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## TITLE PAGE

- Korean Journal for Food Science of Animal Resources -

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ARTICLE INFORMATION	Fill in information in each box below
<b>Article Type</b>	Research article
<b>Article Title</b>	Investigation of taste-related compounds and antioxidative profiles of retorted samgyetang made from fresh and dried <i>Cordyceps militaris</i> mushrooms
<b>Running Title (within 10 words)</b>	Effect of <i>Cordyceps militaris</i> mushrooms on quality improvement of retorted samgyetang
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<b>Conflicts of interest</b>  List any present or potential conflicts of interest for all authors.  (This field may be published.)	The authors declare no potential conflict of interest.
<b>Acknowledgements</b>  State funding sources (grants, funding sources, equipment, and supplies). Include name and number of grant if available.  (This field may be published.)	This study was performed with support from the Korea Institute of Planning and Evaluation for Technology in Food, Agriculture and Forestry through an Export Promotion Technology Development Program (617074-05-3-HD220).
<b>Author contributions</b>  (This field may be published.)	Conceptualization: Sung KL.  Data curation: Barido FH, Kim DY.  Formal analysis: Barido FH.  Methodology: Barido FH, Sung KL, Jang A, Pak JI.

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<p><b>Ethics approval (IRB/IACUC)</b></p> <p>(This field may be published.)</p>	<p>This manuscript does not require IRB/IACUC approval because there are no human and animal participants.</p>

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10 This study was performed to investigate the effects of taste-related compounds and  
11 antioxidatve profiles of retorted samgyetang made from fresh and dried *Cordyceps militaris*  
12 mushrooms. A total of 48 carcasses were prepared from commercial broilers (CB; Ross, 4  
13 weeks old) and randomly distributed into eight different treatments. Each treatment group  
14 consisted of 6 chicken carcasses made with the addition of broth in different condition and  
15 concentration of *Cordyceps militaris* mushrooms. The addition concentration was based on  
16 the broth volume (v/w) under either fresh or dried conditions ranging from 0% as a control to  
17 1%, 2% and 3% of *Cordyceps militaris* mushrooms. *Cordyceps militaris* mushrooms  
18 contributed to an improvement of meat tenderness and the antioxidative profile that led to a  
19 greater suppression of lipid oxidation. The addition of *Cordyceps militaris* mushrooms at 2%  
20 could also enrich the flavor and taste-related compounds, particularly the increase in 5'-AMP  
21 and umami-related free amino acid compounds, L-aspartic acid and L-glutamic acid.  
22 Different addition forms of *Cordyceps militaris* mushrooms, particularly fresh or dried  
23 mushrooms, had only small effects on bioactive compounds, where the dried addition could  
24 possibly enrich samgyetang broth with higher cordycepin and adenosine contents than the  
25 fresh addition. Besides, the addition of *Cordyceps militaris* mushrooms in the dried form  
26 could also contribute to a higher antioxidative profile. Eventually, the addition of *Cordyceps*  
27 *militaris* mushrooms with a minimum addition of 2% contributed to an improvement of meat  
28 quality, antioxidative profile and flavor improvement of samgyetang.

29

30 **Keywords:** Samgyetang, *Cordyceps militaris* mushroom, Meat quality, Flavor.

31

## 32 Introduction

33

34 Numerous studies have suggested that the potential health-promoting function of  
35 *Cordyceps militaris* mushrooms is potentially as good as that of the well-known *Cordyceps*  
36 *sinensis* (Jing et al., 2015). This mushroom from the Clavicipitaceae family is widely  
37 cultivated in East Asian countries, such as China, Korea and Japan, with a main interest for  
38 its rich nutritional content, particularly amino acids, polysaccharides, cordycepin, adenosine,  
39 adenosine monophosphate and protein (Dong et al., 2013). Studies have reported that the  
40 extract from *Cordyceps militaris* mushroom possesses a broad range of pharmacological  
41 functions, such as anticancer, antiviral, anti-inflammatory, immunomodulatory and  
42 antifatigue activities (Won et al., 2005). The major contributors to most pharmacological  
43 functions in *Cordyceps militaris* mushrooms are regulated by the existence of active  
44 biological compounds, such as cordycepin, adenosine, adenine, polysaccharide and  
45 cordyheptapeptide, as well as D-mannitol (Chen et al., 2012). In today's modern and busy  
46 lives, the utilization of *Cordyceps militaris* mushrooms, as an excellent nutritious food, for  
47 the healthy improvement of retorted samgyetang is considered helpful for consumer health  
48 (Jayasena et al., 2014).

49 Samgyetang is a traditional Korean chicken soup made with numerous health-promoting  
50 ingredients, such as glutinous rice, ginseng (*Panax ginseng* C. A. Meyer), garlic (*Allium*  
51 *sativum* L. var. *pekinense*), and jujube (*Ziziphus jujuba* Miller), in which internal organs from  
52 chicken are removed (Chen et al., 2012). Most traditional Korean people consume  
53 samgyetang during the summer season for health purposes. However, with the more  
54 advanced processing technology, the utilization of retort pouches will extend the preservation  
55 period of samgyetang. Numerous studies have mentioned that the health-promoting function  
56 of samgyetang comes from the ingredients used. Ginseng may improve healthy function by

57 lowering blood pressure and glucose levels, reinforcing insulin function, and increasing  
58 antitumor activity and antistress activity (Dong et al., 2013). It is expected that the addition of  
59 functional compounds such as *Cordyceps militaris* mushrooms would promote a healthier  
60 retorted samgyetang product.

61 In terms of bioactive compounds, efforts are made either chemically or physically with the  
62 intention of improving their presence within a wide variety of plants and mushrooms.  
63 Physically, drying is a common method for the improvement of bioactive compounds, since it  
64 can be applied in a vast array of plants and may lead to positive changes in chemical contents  
65 and pharmacological properties (Meng et al., 2008). Drying positively changes the chemical  
66 contents, especially of bioactive compounds, such as thymol in thyme (Diaz et al., 2000),  
67 eugenol in bay leaves (Venskutonis, 1997), lycopene in tomato (Chen et al., 2000), and even  
68 adenosine and cordycepine in *Cordyceps militaris* mushrooms (Wu et al., 2019). However, in  
69 some cases, the drying process has a detrimental effect on bioactive compounds due to the  
70 loss of polysaccharides, polyphenols or flavonoids during the drying stage (Elhamirad and  
71 Zamanipoor, 2012).

72 Flavor is an important factor that plays an important role in eating satisfaction and  
73 eventually promotes increased consumption (Chiang and Yen, 2007). In chicken soup,  
74 including samgyetang, flavor variations are mainly affected by 5'-nucleotides, free amino  
75 acids, soluble sugars and volatile compounds (Jayasena et al., 2015). Several studies were  
76 performed to improve samgyetang flavor, such as through the increase in soluble protein  
77 dissolution (Kong et al., 2017) and the addition of several herbs and spices (Jeong et al.,  
78 2012), and were proven to exhibit better eating satisfaction, especially flavor, than traditional  
79 chicken soup (Kong et al., 2017). The addition of *Cordyceps militaris* mushrooms potentially  
80 improves the quality traits of samgyetang. This is due to its rich umami taste from ingredients  
81 such as MSG-like amino acids, including glutamic and aspartic acids, and 5'-nucleotides,

82 including 5'-adenosine monophosphate (5'-AMP), 5'-guanosine monophosphate (5'-GMP)  
83 and 5'-inosine monophosphate (5'-IMP) (Dermiki et al., 2013).

84 Limited information is accessible related to the effects of *Cordyceps militaris* mushroom  
85 addition, especially the effects of taste-related compounds, antioxidative profile and sensory  
86 properties of either fresh or dried *Cordyceps militaris* mushrooms, on retorted samgyetang.  
87 Therefore, the aim of this research was to investigate the taste-related compounds and  
88 antioxidative profile of retorted samgyetang made with the addition of fresh and dried  
89 *Cordyceps militaris* mushrooms.

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## 91 **Materials and Methods**

### 92 **Sample preparation**

93 A total of 48 carcasses were prepared from commercial broilers (CB; Ross, 4 weeks old)  
94 and were purchased 24 h post slaughter from a local slaughterhouse, with an average weight  
95 of  $550 \pm 20$  g. Each samgyetang pouch was filled with  $450 \pm 50$  ml of broth that had been  
96 previously boiled for 45 min. The boiled broth was prepared with the addition of samgyetang  
97 ingredient pouches consisting of 5 g *Astragalus membranaceus* root, 8.5 g of Mulberry  
98 branch, 8 g of *Kalopanax septemlobus* branch, 2 g of licorice, 9 g of Siberian ginseng and 6 g  
99 of salt. The broth was also augmented with *Cordyceps militaris* mushrooms under either fresh  
100 or dried conditions. Both fresh and dried *Cordyceps militaris* mushroom was purchased from  
101 Mushtech (Gangwon, Korea). The fresh mushroom was prepared from 5 months old of  
102 *Cordyceps militaris* mushroom, while for the dried one was prepared from 5 months old  
103 mushroom subjected to drying process at  $80^{\circ}$  C for 12 H. The addition concentration was  
104 determined based on the broth volume (w/v) ranging from 0% as a control to 1%, 2% and 3%  
105 of *Cordyceps militaris* mushrooms.

106 To prepare the stuffing, glutinous rice was soaked for 1 h and rinsed prior to use. Garlic,  
107 ginseng, and dried jujube were rinsed with cold water. Approximately 35 g of glutinous rice,  
108 8 g of garlic, 5 g of jujube and 7 g of ginseng were placed in rice paper, which was soaked  
109 with warm water and wrapped. The wrapped rice, breast and thigh meat were then stuffed  
110 into a retort pouch [length 19 cm (length)  $\times$  25 cm (height), polyethylene terephthalate (PET)  
111 = 16  $\mu$ m, aluminum = 9  $\mu$ m, nylon = 15  $\mu$ m, polypropylene (PP) = 100  $\mu$ m] gifted from  
112 Sunbong Food (Incheon, Korea). The pouches were then sealed (WB-1150VP; Woobin Tech  
113 Co., Ltd., Incheon, Korea). Two extra pouches were used to measure the  $F_0$  value during the  
114 retorting process. The retort process was performed using a steam-type retort sterilization

115 chamber (Steri-ace, Kyungshan Co., Ltd., Gyeongsan, Korea). The  $f_0$  value of the retorting  
116 process was set to (f0 8).

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118 **Proximate composition**

119 The sample was ground using a food grinder (HMF-1600 PB, Hanil Electric, Seoul, South  
120 Korea) at medium speed for 10 s. The proximate composition was determined according to  
121 the Association of Official Analytical Chemists (AOAC) method (AOAC, 2002). The  
122 percentage of moisture in the sample was determined by drying the samples in an oven at  
123 105 °C for 24 h. The crude fat content was determined according to the ether extraction by  
124 the Soxhlet system. The nitrogen content was determined using the Kjeltex system (2200  
125 Kjeltex Auto Distillation Unit, Foss, Hilleroed, Sweden), and crude protein was calculated as  
126 nitrogen content multiplied by 6.25. Crude ash was determined by burning the samples in the  
127 muffle furnace at 550 °C for 12 h.

128

129 **Cooking loss**

130 Cooking loss is defined as weight loss after being subjected to the retorting process.  
131 Briefly, samples were weighed to obtain an initial weight (W1) before being subjected to  
132 retorting. Samples, in triplicate, were then weighed to obtain the weight after manufacturing  
133 (W2). The percentage of cooking loss was obtained by calculating weight loss (W1-W2)  
134 against W1.

135

136 **Shear force value**

137 Samples from cooking loss experiments were subsequently used to measure tenderness  
138 (toughness) of the meat by performing the Warner-Bratzler Shear Force test using TA-XT2i  
139 Plus (Stable Micro Systems, Surrey, UK). The method was performed according to Jeong et  
140 al. (2020) with a slight modification, where the sample was made into a 1.5 cm × 1 cm size. It  
141 was then placed under the V blade and cut with a constant speed through the gap in the

142 instrument table (assay parameters were as follows: pretest speed: 2.0 mm/s; test speed: 1.0  
143 mm/s; posttest speed: 10 mm/s). Each sample was repeated five times.

144

#### 145 **Lipid oxidation**

146 The measurement of lipid oxidation was performed using 2-thiobarbituric acid reactive  
147 substances (TBARS). A sample of 0.5 grams in a 25-mL TBARS test tube was prepared with  
148 three repetitions, and 0.1 g of antioxidant mixture (consisting of 54% propylene glycol, 40%  
149 Tween 20, 3% butylated hydroxytoluene and 3% butylated hydroxyanisole) was transferred  
150 to the tube. Subsequently, 3 mL of 1% thiobarbituric acid in 0.3% NaOH was added to the  
151 mixture. Immediately after vortexing, 17 mL of 2.5% trichloroacetic acid in 36 mM HCl was  
152 added, and the tube was closed. The sample was subjected to heating in a water bath (BW-  
153 20G, Biotechnical Services, Inc., North Little Rock, AR, USA) at a temperature of 100 °C for  
154 30 min. The tube was then immersed in cold water for another 15 min. A 5 mL aqueous  
155 sample was transferred to a new 15 mL centrifuge tube and mixed with 3 mL of chloroform.  
156 The mixture was then subjected to centrifugation at 2,400×g for 30 min at 4 °C (1248R,  
157 Labogene, Lyngø, Denmark) to separate it from the pellet. The absorbance was measured at  
158 532 nm by using a UV-spectrophotometer (UV-mini 1240 PC, Shimadzu Corp., Kyoto,  
159 Japan) against a blank (distilled water was used to replace the sample). Each sample was  
160 repeated three times. Data are expressed in the form of mg malondialdehyde/kg sample.

161

#### 162 **Cordycepin and adenosine**

163 The determination of bioactive compounds from *Cordyceps militaris* mushrooms,  
164 particularly adenosine and cordycepin, was performed according to a method by Wang et al.  
165 (2016) with slight modifications. For the extraction of adenosine and cordycepin, 1.00 g of  
166 freeze-dried sample was mixed with double-distilled water (100 mL) and extracted for 30

167 min in an ultrasonic bath at 60 kHz. Then, the supernatant was filtered through a membrane  
168 filter (0.45  $\mu\text{m}$ ). A high-performance liquid chromatography (HPLC) system (Model 1525,  
169 Waters, USA) furnished with a reverse C18 column (150 $\times$ 4.6 mm) was used for adenosine  
170 and cordycepin analysis. The mobile phase, whose flow rate was 0.5 mL min<sup>-1</sup> (isocratic  
171 elution), was composed of acetonitrile and double-distilled water (20:80, v v<sup>-1</sup>). The column  
172 temperature was 35 °C, and a UV-visible detector was applied for detection at a wavelength  
173 of 260 nm. HPLC adenosine and cordycepin standards (Sigma) were used for quantitation by  
174 the external standard method.

175

#### 176 **Antioxidant activity assay**

177 The determination of antioxidant activity was performed according to a method by Islam  
178 et al. (2016) with a minor modification. Briefly, a reaction mixture of 1 ml 0.15 mM DPPH-  
179 methanol solution, 4 ml methanol, and 2  $\mu\text{l}$  of test samples (10/ml), BHA, BHT, or water  
180 (control) was incubated at room temperature for 30 min, and absorbance values were  
181 measured at 517 nm using a spectrophotometer (V530, Jasco Co., Japan). The experiment  
182 was repeated three times for each compound. Electron donation ability was defined as the  
183 total reduction of absorbance through a spectrophotometer.

184

#### 185 **Taste-related compounds**

186 The method for analyzing 5'-nucleotide contents (adenosine monophosphate, inosine  
187 monophosphate and guanine monophosphate) was modified from the method of Jayasena et  
188 al. (2015). The 5'-nucleotide analysis was carried out using HPLC (Shimadzu Nexera X2  
189 HPLC, Kyoto, Japan) equipped with an SPD-M20A diode array detector (DAD) at a  
190 wavelength of 254 nm. Freeze-dried broth samples (1.00 g) were diluted with distilled water  
191 and filtered using a 0.22- $\mu\text{m}$  RC syringe filter (Phenomenex, Torrance, CA). Analytes were

192 separated using a Synergi Hydro-RP column (150 x 3.0 mm, 4  $\mu$ m; Phenomenex) with  
193 precolumn AQ C18 (4 x 2.0 mm). The mobile phases were A: 20 mM phosphate buffer (pH  
194 5.9) and B: 100% methanol. The gradient program used was as follows: 0–3 min, 0% B; 3–12  
195 min, 0 to 30% B; 12–13.5 min, 30% B; 13.5–16 min, 30 to 0% B. The total analysis time per  
196 sample was 25 min. After every injection, 20% ACN solution and ultrapure H<sub>2</sub>O were used to  
197 rinse the needle. The flow rate was 0.4 mL/min, the injection volume was 5  $\mu$ L, and the  
198 column oven temperature was 25 °C. 5'-Nucleotide concentrations were expressed as mg of  
199 compound per 100 g of cooked matter (mg/100 g FM).

200 Two MSG-related amino acids (L-aspartic acid, L-glutamic acid) were analyzed and  
201 identified simultaneously using the HPLC method described in the manufacturer's technical  
202 notes (Shimadzu Corporation). A Nexera SIL-30AC autosampler with automated  
203 pretreatment functions was used for the derivatization of amino acids into fluorescent  
204 substances. The following derivatization reagents were used: OPA and 3-MPA in 0.1 M  
205 borate buffer, FMOC in acetonitrile, and acidic phosphate buffer (pH 2.1). After the  
206 derivatization procedure, one microliter (1  $\mu$ L) of the derivatized standard or sample was  
207 injected. The solvents used in the gradient program were A: 20 mM phosphate buffer (pH  
208 6.5) and B: 45/40/15 ACN/MeOH/H<sub>2</sub>O. The gradient was as follows: 0–2 min, 11% B; 2–4  
209 min, 11 to 17% B; 4–5.5 min, 17 to 31% B; 5.5–10 min, 31 to 32.5% B; 10–12 min, 32.5 to  
210 46.5% B; 12–15.5 min, 46.5 to 55% B; 15.5–16 min, 55 to 100% B; 16–19.5 min, 100% B;  
211 19.5–20 min, 100 to 11% B. The total analysis time per sample was 25 min. After every  
212 injection, 80% MeOH and 20% ACN were used to wash the needle. The flow rate was set to  
213 1.0 mL/min, and the column temperature was maintained at 35 °C.

214

215

216 **Statistical analysis**

217 The data results were analyzed by two-way multivariate of variance (MANOVA) using R-version  
218 3.6.1 (The R-foundation for Statistical Computing, Vienna, Austria). The mean value of each group  
219 was separated using Duncan's multiple range test. Differences was considered as significant for p  
220 values lower than 0.05.

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## 223 **Results and Discussion**

### 224 **Proximate composition**

225 Table 1 displays the proximate composition of samgyetang breast and thigh meat after  
226 treatment with either fresh or dried *Cordyceps militaris* mushrooms. Different condition of  
227 *Cordyceps militaris* mushroom and the level of addition had no significant interaction on  
228 overall proximate composition traits ( $p>0.05$ ). In particular, no significant difference ( $p>0.05$ )  
229 was observed for the proximate composition of moisture content in breast and thigh meat.  
230 These results were in line with the study by Triyannanto et al. (2014), who did not find any  
231 difference after applying additional ingredients to samgyetang. The moisture content in this  
232 study was still within the range of previously reported studies (Jeong et al., 2020).

233 The protein, fat and ash percentages were also found to be insignificant for all observed  
234 samples ( $p>0.05$ ). The addition of *Cordyceps militaris* mushrooms at different levels did not  
235 significantly contribute to changes in protein, fat and ash percentages in this study. The  
236 protein percentage for breast meat ranged from 28.1-28.9%, while it ranged from 24.1-25.6%  
237 for thigh meat. This value was higher than that found in a previous study by Ali et al. (2007)  
238 and still within the range reported in a study by Jeong et al. (2020). However, no effect was  
239 observed ( $p>0.05$ ) by *Cordyceps militaris* mushrooms in this study, which might be due to  
240 the small addition percentage.

241

### 242 **Cooking loss**

243 Cooking loss can be defined as the percentage of cooking yield loss after being subjected  
244 to processing stages, and it significantly correlates with economic traits (Barido et al., 2020).  
245 Cooking loss is closely correlated with the capability of myofibrillar protein to retain muscle  
246 water during processing. Therefore, meat quality traits are important for meat shrinkage  
247 prevention. Cooking loss also has a strong relationship with eating satisfaction since muscle

248 water contributes to the juiciness level of meat. Higher cooking loss will lead to less meat  
249 product juiciness (Jeong et al., 2020). Regarding cooking loss, we did not observe a  
250 statistically significant difference ( $p>0.05$ ) either for the condition of *Cordyceps militaris*  
251 mushroom added and the level of additions, which implies that the addition of *Cordyceps*  
252 *militaris* mushrooms did not significantly affect the cooking yield percentage. The percentage  
253 of cooking loss ranged from 24.4% to 26.1%.

254

### 255 **Shear force value**

256 Meat tenderness is directly correlated with the acceptability of meat by consumers and  
257 leads to consumers' repurchase intent (Kemp and Wheeler, 2011). Tenderness is determined  
258 by complex factors, including muscle water, the degradation of muscle skeleton proteins and  
259 protease enzymes that work postmortem (He et al., 2019). Therefore, several attempts were  
260 made to improve the tenderness level to increase the eating quality. In this study, *Cordyceps*  
261 *militaris* mushrooms were added at different levels to improve the tenderness of samgyetang  
262 meat.

263 As seen in Table 2, it was found that the tenderness could be improved by the addition of  
264 *Cordyceps militaris* mushrooms. The shear force value, which reflects the degree of meat  
265 tenderness, seemed to be significantly affected by the addition of *Cordyceps militaris*  
266 mushrooms at a level of more than 2%. Both breast and thigh meat with the addition of  
267 *Cordyceps militaris* mushrooms at levels of 2% and 3% in fresh and dried conditions  
268 contributed to a lower shear force value compared to the control and 1% treatments. However,  
269 no significant difference was found ( $p>0.05$ ) between fresh and dried *Cordyceps militaris*  
270 mushroom addition. The increment in tenderness level was seemed only affected by the level  
271 of *Cordyceps militaris* mushroom addition as reflected by no significant interaction effect  
272 ( $p>0.05$ ) between different condition and addition level of *Cordyceps militaris* mushroom. The

273 tenderization effect on both breast and thigh meat might be due to the existence of adenosine  
274 monophosphate within the *Cordyceps militaris* mushroom, which in turn leads to the  
275 increased activation of protease enzymes (Wang et al., 2015). The addition of adenosine  
276 monophosphate is often used to improve meat tenderness (Wang et al., 2013).

277

## 278 **Lipid oxidation**

279 TBARS measures the degree of lipid deterioration from the sample. It serves to understand  
280 the concentration of malondialdehyde, a marker for oxidative stress (Mc Millin, 2008).  
281 Therefore, it can be a useful assay for the determination of a product's rancidity (Das et al.,  
282 2010). Table 3 displays the lipid oxidation rate of the samgyetang meat treated with the  
283 addition of *Cordyceps militaris* mushrooms. The malondialdehyde content as an oxidative  
284 stress marker was significantly suppressed ( $p < 0.05$ ) by the addition of *Cordyceps militaris*  
285 mushrooms at levels of 2% and 3%. However, no significant effect was recorded ( $p > 0.05$ )  
286 from a different condition of *Cordyceps militaris* mushroom added. In addition, insignificant  
287 interaction effect between different conditions and addition mushroom level ( $p > 0.05$ ) was  
288 observed on malondialdehyde content. *Cordyceps militaris* mushroom extracts could be a  
289 strong source of natural antioxidants. The major contributors to most pharmacological  
290 functions in *Cordyceps militaris* mushrooms are regulated by the existence of active  
291 biological compounds, such as cordycepin, adenosine, adenine, polysaccharide and  
292 cordyheptapeptide, as well as D-mannitol (Jayasena et al., 2014). This finding was also  
293 supported by Das et al. (2010) who explained that the strong antiradical potency possessed by  
294 *Cordyceps militaris* might be the basis of their strong therapeutic efficacy in traditional  
295 medicine. However, no effect on the lipid oxidation rate of both breast and thigh meat was  
296 found for fresh or dried *Cordyceps militaris* mushroom addition ( $p > 0.05$ ).

297



298 **Antioxidant activity**

299 DPPH is known as a stable radical at room temperature that accepts electrons or hydrogen  
300 radicals to become a stable diamagnetic molecule (Islam et al., 2016). It has been used to  
301 determine the antioxidant activity of various neutral products. The effect of antioxidants on  
302 DPPH has been thought to be due to their hydrogen donating ability (Adebayo et al., 2012).  
303 Antioxidant content within a product is an important variable for consumers because higher  
304 antioxidant levels positively correlate with health-promoting functions after consumption.  
305 The effect of *Cordyceps militaris* mushroom addition, either fresh or dried, at different  
306 concentrations on the antioxidant activity of retorted samgyetang is shown in Table 3. The  
307 highest scavenging activities were observed with the addition of dried *Cordyceps militaris*  
308 mushrooms at a level of 3% in both breast and thigh samples, with values of 59.96% and  
309 50.16%, respectively. The lowest scavenging activities were recorded for the control sample,  
310 with values of 32.92% and 34.92% for thigh and breast meat, respectively. The addition of  
311 *Cordyceps militaris* mushrooms at a level of more than 2% significantly improved the  
312 antioxidant activities of retorted samgyetang, as noted by a significant difference from the  
313 control and 1% addition treatments ( $p < 0.05$ ). *Cordyceps militaris* mushrooms under dried  
314 conditions contributed to higher antioxidant activities than those under fresh conditions.  
315 Furthermore, this study did not found significant interaction between different condition and  
316 addition level of *Cordyceps militaris* mushroom ( $p > 0.05$ ) on DPPH scavenging activity.  
317 Drying might lead to important changes in several pharmacological-related compounds  
318 (Akinmoladun et al., 2010) through a cell destruction mechanism that inactivates  
319 peroxidation enzymes, releases more short bioactive chains and improves their activities (Yu  
320 et al., 2009). However, no significant difference was found between the 2% and 3%  
321 treatments.

322

### 323 **Cordycepin and Adenosine content**

324 For centuries, *Cordyceps militaris* mushrooms have been widely used as versatile  
325 medicines for curing diseases, including liver disease, renal dysfunction, hyperlipidemia, and  
326 hyperglycemia (Zhu et al., 2013). Moreover, bioactive compounds, mainly cordycepin,  
327 adenosine, adenine, polysaccharide and cordyheptapeptide, as well as D-mannitol, are  
328 essential for immunostimulation, a mechanism of improving body defense in elderly people  
329 as well as in cancer patients (Katsube et al., 2009). This study investigated the functional  
330 effect of samgyetang products after the addition of either fresh or dried *Cordyceps militaris*  
331 mushroom, especially the existence of major bioactive compounds, mainly cordycepin and  
332 adenosine, after cooking. As displayed in Table 4, the higher concentration of *Cordyceps*  
333 *militaris* mushroom addition significantly improved the contents of bioactive compounds  
334 within the broth sample. For the cordycepin content, the highest level was observed in the  
335 treatment with the addition of dried *Cordyceps militaris* mushrooms at 3% with 0.66 mg/g db.  
336 The dried *Cordyceps militaris* mushroom addition had a higher level of cordycepin than the  
337 fresh mushroom addition ( $p < 0.05$ ). However, insignificant interaction between different  
338 conditions and an additional level of *Cordyceps militaris* mushroom ( $p > 0.05$ ) was  
339 documented, which implies that each treatment variable had a certain effect on samgyetang.  
340 High temperature promoted cell wall destruction. This destruction of cells contributed to the  
341 exposure of the substrates and the enzymes, while the decrease in moisture content also  
342 increased their concentrations. The autocatalytic oxidation triggered by the oxygen contact  
343 and decrease in moisture content promoted the increase in cordycepin content (Ke et al.,  
344 2011). It also revealed that cordycepin is apparently thermally stable. In addition, a similar  
345 trend was found for adenosine, where the highest addition level of *Cordyceps militaris*  
346 mushrooms had the highest adenosine content among the samples. However, for the

347 adenosine content, no difference was found between fresh and dried additions in the  
348 samgyetang broth sample.

349

### 350 **Taste-related compounds**

351 Flavor plays an important role in eating satisfaction and eventually promotes more  
352 consumption (Chiang and Yen, 2007). In samgyetang, flavor variations are mainly affected  
353 by 5'-nucleotides, free amino acids, soluble sugars and volatile compounds (Jayasena et al.,  
354 2015). However, even after being subjected to the processing stage, other essential nutrients,  
355 such as carbohydrates, lipids, micronutrients and soluble proteins, are less dissolved into  
356 broth (Takakura et al., 2014; Zhang et al., 2017; Beluhan and Ranogajec, 2011). Therefore,  
357 this study aimed to enrich the Samgyetang flavor through the addition of *Cordyceps militaris*  
358 mushroom. Since, aside from its nutrient contents, mushrooms are consumed because of their  
359 unique taste and aroma (Tsai et al., 2008; Mau et al., 1998). The effect of *Cordyceps militaris*  
360 mushrooms on taste-related compounds in this study is shown in Table 5. Adenosine  
361 monophosphate (5'-AMP) was strongly affected by the addition of *Cordyceps militaris*  
362 mushrooms with no significant difference between fresh and dried mushroom addition. In  
363 this study, the AMP in 2% and 3% was significantly higher compared to that of control and 1%  
364 group, while regardless the condition and addition level of *Cordyceps militaris* mushrooms,  
365 inosine monophosphate was not significantly affected. It might indicate that high content of  
366 AMP within the *Cordyceps militaris* mushroom could contribute to a higher generation of  
367 meat AMP. The expected mechanism is that exogenous AMP, which penetrates into the meat  
368 muscle was not completely converted into IMP and remain unchanged from a state of AMP  
369 residue. AMP is one of the major bioactive compounds within the *Cordyceps militaris*  
370 mushroom with a broad range of pharmacological functions (Gamage et al., 2018). Through  
371 adenosine monophosphate deaminase (AMPD) pathway, AMP could be converted into

372 ammonia and IMP with the rate of conversion is depend on various factors including heat  
373 (Wang et al., 2015). In addition, guanosine monophosphate was not affected regardless the  
374 concentration of *Cordyceps militaris* mushroom added ( $p>0.05$ ). There was no significant  
375 interaction effect between different conditions and an additional level of *Cordyceps militaris*  
376 mushroom ( $p>0.05$ ) on taste-related 5'-nucleotide compounds in this study.

377 L-Aspartic acid and L-glutamic acid were the predominant free amino acids related to  
378 MSG-like flavor in this study. As seen in Table 6, compared to the control and 1% addition  
379 treatments, L-aspartic acid was significantly affected by the addition of either fresh or dried  
380 *Cordyceps militaris* mushrooms at levels of 2% and 3% ( $p<0.05$ ). In contrast, the highest  
381 concentration of L-glutamic acid was observed in the treatment with 3% of either fresh or  
382 dried *Cordyceps militaris* mushrooms ( $p<0.05$ ). The L-glutamic acid level was 9.92 g/100 g  
383 dry weight and 9.90 g/100 g dry weight for fresh and dried mushrooms, respectively.  
384 However, this study did not found any significant difference between the condition and the  
385 addition level of mushroom added ( $p>0.05$ ) on both L-Aspartic acid and L-glutamic acid.  
386 Numerous studies have mentioned that mushrooms are rich in umami flavors (Zhang et al.,  
387 2013). The umami taste comes from the rich contents of sodium salts, namely, glutamic acids  
388 and aspartic acids, also known as umami amino acids (Yang et al., 2001). Studies have also  
389 characterized other free amino acids as having a sweet, bitter or neutral taste (Zhang et al.,  
390 2013). Therefore, apart from giving the umami taste, mushroom addition into samgyetang  
391 could also possibly enrich the overall flavor of samgyetang.

392

### 393 **Conclusions**

394 This study aimed to investigate the taste-related compounds and antioxidative profile of  
395 retorted samgyetang made with the addition of fresh and dried *Cordyceps militaris*  
396 mushrooms. The addition of *Cordyceps militaris* mushrooms with a minimum addition of 2%

397 contributed to an improvement in meat tenderness and the antioxidative profile that led to a  
398 greater suppression of lipid oxidation. Besides, the utilization of *Cordyceps militaris*  
399 mushrooms as an additional functional ingredient at 2% either in fresh or dried state could  
400 also enrich the flavor and taste-related compounds, as reflected by the increase in 5'-AMP  
401 and umami-related free amino acid compounds, especially L-aspartic acid and L-glutamic  
402 acid. Different addition forms of *Cordyceps militaris* mushrooms, particularly fresh or dried  
403 mushrooms, had only small effects on bioactive compounds, where the dried addition could  
404 possibly enrich samgyetang broth with higher cordycepin and adenosine contents than the  
405 fresh addition. In addition, the addition of *Cordyceps militaris* mushrooms in the dried form  
406 could also contribute to a higher antioxidative profile.

407

#### 408 **Acknowledgement**

409

410 This study was performed with support from the Korea Institute of Planning and  
411 Evaluation for Technology in Food, Agriculture and Forestry through an Export Promotion  
412 Technology Development Program (617074-05-3-HD220).

413

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518 dry *Cordyceps militaris* in vivo and in vitro. *J Ethnopharmacol* 149:713-719.

519 Table 1. Effect of fresh and dried *Cordyceps militaris* mushroom addition on proximate composition (%) of retorted Samgyetang

Sample	Variables	Condition	Treatments <sup>1)</sup>				SEM <sup>2)</sup>	
			C	1 %	2 %	3 %		
Breast	Moisture (%)	Fresh	65.9	65.4	65.8	65.6	0.21	
		Dried	65.7	66.22	66.29	66.31	0.41	
	Crude protein (%)	Fresh	28.2	28.8	28.1	28.9	0.52	
		Dried	28.45	28.12	28.53	28.41	0.18	
	Crude fat (%)	Fresh	2.3	2.1	2.4	2.3	0.24	
		Dried	2.14	2.23	2.42	2.43	0.21	
	Ash (%)	Fresh	0.72	0.73	0.74	0.77	0.01	
		Dried	0.61	0.65	0.68	0.62	0.03	
	Thigh	Moisture (%)	Fresh	65.4	64.9	65.3	65.1	0.31
			Dried	65.6	65.1	65.5	65.3	0.71
Crude protein (%)		Fresh	24.7	25.3	24.6	25.4	0.51	
		Dried	24.4	25.9	24.1	25.6	0.51	
Crude fat (%)		Fresh	8.89	8.69	8.99	8.89	0.19	
		Dried	9.02	8.81	9.11	9.00	0.32	
Ash (%)		Fresh	0.91	0.92	0.93	0.96	0.02	
		Dried	0.94	0.95	0.86	0.99	0.06	

520

521 <sup>1</sup>C, control; 1%, addition of 1% (w/v) of either fresh or dried *Cordyceps militaris* mushroom; 2%, addition of 2% (w/v) of either fresh or dried  
522 *Cordyceps militaris* mushroom; 3%, addition of 3% (w/v) of either fresh or dried *Cordyceps militaris* mushroom.

523 <sup>2</sup>SEM, standard error of the mean.

524

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525 **Table 2.** Effect of fresh and dried *Cordyceps militaris* mushroom addition on texture properties and cooking loss of retorted Samgyetang

Sample	Variables	Condition	Treatments <sup>1)</sup>				SEM <sup>2)</sup>
			C	1 %	2 %	3 %	
Breast	Shear force (kg)	Fresh	1.97 <sup>a</sup>	1.98 <sup>a</sup>	1.55 <sup>b</sup>	1.57 <sup>b</sup>	0.11
		Dried	1.92 <sup>a</sup>	1.91 <sup>a</sup>	1.52 <sup>b</sup>	1.54 <sup>b</sup>	0.18
Thigh	Shear force (kg)	Fresh	1.17 <sup>a</sup>	1.08 <sup>a</sup>	0.80 <sup>b</sup>	0.79 <sup>b</sup>	0.10
		Dried	1.13 <sup>a</sup>	1.11 <sup>a</sup>	0.89 <sup>b</sup>	0.82 <sup>b</sup>	0.03
Whole carcass	Cooking loss (%)	Fresh	24.5	25.0	25.1	24.4	0.71
		Dried	25.9	25.2	26.1	26.1	0.91

526 <sup>1)</sup>C, control; 1%, addition of 1% (w/v) of either fresh or dried *Cordyceps militaris* mushroom; 2%, addition of 2% (w/v) of either fresh or dried  
 527 *Cordyceps militaris* mushroom; 3%, addition of 3% (w/v) of either fresh or dried *Cordyceps militaris* mushroom

528 <sup>2)</sup>SEM, standard error of the mean

529 <sup>a-c</sup> Means within each row are significantly different (p<0.05).

530

531 **Table 3.** Effect of fresh and dried *Cordyceps militaris* mushroom addition on TBARS value and antioxidant activity of retorted Samgyetang

Sample	Variables	Condition	Treatments <sup>1)</sup>				SEM <sup>2)</sup>
			C	1 %	2 %	3 %	
Breast	TBARS (MDA mg/kg meat)	Fresh	0.51 <sup>a</sup>	0.49 <sup>a</sup>	0.29 <sup>b</sup>	0.31 <sup>b</sup>	0.02
		Dried	0.51 <sup>a</sup>	0.50 <sup>a</sup>	0.32 <sup>b</sup>	0.37 <sup>b</sup>	0.08
	DPPH (% inhibition)	Fresh	34.92 <sup>b</sup>	30.12 <sup>by</sup>	46.93 <sup>ay</sup>	46.96 <sup>ay</sup>	4.11
		Dried	35.12 <sup>b</sup>	43.92 <sup>bx</sup>	56.93 <sup>ax</sup>	59.96 <sup>ax</sup>	3.46
Thigh	TBARS (MDA mg/kg meat)	Fresh	0.61 <sup>a</sup>	0.59 <sup>a</sup>	0.49 <sup>ax</sup>	0.41 <sup>bx</sup>	0.19
		Dried	0.63 <sup>a</sup>	0.60 <sup>a</sup>	0.30 <sup>by</sup>	0.31 <sup>by</sup>	0.07
	DPPH (% inhibition)	Fresh	32.92 <sup>b</sup>	32.29 <sup>by</sup>	36.18 <sup>aby</sup>	40.87 <sup>ay</sup>	7.21
		Dried	36.11 <sup>b</sup>	37.04 <sup>bx</sup>	38.13 <sup>abx</sup>	50.16 <sup>ax</sup>	5.16

532 <sup>1)</sup>C, control; 1%, addition of 1% (w/v) of either fresh or dried *Cordyceps militaris* mushroom; 2%, addition of 2% (w/v) of either fresh or dried  
533 *Cordyceps militaris* mushroom; 3%, addition of 3% (w/v) of either fresh or dried *Cordyceps militaris* mushroom

534 <sup>2)</sup>SEM, standard error of the mean

535 <sup>a-c</sup> Means within each row are significantly different (p<0.05).

536 <sup>x-y</sup> Means within each column are significantly different (p<0.05).

537

538 **Table 4.** Effect of fresh and dried *Cordyceps militaris* mushroom addition on the existence of cordycepin and adenosine content of retorted  
 539 samgyetang

Sample	Variables	Condition	Treatments <sup>1)</sup>				SEM <sup>2)</sup>
			C	1 %	2 %	3 %	
Broth	Cordycepin (mg/g db)	Fresh	Nd <sup>3)</sup>	0.29 <sup>ay</sup>	0.48 <sup>a</sup>	0.49 <sup>ay</sup>	0.05
		Dried	Nd	0.37 <sup>cx</sup>	0.51 <sup>b</sup>	0.66 <sup>ax</sup>	0.07
	Adenosine (mg/g db)	Fresh	Nd	0.21 <sup>c</sup>	0.38 <sup>b</sup>	0.45 <sup>ay</sup>	0.02
		Dried	Nd	0.22 <sup>c</sup>	0.45 <sup>b</sup>	0.55 <sup>ax</sup>	0.02

540 <sup>1)</sup>C, control; 1%, addition of 1% (w/v) of either fresh or dried *Cordyceps militaris* mushroom; 2%, addition of 2% (w/v) of either fresh or dried  
 541 *Cordyceps militaris* mushroom; 3%, addition of 3% (w/v) of either fresh or dried *Cordyceps militaris* mushroom

542 <sup>2)</sup>SEM, standard error of the mean

543 <sup>3)</sup>ND, Not detected

544 <sup>a-c</sup> Means within each row are significantly different (p<0.05).

545 <sup>x-y</sup> Means within each column are significantly different (p<0.05).

546

547 **Table 5.** Effect of fresh and dried *Cordyceps militaris* mushroom addition on taste related 5'-nucleotide compound of retorted Samgyetang

Sample	Variables	Condition	Treatments <sup>1)</sup>				SEM <sup>2)</sup>
			C	1 %	2 %	3 %	
Broth	5'-AMP (mg/100 g dry weight)	Fresh	0.35 <sup>b</sup>	0.39 <sup>b</sup>	0.81 <sup>a</sup>	0.92 <sup>a</sup>	0.07
		Dried	0.39 <sup>b</sup>	0.39 <sup>b</sup>	0.92 <sup>a</sup>	0.93 <sup>a</sup>	0.11
	5'-IMP (mg/100 g dry weight)	Fresh	0.07	0.08	0.12	0.09	0.01
		Dried	0.07	0.07	0.08	0.09	0.00
	5'-GMP(mg/100 g dry weight)	Fresh	0.22	0.23	0.23	0.31	0.03
		Dried	0.29	0.32	0.33	0.21	0.01

548 <sup>1)</sup>C, control; 1%, addition of 1% (w/v) of either fresh or dried *Cordyceps militaris* mushroom; 2%, addition of 2% (w/v) of either fresh or dried  
 549 *Cordyceps militaris* mushroom; 3%, addition of 3% (w/v) of either fresh or dried *Cordyceps militaris* mushroom

550 <sup>2)</sup>SEM, standard error of the mean

551 <sup>a-b</sup> Means within each row are significantly different (p<0.05).

552



553 **Table 6.** Effect of fresh and dried *Cordyceps militaris* mushroom addition on taste related free amino acid of retorted Samgyetang

Sample	Variables	Condition	Treatments <sup>1)</sup>				SEM <sup>2)</sup>
			C	1 %	2 %	3 %	
Broth	L-aspartic acid (g/100 g dry weight)	Fresh	5.11 <sup>b</sup>	5.38 <sup>b</sup>	6.43 <sup>a</sup>	6.40 <sup>a</sup>	0.15
		Dried	5.34 <sup>b</sup>	5.36 <sup>b</sup>	6.41 <sup>a</sup>	6.48 <sup>a</sup>	0.11
	L-glutamic acid (g/100 g dry weight)	Fresh	9.11 <sup>b</sup>	9.16 <sup>b</sup>	9.19 <sup>b</sup>	9.92 <sup>a</sup>	0.01
		Dried	9.09 <sup>b</sup>	9.12 <sup>b</sup>	9.22 <sup>b</sup>	9.90 <sup>a</sup>	0.12

554 <sup>1)</sup>C, control; 1%, addition of 1% (w/v) of either fresh or dried *Cordyceps militaris* mushroom; 2%, addition of 2% (w/v) of either fresh or dried  
 555 *Cordyceps militaris* mushroom; 3%, addition of 3% (w/v) of either fresh or dried *Cordyceps militaris* mushroom

556 <sup>2)</sup>SEM, standard error of the mean

557 <sup>a-b</sup> Means within each row are significantly different (p<0.05).

558

559