Uncooked	CIE L^*	71.44 ± 0.29^b	72.04 ± 0.29^a	68.98 ± 0.45^c	69.08 ± 0.18^c	67.52 ± 0.25^d
		CIE a^*	7.42 ± 0.38^e	9.54 ± 0.30^d	11.30 ± 0.60^c	12.70 ± 0.56^b	14.32 ± 0.41^a
		CIE b^*	17.46 ± 1.01^e	21.56 ± 0.42^d	23.80 ± 0.99^c	26.36 ± 1.54^b	28.36 ± 0.83^a
	Cooked	CIE L^*	73.86 ± 0.79^a	72.24 ± 0.21^b	70.82 ± 0.24^c	69.52 ± 0.74^d	68.18 ± 0.76^e
		CIE a^*	7.84 ± 0.38^e	10.06 ± 0.33^d	11.48 ± 0.28^c	13.16 ± 0.58^b	14.04 ± 0.69^a
		CIE b^*	14.30 ± 0.35^d	18.26 ± 0.55^c	20.16 ± 0.36^b	22.68 ± 0.82^a	23.24 ± 0.99^a

All values are mean±SD.

[a-e] Mean in the same row with different letters are significantly different (p<0.05).

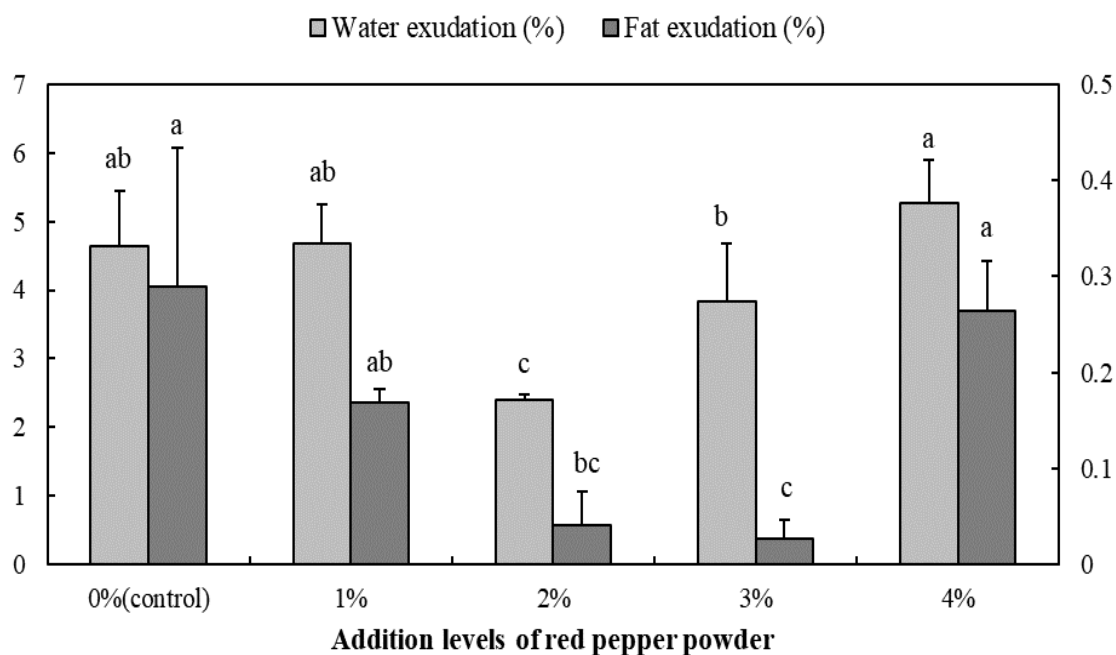332 **Table 2. Texture properties of emulsion model sausages with red pepper seed pow**

333 **der**

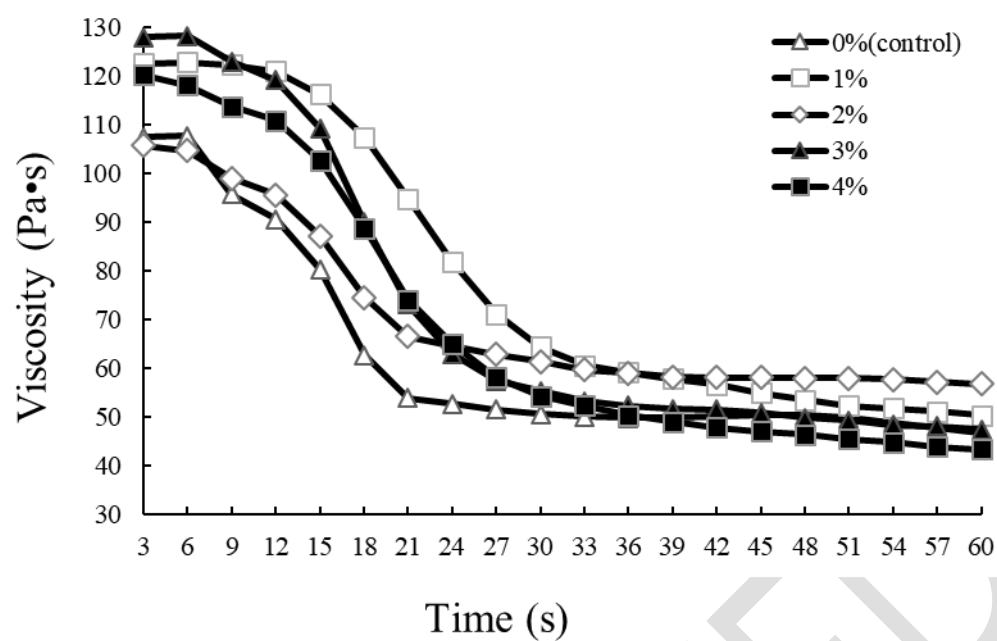| Traits	Addition levels of red pepper seed powder (%)				
	0(control)	1	2	3	4
Hardness (N)	49.7 ± 1.96^b	53.3 ± 3.04^{ab}	55.2 ± 4.70^b	56.0 ± 2.06^{ab}	57.6 ± 5.88^a
Springiness	0.93 ± 0.01^b	0.94 ± 0.02^{ab}	0.95 ± 0.02^a	0.93 ± 0.01^b	0.94 ± 0.02^{ab}
Gumminess (N)	8.5 ± 1.18^b	11.8 ± 0.69^a	12.0 ± 1.18^a	10.0 ± 0.69^b	12.8 ± 1.18^a
Chewiness (N)	7.9 ± 1.08^b	11.2 ± 0.59^a	11.4 ± 1.08^a	9.4 ± 0.78^b	12.1 ± 1.08^a
Cohesiveness	0.17 ± 0.02^b	0.22 ± 0.02^a	0.22 ± 0.01^a	0.18 ± 0.01^b	0.22 ± 0.01^a

334 All values are mean±SD.

335 [a-b] Mean in the same row with different letters are significantly different ($p<0.05$).

336

Fig. 1. Moisture and fat contents of emulsion model sausages with red pepper seed powder. [a-c] Means within different letters in each parameter indicate differences between treatments ($p < 0.05$). Bar (SD) was determined using three independent experiments each assayed in triplicates.
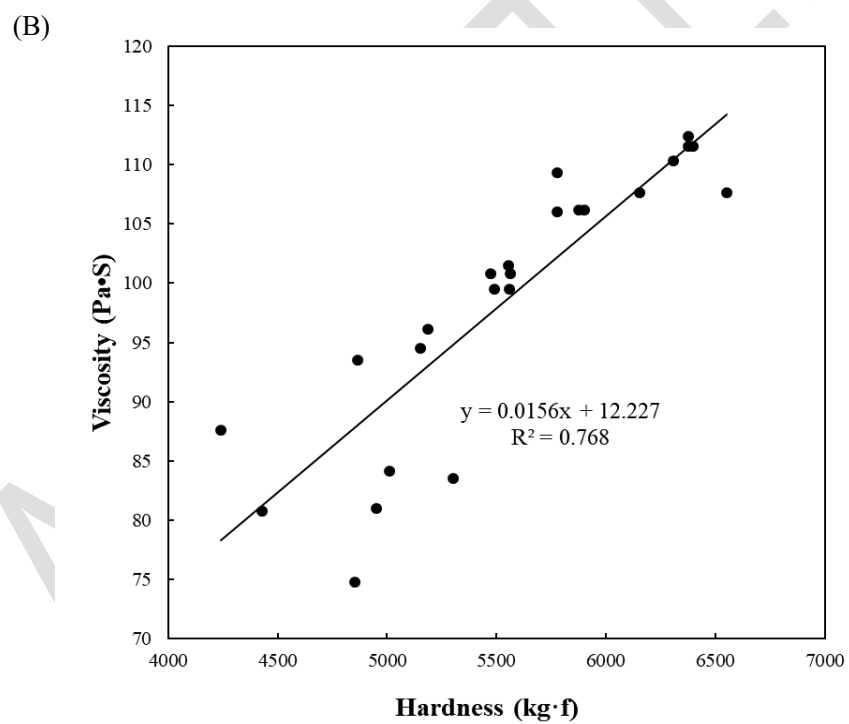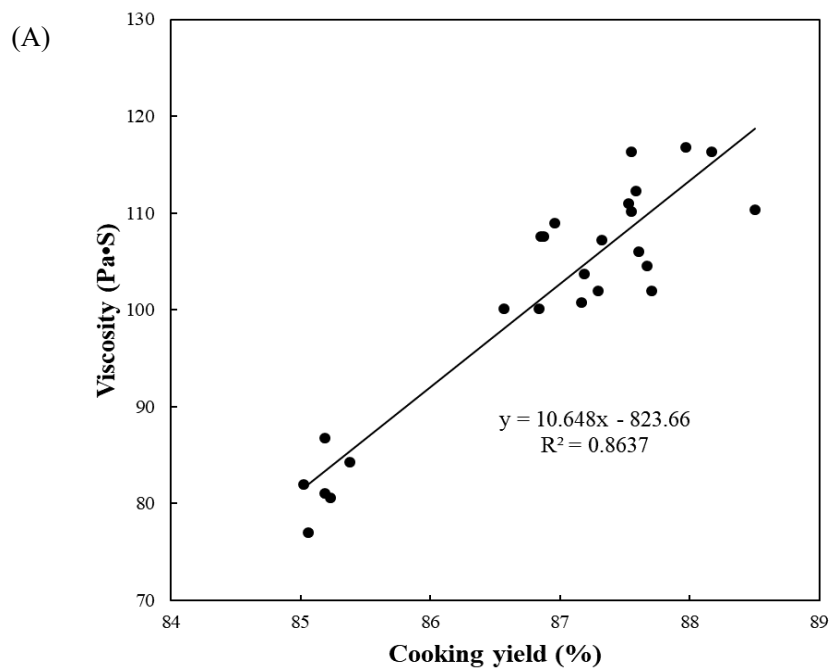
☐ Water exudation (%) ☐ Fat exudation (%)

343

Fig. 2. Emulsion stability of emulsion model sausages with red pepper seed powder. [a-c]
Means within different letters in each parameter indicate differences between treatments
($p<0.05$). Bar (SD) was determined using three independent experiments each assayed in
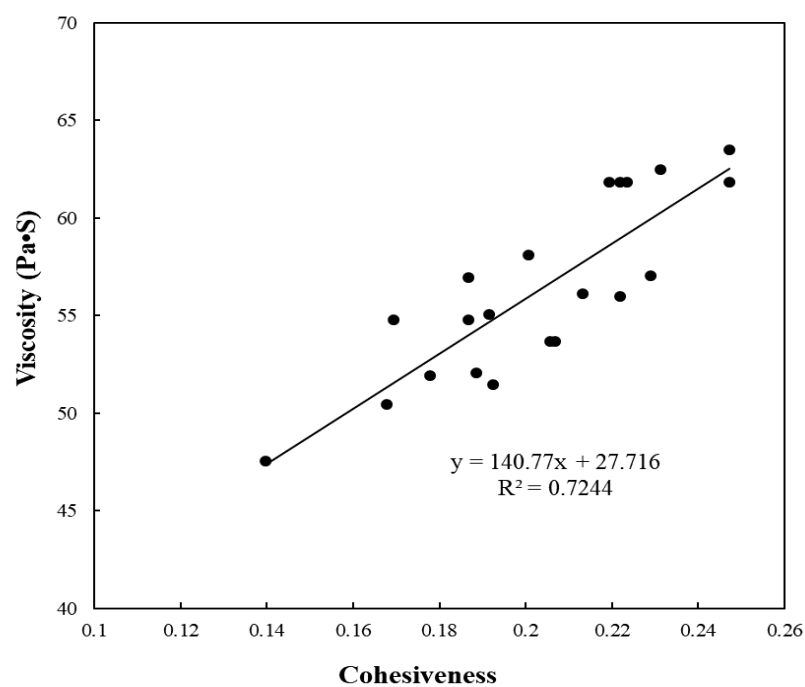triplicates.

349

350 **Fig. 3. Apparent viscosity of meat emulsion with red pepper seed powder.**

351

(A)

y = 10.648x - 823.66
R² = 0.8637

Cooking yield (%)

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(B)

y = 0.0156x + 12.227
R² = 0.768

Hardness (kg·f)

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(C)

y = 140.77x + 27.716
R² = 0.7244

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363 **Fig. 4. Relationship between viscosity and each parameter [(A) cooking yield (B)**
364 **hardness (C) cohesiveness] of meat emulsion with red pepper seed powder.**