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9 **Abstract**

10 This study investigated freeze- or oven-dried winter mushroom powder (FDP or ODP,
11 respectively) as an alternative to phosphate in beef patties. The beef patties were prepared with
12 four treatments: no addition of phosphate and winter mushroom (control), addition of 0.3%
13 sodium pyrophosphate (BP), addition of 1% FDP (BFW), and addition of 1% ODP (BOW).
14 The pH of FDP and ODP was 6.73, and 7.00, respectively. FDP and ODP contained phenolic
15 compound at a level of 3.50 and 5.45 g gallic acid equivalent/kg, respectively. The cooking loss
16 of beef patties was the highest in the control and lowest in BP ($p < 0.05$). BFW had lower cooking
17 loss than the control ($p < 0.05$), and BOW showed similar cooking loss as that of the control
18 ($p > 0.05$). Inhibition of lipid oxidation was found in BP and BOW as compared with control
19 ($p < 0.05$). BFW was similar to the control in terms of the degree of lipid oxidation ($p > 0.05$).
20 BOW showed lower L^* and higher a^* values than those of the control, BP, and BFW ($p < 0.05$).
21 Texture properties such as hardness, springiness, cohesiveness, gumminess, and chewiness
22 were the highest in BP ($p < 0.05$). A slight increase in hardness and springiness was observed in
23 BOW compared to those of the control ($p < 0.05$). The results showed that FDP and ODP did
24 not exhibit all the properties of phosphate in beef patties. Therefore, FDP and ODP can be used
25 for partial substitution of phosphate in beef patties.

26

27 Keywords: beef patty, phosphate, winter mushroom, drying method

28

29 **1. Introduction**

30 The increase in consumer demand for healthy foods has led to a growing interest in the
31 clean-label food market (Asioli et al., 2017). Clean labels are granted to foods that meet several
32 requirements (Yong et al., 2020), including the absence of synthetic additives. Synthetic nitrite,
33 phosphate, and antioxidants have been used to improve the shelf life and sensorial quality of
34 processed meat products (Sebranek, 2009; Jo et al., 2020c). Although synthetic food additives
35 have advantageous effects on the quality of processed foods, natural-based ingredients have
36 been developed for the substitution of synthetic additives in processed meat products (Jo et al.,
37 2020b; Yong et al., 2020).

38 Phosphate is an essential additive for meat products. It increases product yield and
39 improves sensorial properties by increasing water retention and gel strength and inhibiting lipid
40 oxidation (Chen et al., 2019; Choe et al., 2018; Thangavelu et al., 2019). Winter mushrooms
41 have plenty of antioxidative substances such as phenolic compounds and ergothioneine, which
42 show activities of metal ion chelation and free radical scavenging (Jo et al., 2020b). In addition,
43 the water retention in the meat products was improved with the addition of winter mushrooms
44 because of high dietary fiber and pH in winter mushrooms (Choe et al., 2018). Recent studies
45 reported that winter mushroom could be a suitable alternative to phosphate in pork sausage,
46 chicken sausage, and enhanced beef (Choe et al., 2018; Choi et al., 2020; Jo et al., 2018; Jo et
47 al., 2020b).

48 Drying is a general process to increase the shelf life and ease-of-use of natural products.
49 Freeze drying is a well-known method that can be widely used for the preservation of various
50 natural products without the deterioration of their functional properties, such as antioxidant and
51 antimicrobial activities (Thamkaew et al., 2020). However, this method involves considerable
52 time and cost (Soysal and Ö ztekin, 2001). Oven drying is a relatively inexpensive and simple
53 process that is commonly used in the food industry (Soysal and Ö ztekin, 2001). In addition,

54 some previous studies reported that drying of various natural substances with hot air at
55 approximately 170°C increased the antioxidant activity with the Maillard reaction (Chang et al.,
56 2006; Lin et al., 2016). Maillard reaction is a type of non-enzymatic browning which involves
57 the chemical reaction of an amino acid with carbonyl group of a reducing sugar, and the reaction
58 product exhibits an antioxidant activity owing to its hydrogen-donating ability (Namiki, 1988).
59 In addition, it can be a major factor in the formation of a desirable flavor in heated foods by
60 generating various flavoring compounds (Van Boekel, 2006).

61 Beef patty is a popular food consumed globally and a major meat product in the home meal
62 replacement market. Beef is highly nutritious because it contains balanced amino acids,
63 vitamins, and minerals (Lee et al., 2020b). However, beef products are easily oxidized because
64 of the abundant iron content, which is a critical prooxidant in meat (Baron and Andersen, 2002;
65 Choi et al., 2020; Lee et al., 2020a). Lipid oxidation affects the sensory and nutritional value of
66 meat products, and lipid oxidation products, including malondialdehyde (MDA), are negatively
67 recognized by consumers for their genotoxicity and cytotoxicity (Jung et al., 2016; Min et al.,
68 2010).

69 We hypothesized that winter mushrooms could improve the quality of beef patties owing
70 to their antioxidant activity, dietary fiber, and pH increase effect and exhibit actions similar to
71 those of phosphate in beef patties. In addition, oven-dried winter mushrooms may be more
72 effective in improving the quality of beef patties by maximizing antioxidant activity and
73 enhancing sensorial properties. Therefore, the antioxidant activity and additional effects of
74 freeze- and oven-dried winter mushroom powder in beef patties and its use as an alternative to
75 phosphate were investigated in this study.

76

77 **2. Materials and Methods**

78 **2.1. Properties of the freeze- or oven-dried winter mushroom powder**

79 **2.1.1. Preparation of the winter mushroom powder**

80

81 Winter mushrooms (*Flammulina velutipes*) were purchased from a local market (Daejeon,
82 Korea). The mushrooms were frozen at -70°C for 24 h, and lyophilized using a freeze dryer
83 (Bondiro, Ilshin Co., Seoul, Republic of Korea) at room temperature and the 5 mTorr of
84 chamber pressure, and then pulverized using a food processor (FPM250, Kenwood Ltd., Havant,
85 UK), yielding the freeze-dried powder (FDP). The oven-dried winter mushroom powder (ODP)
86 was prepared using a drying oven (JSOF-150, JS Research Inc., Gongju, Republic of Korea) at
87 170°C for 15 min and pulverized using a food processor (FPM250). The moisture contents of
88 FDP and ODP were 5.72% and 4.67%, respectively. The powders were stored at -20°C until
89 their use.

90

91 **2.1.2. Antioxidant potential**

92

93 The methanolic extracts of winter mushroom powder were prepared by the extraction of
94 0.5 g mushroom powder in 49.5 mL of 70% (v/v) methanol while shaking for 60 min.

95 The total phenolic content in the methanolic extracts of FDP and ODP was measured using
96 the Folin–Ciocalteu method (Subramanian et al., 1965) and is expressed as gallic acid
97 equivalents (g/kg).

98 The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of the methanolic
99 extract was estimated using the method described by Jung et al. (2017), and the half-maximal
100 effective concentration (EC_{50}) for scavenging DPPH radicals was calculated.

101 Reducing power was determined based on the reduction rate of ferric iron to ferrous iron
102 using the method described by Oyaizu (1986). The sample solutions of FDP and ODP (15, 12.5,
103 10, 7.5, and 5 mg/100 mL in 70% (v/v) methanol and 7, 5.75, 4.5, 3.25, and 2 mg/100 mL in

104 70% (v/v) methanol, respectively) were used to measure the reducing power. The half-maximal
105 effective concentration (EC₅₀) for 0.5 absorbance at 700 nm was calculated using a
106 spectrophotometer (DU[®] 530, Beckman Instruments Inc., Fullerton, CA, USA).

107

108 **2.1.3. pH and color**

109

110 The 1 g of FDP and ODP were homogenized with 9 mL distilled water using a homogenizer
111 (T25 basic, IKA[®] -Werke GmbH & Co. KG, Staufen, Germany) and centrifuged at 2,090×g for
112 10 min using a centrifuge machine (ScanSpeed 1580R, Labogene ApS, Lillerød, Denmark).
113 The supernatant was filtered using filter paper (No. 4 filter paper, Whatman, Maidstone,
114 England) and the pH value was measured using a pH meter (SevenEasy, Mettler-Toledo Intl
115 Inc., Schwerzenbach, Switzerland).

116 The instrumental color (CIE L*, a*, b*) of FDP and ODP was measured using a
117 spectrophotometer (CM-3500d, Konica Minolta Inc., Tokyo, Japan). The measurements were
118 taken perpendicular to the measuring mini petri dish (CM-A128, Konica Minolta Inc., Tokyo,
119 Japan) containing powder with an illumination area of 30 mm, illuminant D65, and 10 °
120 standard observer. The results were analyzed using SpectraMagic Software (SpectraMagic[™]
121 NX, Konica Minolta Inc., Tokyo, Japan).

122

123 **2.2. Qualitative properties of beef patties**

124 **2.2.1. Manufacture of beef patty**

125

126 Three fresh bottom rounds from three heifer carcasses were prepared, and the bottom round
127 from each carcass was allocated to each batch. The bottom round of beef (390 g) was mixed
128 with pork back fat (60 g), bread powder (30 g), chopped onion (30 g), chopped garlic (12 g),
129 isolated soybean powder (12 g), salt (6 g), and water (60 g) in a mixer for 5 min. The formula

130 for each of the four treatments are as follows: 1) control: patty manufactured without sodium
131 pyrophosphate, 2) Positive control (BP): patty manufactured with sodium pyrophosphate
132 (0.3%); 3) BFW: patty manufactured with FDP (1%); and 4) BOW: patty manufactured with
133 ODP (1%). The meat batter was prepared thrice for each treatment, and the patty (100 g meat
134 batter) was molded with a stainless container (80 mm × 45 mm). Five patties were made from
135 each meat batter and cooked in an electric oven (COR-055KE, SK magic Co., Ltd., Seoul,
136 Republic of Korea) at 170°C for 15 min. Total nine beef patties (three patties /each batch) were
137 used for the analysis of physicochemical properties.

138

139 **2.2.2. pH of meat batter and cooking loss of beef patty**

140

141 Meat batter (1 g) was homogenized with distilled water (9 mL) using a homogenizer (T25
142 basic). The homogenates were centrifuged at 2,090×g for 10 min (1580R) and filtered using
143 filter paper (No. 4 filter paper). The pH value of the filtrate was measured using a pH meter
144 (SevenEasy).

145 Cooking loss of beef patties was calculated by comparing the weight difference between
146 the cooked beef patties and raw beef patties.

147

148 **2.2.3. Lipid and protein oxidation**

149

150 Lipid oxidation in beef patties was detected by measuring the MDA content. MDA was
151 extracted from beef patties and then analyzed using a high-performance liquid chromatography
152 (HPLC) system (1200 series, Agilent Technologies Inc., USA) with an Atlantis T3 C18 RP
153 column (Waters corp., Milford, MA, USA) (4.6 × 250 mm, 5 µm-particles). The analysis
154 procedures were performed in accordance with the method described by Jung et al. (2016).
155 1,1,3,3-tetraethoxypropane was used as the standard compound, and the MDA content in beef

156 patties was expressed as mg MDA/kg beef patties.

157 Protein oxidation in beef patties was measured by analyzing the total carbonyl content
158 using the derivatization method of 2,4-dinitrophenylhydrazine (DNPH), as described by Jo et
159 al. (2020b). The carbonyl content was expressed as nmol/mg of protein using an absorption
160 coefficient of $21.0 \text{ nM}^{-1} \text{ cm}^{-1}$ at 370 nm for protein hydrazones.

161

162 **2.2.4. Instrumental color measurements**

163

164 A colorimeter (CM-3500d) was used to measure the color (CIE L*, a*, b*) of each beef
165 patty. The measurements were taken perpendicular to the inner surface of the patty at two
166 different locations per sample with an illumination area of 30 mm, illuminant D65, and 10°
167 standard observer. The results were then analyzed using SpectraMagic Software
168 (SpectraMagic™ NX).

169

170 **2.2.5. Texture profile analysis (TPA)**

171

172 The texture of beef patties was measured with a two-bite system using a texture analyzer
173 (Model A-XT2, Stable Micro Systems Ltd., Godalming, UK) with a compression probe that
174 had a diameter of 70 mm. The patties were cut to regular size ($2 \times 2 \times 1.5 \text{ cm}^3$) and subjected
175 to two cycles of 70% compression at a test speed of 2 mm/s. The texture characteristics of beef
176 patties were expressed as hardness, springiness, cohesiveness, gumminess, and chewiness.

177

178 **2.3. Statistical analysis**

179

180 This study was independently repeated thrice (three batches). The data from the quality
181 measurements of beef patties were statistically analyzed using the mixed model under a
182 randomized complete block design (with a batch corresponding to a block). The least-square

183 mean values and standard error of the least-square means (SEM) were reported, and Tukey's
184 multiple range test was performed to draw specific comparisons. SAS software (version 9.4,
185 SAS Institute Inc., Cary, NC, USA) was used for the statistical analyses.

186

187 **3. Results and discussion**

188 **3.1. Properties of the winter mushroom powder**

189 **3.1.1. Antioxidant potential**

190

191 Winter mushrooms are fungi that exhibit a high antioxidant activity (Shah et al., 2018; Zhang
192 et al., 2013). Phenolic compounds are anti-oxidizing substances that remove reactive oxygen
193 species existing in plants and play a role in reducing oxidative stress. The total phenolic content
194 of the oven-dried winter mushroom powder (ODP) was 5.45 g GAE/kg that was significantly
195 higher than that of the freeze-dried winter mushroom powder (FDP) at 3.50 g GAE/ kg (Table
196 1, $p < 0.05$). The EC_{50} values of FDP and ODP for DPPH radical scavenging were 16.89 g/kg
197 and 11.52 g/kg, respectively, which were significantly different between treatments ($p < 0.05$).
198 In addition, ODP had a significantly lower EC_{50} value of reducing power than that of FDP
199 ($p < 0.05$), indicating that ODP has a higher antioxidant potential than FDP. This is analogous to
200 the phenomenon that previous studies report of an increase in phenolic compound content and
201 antioxidant activity of natural products subjected to heat treatment (Li and Shah, 2013; Saad et
202 al., 2014). Another study suggests that heat treatment of plants could improve the antioxidant
203 activity by releasing phenolic compounds from the collapsed cell wall and the rapid inactivation
204 of polyphenol oxidase (PPOs) such as tyrosinase, catecholase, and laccase (Nguyen et al., 2018).
205 In addition, Maillard reaction products have antioxidant activity, which might be due to the
206 action of reductones and melanoidins formed in the reaction process (Namiki, 1988). The
207 reductone can effectively donate hydrogen, and melanoidin can block the reaction of metal ions
208 or residues generating radicals (Namiki, 1988).

210 **3.1.2. pH and color**

211

212 The pH of the ingredients is an important property of meat products because the increase in
213 pH with the addition of ingredients can improve water retention in meat products (Choe et al.,
214 2018; Jo et al., 2020a). When the pH of fresh winter mushroom was 6.81 (data not shown), the
215 pH of ODP and FDP was 7.00 and 6.73, respectively, and the pH of ODP was significantly
216 higher than that of FDP (Table 1, $p < 0.05$). Evidently, the oven-drying process resulted in the
217 increased pH of winter mushrooms. However, these results may not be common. Brands and
218 Van Beekel (2002) reported that formic acid and acetic acid are the main products of the
219 Maillard reaction. Lertittikul et al. (2007) also reported a decrease in the pH value of porcine
220 plasma protein–glucose Maillard reaction products with increasing heating time. Previous
221 studies found that the pH of foods containing proteins increased after heat treatment, which
222 might be attributed to the release of basic amino acids from heat-denatured proteins (Vasanthi
223 et al., 2007; Oz et al., 2017). Thus, a similar effect may occur in winter mushrooms subjected
224 to heat treatment.

225 The application of oven-drying altered the color of winter mushrooms to brown by the
226 Maillard reaction. ODP had a lower L^* value and higher a^* and b^* values than that of FDP
227 (Table 1, $p < 0.05$), and this change could be an effect of the brown pigment melanoidin as the
228 final Maillard reaction product (Namiki, 1988).

229 **3.2. Properties of beef patty**

230 **3.2.1. pH of meat batter and cooking loss**

231

232 The high pH of meat products is one of the major factors determining the quality and
233 increasing the economic value of meat products. Meat batter containing no phosphate and
234 winter mushroom powder (control) showed the lowest pH among the treatments (Table 2,

235 $p < 0.05$). Although the addition of winter mushroom powders (BFW and BOW) to meat batter
236 at a concentration of 10 g/kg increased the pH compared to the control ($p < 0.05$), the effect of
237 the winter mushroom powder on the pH of the meat batter was not notable. In addition, no
238 difference in pH between BFW and BOW was found despite the higher pH of ODP than that
239 of FDP. The pH of BFW and BOW was significantly lower than that of BP, which was
240 manufactured with the addition of 0.3% sodium pyrophosphate ($p < 0.05$). Choe et al. (2018)
241 reported no difference in pH between pork batter containing 1% winter mushroom powder and
242 0.3% sodium pyrophosphate. However, chicken meat batter containing 1% winter mushroom
243 powder had a lower pH than that of chicken meat batter containing 0.3% sodium pyrophosphate
244 (Jo et al., 2018). The different effects of winter mushroom powder addition on the pH of meat
245 batter may be caused by the differences in buffering capacity of meats such as beef, pork, and
246 chicken.

247 The increase in pH of meat batter for comminuted meat products improves their water and
248 fat retention because the increased electrical repulsion forces among proteins increases the
249 space available for water in myofibrillar filaments and gel structure and fat droplets in the gel
250 structure (Chen et al., 2019). The highest and lowest cooking loss of beef patties occurred in
251 the control group and the BP, respectively (Table 2, $p < 0.05$). BFW showed a significantly lower
252 cooking loss than the control group ($p < 0.05$). However, the cooking loss in BOW was similar
253 to that in the control ($p > 0.05$). Previous studies found that the addition of winter mushroom
254 powder to pork and chicken sausages with the same level of concentration as that used in the
255 present study resulted in a decrease in cooking loss, which was attributed to the increase in pH
256 and the water holding and fat binding capacity of dietary fiber in winter mushroom powder
257 (Choe et al., 2018; Jo et al., 2018). In the present study, there were no notable pH changes in
258 meat batter with the addition of winter mushroom powder. The decrease in the cooking loss of
259 BFW might be due to dietary fiber. However, the dietary fiber in ODP might lose its water

260 holding capacity and fat binding capacity by heat treatment. A previous study reported a
261 reduced water-holding capacity of dietary fiber with structural damage induced by heat
262 treatment (Miranda et al., 2010; Vega-Gálvez et al., 2015).

263

264 **3.2.2. Lipid and protein oxidation**

265

266 The content of MDA, a secondary product of lipid oxidation, in beef patties was the highest
267 in the control and the lowest in the BP (Table 3, $p < 0.05$). This result was attributed to the
268 antioxidant activity of phosphate in the BP. Phosphate suppresses lipid oxidation through metal-
269 ion chelating activity in the meat system (Choe et al., 2018). Beef contains considerable
270 myoglobin, and the iron ions released from myoglobin can generate hydroxyl radicals via the
271 Fenton reaction (Lloyd et al., 1997). Therefore, the antioxidant activity of phosphate by
272 chelating metal ions may be maximized in beef compared to that in chicken and pork. Previous
273 studies found that freeze-dried winter mushroom at 1.0% level of concentration in pork and
274 chicken sausages served as an alternative to phosphate and decreased lipid oxidation (Jo et al.,
275 2018; Jo et al., 2020b). However, there was no inhibition of lipid oxidation in BFW compared
276 to that in the control used in the present study ($p > 0.05$).

277 This result might be attributed to the different lipid oxidation potential with the major
278 oxidants and substrates among beef, pork, and chicken (Rhee et al., 1996). Phenolic compounds
279 in winter mushroom powder can effectively suppress lipid oxidation in pork and poultry.
280 However, its effect as a prooxidant might be less in beef because the main factor of lipid
281 oxidation in beef is metal ions (Min et al., 2010). Nonetheless, the MDA content of BOW was
282 lower than that of the control and BFW ($p < 0.05$), although it was higher than that of BP
283 ($p < 0.05$). These results were attributed to the increased antioxidant activity of winter
284 mushrooms after oven-drying. The ODP showed an increase in antioxidant activity with an
285 increase in phenolic content and the generation of Maillard reaction products (Table 1). Alfawaz

286 et al. (1994) and Smith and Alfawaz (1995) reported the inhibitory effects of Maillard reaction
287 products on lipid oxidation in cooked ground beef.

288 There were no significant differences in the carbonyl content among the treatments (Table
289 3, $p>0.05$). Phosphate and winter mushroom powders had no inhibitory effect on protein
290 oxidation in beef patties. This result is in line with that of a previous study. Jo et al. (2018)
291 reported that phosphate and winter mushroom powder had no effect on the protein oxidation in
292 chicken sausages. However, the effect of winter mushrooms on protein oxidation in meat
293 products should be further studied with other protein oxidation parameters, such as free
294 sulfhydryl content and tryptophan fluorescence intensity. The carbonyl content might not be a
295 suitable indicator of protein oxidation in beef patties. In this study, the carbonyl content of beef
296 patties that included winter mushroom powders, tended to be higher than that of control and
297 BP. This might be due to the action of quinones generated by the oxidation of phenolic
298 compounds (Pourcel et al., 2007). Quinones can accelerate the oxidative deamination of
299 responsive amino acids and consequently produce applicable carbonyl compounds (Estévez and
300 Heinonen, 2010). Cando et al. (2014) reported an increase in the carbonyl content and inhibition
301 of lipid oxidation in beef patties with the addition of the phenolic-rich extract of Willowherb
302 (*Epilobium hirsutum L.*).

303 **3.2.3. Color and texture**

304
305 The intense color of natural ingredients incorporated in meat products results in color
306 changes in meat products (Jeong et al., 2020; Lee et al., 2019). There were no significant
307 differences in L^* and a^* values, except for b^* value among control, BP, and BFW (Table 4,
308 $p>0.05$). Previous study reported that the lack of an effect of winter mushroom based on its
309 white color on the color of meat products could be a suitable property as a natural ingredient
310 for meat products (Choe et al., 2018; Choi et al., 2020; Jo et al., 2018; Jo et al., 2020b). BP had
311 a higher b^* value than control and BFW ($p<0.05$). This result might be attributed to the increase

312 in Maillard reaction by the increase of pH with the phosphate addition. Maillard reaction rate
313 is faster in high pH environments, and some studies reported that phosphate accelerates Maillard
314 reaction (Bell, 1997). The ODP showed lower L* and higher a* values with the Maillard
315 reaction compared to FDP (Table 1). From the results, BOW showed the lower L* and the
316 higher a* values compared to those of control, BP, and BFW ($p < 0.05$). However, the difference
317 in color among the treatments was small.

318 The texture properties of beef patties were presented as hardness, springiness, cohesiveness,
319 gumminess, and chewiness (Table 5). BP showed the highest values for all the texture properties
320 ($p < 0.05$). The addition of phosphates in meat products increases the solubility of myofibrillar
321 proteins by the dissociation of actomyosin and an increase in ion strength (Chen et al., 2019).
322 The increase in solubilized myofibrillar proteins results in the strong and elastic gel structure
323 of comminuted meat products (Choe et al., 2018). In addition, the phosphorylation of
324 myofibrillar proteins leads to an increase in the interaction between insolubilized and
325 solubilized myofibrillar proteins, thereby increasing the gel strength (Chen et al., 2019). There
326 were no differences in cohesiveness, gumminess, and chewiness among the control, BFW, and
327 BOW groups ($p > 0.05$). The hardness of BOW and springiness of BFW and BOW were higher
328 than those of the control ($p < 0.05$). The effect of winter mushroom powder addition on the
329 texture of meat products differed between beef patties and sausages. Choe et al. (2018) and Jo
330 et al. (2018) reported a decrease in hardness and springiness of pork and chicken sausages with
331 the addition of winter mushroom powder and reported that these results were caused by the
332 hindrance of gelling by dietary fiber in winter mushroom. The difference in texture changes in
333 meat products with winter mushroom addition might be different in the organization of structure
334 among meat products. The structure of sausages, especially emulsion sausages, is densely
335 organized by the interaction of solubilized proteins, insoluble proteins, and small particles of
336 myofibrillar filaments (Chen et al., 2019). However, the structure of beef patties is relatively

337 less dense compared to that of sausage because it is organized by the interaction of partly
338 solubilized protein and meat particles. Therefore, the interactions among structural components
339 in beef patties might not be affected by dietary fiber from winter mushrooms. In the present
340 study, the pH of the meat batter increased for BFW and BOW (Table 2). The increase in pH of
341 meat batter results in a decrease of the electrical attraction force among myofibrillar proteins,
342 resulting in increased solubility of myofibrillar proteins (Thangavelu et al. 2019). Therefore,
343 the myofibrillar proteins in BFW and BOW might be more solubilized and form a more intense
344 structure than the control.

345

346 **4. Conclusion**

347 The antioxidant activity and effects of freeze- and oven-dried winter mushroom powder in
348 beef patties were observed to investigate its potential as a phosphate substitute.

349 Phosphate reduced the cooking loss and lipid oxidation and increased the texture properties
350 including hardness, springiness, cohesiveness, gumminess and chewiness of beef patties. The
351 addition of FDP in beef patties reduced the cooking loss, whereas it had no antioxidant effect.
352 However, the addition of ODP inhibited lipid oxidation without affecting the cooking loss of
353 beef patties. Both FDP and ODP showed no notable effects on the texture of beef patties. These
354 results showed that winter mushroom powder showed different activities based on the drying
355 processes as an alternative to phosphate in beef patties. Furthermore, winter mushroom powder
356 did not reproduce all the actions of phosphate in beef patties. Therefore, FDP and ODP can be
357 used for the partial substitution of phosphate activity in beef patties, and the combined effects
358 with other natural ingredients should be further studied in beef patties.

359

360 **Conflict of interest**

361 The authors declare that they have no conflicts of interest.

362

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372

373 **Ethics approval**

374 This study did not require IRB/IACUC approval because it did not involve human or animal
375 participants.

376

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490 **Tables**

491 **Table 1. Properties of the winter mushroom powder**

Properties	FDP ³	ODP ⁴	SEM ⁵
Total phenolic content (g GAE ¹ /kg)	3.50 ^b	5.45 ^a	0.157
EC ₅₀ ² value of scavenging			
DPPH (g/kg)	16.89 ^a	11.52 ^b	0.105
Reducing power (g/kg)	6.03 ^a	3.63 ^b	0.109
pH	6.73 ^b	7.00 ^a	0.011
L*	87.02 ^a	52.65 ^b	0.165
a*	0.16 ^b	8.78 ^a	0.076
b*	20.98 ^b	23.48 ^a	0.150

492 ¹Gallic acid equivalent.

493 ²Half-maximal effective concentration.

494 ³FDP: freeze-dried winter mushroom powder.

495 ⁴ODP: oven-dried (170°C) winter mushroom powder.

496 ⁵Standard error of the least square mean (n=6).

497 ^{a,b} Different letters in the same row indicate significant differences between means
 498 (p<0.05).

499

500 **Table 2. pH of meat batter and cooking loss of beef patty added with winter**
 501 **mushroom powder**

Treatments ¹	pH	Cooking loss (%)
Control	5.73 ^c	15.20 ^a
BP	6.08 ^a	10.23 ^c
BFW	5.78 ^b	12.93 ^b
BOW	5.76 ^b	14.34 ^a
SEM ²	0.006	0.320

502 ¹Control: beef patty manufactured without sodium pyrophosphate; BP: beef patty
 503 manufactured with sodium pyrophosphate; BFW: beef patty manufactured with 1%
 504 freeze-dried winter mushroom powder; BOW: beef patty manufactured with 1% oven-
 505 dried winter mushroom powder.

506 ²Standard error of the least square mean (n=12).

507 ^{a-c}Different small letters in the same column indicate significant differences between
 508 means (p<0.05).



509 **Table 3. Malondialdehyde and carbonyl contents in the beef patty added with winter**
 510 **mushroom powder**

Treatments ¹	Malondialdehyde content (mg/kg)	Carbonyl content (nmol/mg)
Control	2.16 ^a	1.97
BP	1.60 ^c	1.92
BFW	2.14 ^a	2.20
BOW	1.79 ^b	2.07
SEM ²	0.035	0.091

511 ¹Control: beef patty manufactured without sodium pyrophosphate; BP: beef patty
 512 manufactured with sodium pyrophosphate; BFW: beef patty manufactured with 1%
 513 freeze-dried winter mushroom powder; BOW: beef patty manufactured with 1% oven-
 514 dried winter mushroom powder.

515 ²Standard error of the least square mean (n=12).

516 ^{a-c}Different small letters in the same column indicate significant differences between
 517 means (p<0.05).

518



519 **Table 4. Meat color of the beef patty added with winter mushroom powder**

Treatments ¹	L*	a*	b*
Control	39.57 ^a	6.58 ^b	15.37 ^c
BP	39.56 ^a	6.84 ^b	17.48 ^a
BFW	39.07 ^a	6.87 ^b	16.91 ^b
BOW	37.10 ^b	7.50 ^a	17.03 ^{ab}
SEM ²	0.352	0.126	0.316

520 ¹Control: beef patty manufactured without sodium pyrophosphate; BP: beef patty
 521 manufactured with sodium pyrophosphate; BFW: beef patty manufactured with 1%
 522 freeze-dried winter mushroom powder; BOW: beef patty manufactured with 1% oven-
 523 dried winter mushroom powder.

524 ²Standard error of the least square mean (n=12).

525 ^{a-c}Different letters in the same column indicate significant differences between means
 526 (p<0.05).

527



528 **Table 5. Texture properties of the beef patty added with winter mushroom powder**

Treatments ¹	Hardness (N)	Springiness	Cohesiveness	Gumminess (N)	Chewiness (N)
Control	36.41 ^c	0.50 ^c	0.39 ^b	14.07 ^b	7.01 ^b
BP	76.83 ^a	0.68 ^a	0.45 ^a	34.50 ^a	23.40 ^a
BFW	40.59 ^{bc}	0.55 ^b	0.36 ^b	14.51 ^b	8.02 ^b
BOW	43.66 ^b	0.55 ^b	0.36 ^b	15.56 ^b	8.58 ^b
SEM ²	1.450	0.012	0.009	0.815	0.492

529 ¹Control: beef patty manufactured without sodium pyrophosphate; BP: beef patty
 530 manufactured with sodium pyrophosphate; BFW: beef patty manufactured with 1%
 531 freeze-dried winter mushroom powder; BOW: beef patty manufactured with 1% oven-
 532 dried winter mushroom powder.

533 ²Standard error of the least square mean (n=12).

534 ^{a-c}Different small letters in the same column indicate significant differences between
 535 means (p<0.05).

536

