Food Science of Animal Resources

Food Sci. Anim. Resour. 2020 September 40(5):772~784
DOI https://doi.org/10.5851/kosfa.2020.e53

pISSN : 2636-0772 eISSN : 2636-0780
http://www.kosfaj.org

ARTICLE

Investigation of Taste-Related Compounds and Antioxidative Profiles of Retorted Samgyetang Made from Fresh and Dried *Cordyceps militaris* Mushrooms

Farouq Heidar Barido, Aera Jang, Jae In Pak, Do Yeong Kim, and Sung Ki Lee*

Department of Applied Animal Science, College of Animal Life Sciences, Kangwon National University, Chuncheon 24341, Korea

OPEN ACCESS

Received May 31, 2020
Revised July 13, 2020
Accepted July 14, 2020

***Corresponding author** : Sung Ki Lee
Department of Applied Animal Science, College of Animal Life Sciences, Kangwon National University, Chuncheon 24341, Korea
Tel: +82-33-250-8646
Fax: +82-33-251-7719
E-mail: skilee@kangwon.ac.kr

***ORCID**
Farouq Heidar Barido
https://orcid.org/0000-0002-3171-5426
Aera Jang
https://orcid.org/0000-0003-1789-8956
Jae In Pak
https://orcid.org/0000-0003-2563-0642
Do Yeong Kim
https://orcid.org/0000-0002-2882-0735
Sung Ki Lee
https://orcid.org/0000-0002-2989-4787

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

Keywords samgyetang, *Cordyceps militaris* mushroom, meat quality, flavor

Introduction

Numerous studies have suggested that the potential health-promoting function of *Cordyceps militaris* (*C. militaris*) mushrooms is potentially as good as that of the well-known *C. sinensis* (Jing et al., 2015). This mushroom from the Clavicipitaceae family is widely cultivated in East Asian countries, such as China, Korea and Japan, with a

main interest for its rich nutritional content, particularly amino acids, polysaccharides, cordycepin, adenosine, adenosine monophosphate and protein (Dong et al., 2013). Studies have reported that the extract from *C. militaris* mushroom possesses a broad range of pharmacological functions, such as anticancer, antiviral, anti-inflammatory, immunomodulatory and antifatigue activities (Won et al., 2005). The major contributors to most pharmacological functions in *C. militaris* mushrooms are regulated by the existence of active biological compounds, such as cordycepin, adenosine, adenine, polysaccharide and cordyheptapeptide, as well as D-mannitol (Chen et al., 2012). In today's modern and busy lives, the utilization of *C. militaris* mushrooms, as an excellent nutritious food, for the healthy improvement of retorted samgyetang is considered helpful for consumer health (Jayasena et al., 2014).

Samgyetang is a traditional Korean chicken soup made with numerous health-promoting ingredients, such as glutinous rice, ginseng (*Panax ginseng* C. A. Meyer), garlic (*Allium sativum* L. var. *pekinense*), and jujube (*Ziziphus jujuba* Miller), in which internal organs from chicken are removed (Chen et al., 2012). Most traditional Korean people consume samgyetang during the summer season for health purposes. However, with the more advanced processing technology, the utilization of retort pouches will extend the preservation period of samgyetang. Numerous studies have mentioned that the health-promoting function of samgyetang comes from the ingredients used. Ginseng may improve healthy function by lowering blood pressure and glucose levels, reinforcing insulin function, and increasing antitumor activity and antistress activity (Dong et al., 2013). It is expected that the addition of functional compounds such as *C. militaris* mushrooms would promote a healthier retorted samgyetang product.

In terms of bioactive compounds, efforts are made either chemically or physically with the intention of improving their presence within a wide variety of plants and mushrooms. Physically, drying is a common method for the improvement of bioactive compounds, since it can be applied in a vast array of plants and may lead to positive changes in chemical contents and pharmacological properties (Meng et al., 2008). Drying positively changes the chemical contents, especially of bioactive compounds, such as thymol in thyme (Díaz et al., 2002), eugenol in bay leaves (Venskutonis, 1997), lycopene in tomato (Chen et al., 2000), and even adenosine and cordycepine in *C. militaris* mushrooms (Wu et al., 2019). However, in some cases, the drying process has a detrimental effect on bioactive compounds due to the loss of polysaccharides, polyphenols or flavonoids during the drying stage (Elhamirad and Zamanipoor, 2012).

Flavor is an important factor that plays an important role in eating satisfaction and eventually promotes increased consumption (Chiang and Yen, 2007). In chicken soup, including samgyetang, flavor variations are mainly affected by 5'-nucleotides, free amino acids, soluble sugars and volatile compounds (Jayasena et al., 2015). Several studies were performed to improve samgyetang flavor, such as through the increase in soluble protein dissolution (Kong et al., 2017) and the addition of several herbs and spices (Jeong et al., 2012), and were proven to exhibit better eating satisfaction, especially flavor, than traditional chicken soup (Kong et al., 2017). The addition of *C. militaris* mushrooms potentially improves the quality traits of samgyetang. This is due to its rich umami taste from ingredients such as monosodium glutamate (MSG)-like amino acids, including glutamic and aspartic acids, and 5'-nucleotides, including 5'-adenosine monophosphate (5'-AMP), 5'-guanosine monophosphate (5'-GMP) and 5'-inosine monophosphate (5'-IMP) (Dermiki et al., 2013).

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

Materials and Methods

Sample preparation

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

To prepare the stuffing, glutinous rice was soaked for 1 h and rinsed prior to use. Garlic, ginseng, and dried jujube were rinsed with cold water. Approximately 35 g of glutinous rice, 8 g of garlic, 5 g of jujube and 7 g of ginseng were placed in rice paper, which was soaked with warm water and wrapped. The wrapped rice, breast and thigh meat were then stuffed into a retort pouch [20 cm (width)×27 cm (length)]. The pouches were then sealed (WB-1150VP; Woobin Tech., Incheon, Korea). Two extra pouches were used to measure the F_0 value during the retorting process. The retort process was performed using a steam-type retort sterilization chamber (Steri-ace, Gyeongsan, Korea). The f_0 value of the retorting process was set to 8.

Proximate composition

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

Cooking loss

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

Shear force value

Samples from cooking loss experiments were subsequently used to measure tenderness (toughness) of the meat by performing the Warner-Bratzler Shear Force test using TA-XT2*i* Plus (Stable Micro Systems, Surrey, UK). The method was performed according to Jeong et al. (2020) with a slight modification, where the sample was made into a 1.5 cm×1 cm size. It was then placed under the V blade and cut with a constant speed through the gap in the instrument table (assay parameters were as follows: pretest speed: 2.0 mm/s; test speed: 1.0 mm/s; posttest speed: 10 mm/s). Each sample was repeated five times.

Lipid oxidation

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

Cordycepin and adenosine

The determination of bioactive compounds from *C. militaris* mushrooms, particularly adenosine and cordycepin, was performed according to a method by Wang et al. (2016) with slight modifications. For the extraction of adenosine and cordycepin, 1.00 g of freeze-dried sample was mixed with double-distilled water (100 mL) and extracted for 30 min in an ultrasonic bath at 60 kHz. Then, the supernatant was filtered through a membrane filter (0.45 μm). A high-performance liquid chromatography (HPLC) system (Model 1525, Waters, Milford, MA, USA) furnished with a reverse C18 column (150×4.6 mm) was used for adenosine and cordycepin analysis. The mobile phase, whose flow rate was 0.5 mL min^{-1} (isocratic elution), was composed of acetonitrile and double-distilled water (20:80, v v^{-1}). The column temperature was 35℃, and a UV–visible detector was applied for detection at a wavelength of 260 nm. HPLC adenosine and cordycepin standards (Sigma-Aldrich, St. Louis, MO, USA) were used for quantitation by the external standard method.

Antioxidant activity assay

The determination of antioxidant activity was performed according to a method by Islam et al. (2016) with a minor modification. Briefly, a reaction mixture of 1 mL 0.15 mM 1,1-diphenyl-2-picrylhydrazyl (DPPH)-methanol solution, 4 mL methanol, and 2 μL of test samples (10/mL), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), or water (control) was incubated at room temperature for 30 min, and absorbance values were measured at 517 nm using a spectrophotometer (V530, Jasco, Japan). The experiment was repeated three times for each compound. Electron donation ability was defined as the total reduction of absorbance through a spectrophotometer.

Taste-related compounds

The method for analyzing 5’-nucleotide contents (adenosine monophosphate, inosine monophosphate and guanine monophosphate) was modified from the method of Jayasena et al. (2015). The 5’-nucleotide analysis was carried out using HPLC (Shimadzu Nexera X2 HPLC) equipped with an SPD-M20A diode array detector (DAD) at a wavelength of 254 nm. Freeze-dried broth samples (1.00 g) were diluted with distilled water and filtered using a 0.22-μm RC syringe filter (Phenomenex, Torrance, CA, USA). Analytes were separated using a Synergi Hydro-RP column (150×3.0 mm, 4 μm; Phenomenex) with precolumn AQ C18 (4×2.0 mm). The mobile phases were A: 20 mM phosphate buffer (pH 5.9) and B:

100% methanol. The gradient program used was as follows: 0–3 min, 0% B; 3–12 min, 0 to 30% B; 12–13.5 min, 30% B; 13.5–16 min, 30 to 0% B. The total analysis time per sample was 25 min. After every injection, 20% ACN solution and ultrapure H_2O were used to rinse the needle. The flow rate was 0.4 mL/min, the injection volume was 5 μL, and the column oven temperature was 25℃. 5'-Nucleotide concentrations were expressed as mg of compound per 100 g of cooked matter (mg/100 g FM).

Two MSG-related amino acids (L-aspartic acid, L-glutamic acid) were analyzed and identified simultaneously using the HPLC method described in the manufacturer's technical notes (Shimadzu). A Nexera SIL-30AC autosampler with automated pretreatment functions was used for the derivatization of amino acids into fluorescent substances. The following derivatization reagents were used: OPA and 3-MPA in 0.1 M borate buffer, FMOC in acetonitrile, and acidic phosphate buffer (pH 2.1). After the derivatization procedure, 1 μL of the derivatized standard or sample was injected. The solvents used in the gradient program were A: 20 mM phosphate buffer (pH 6.5) and B: 45/40/15 ACN/MeOH/H_2O. The gradient was as follows: 0–2 min, 11% B; 2–4 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 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; 19.5–20 min, 100% to 11% B. The total analysis time per sample was 25 min. After every injection, 80% MeOH and 20% ACN were used to wash the needle. The flow rate was set to 1.0 mL/min, and the column temperature was maintained at 35℃.

Statistical analysis

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

Results and Discussion

Proximate composition

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

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

Cooking loss

Cooking loss can be defined as the percentage of cooking yield loss after being subjected to processing stages, and it

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

Sample	Variables	Condition	Treatments[1)]				SEM
			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

[1)] C, control; 1%, addition of 1% (w/v) of either fresh or dried *C. militaris* mushroom; 2%, addition of 2% (w/v) of either fresh or dried *C. militaris* mushroom; 3%, addition of 3% (w/v) of either fresh or dried *C. militaris* mushroom.

significantly correlates with economic traits (Barido et al., 2020). Cooking loss is closely correlated with the capability of myofibrillar protein to retain muscle water during processing. Therefore, meat quality traits are important for meat shrinkage prevention. Cooking loss also has a strong relationship with eating satisfaction since muscle water contributes to the juiciness level of meat. Higher cooking loss will lead to less meat product juiciness (Jeong et al., 2020). Regarding cooking loss, we did not observe a statistically significant difference ($p>0.05$) either for the condition of *C. militaris* mushroom added and the level of additions, which implies that the addition of *C. militaris* mushrooms did not significantly affect the cooking yield percentage. The percentage of cooking loss ranged from 24.4% to 26.1%.

Shear force value

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

As seen in Table 2, it was found that the tenderness could be improved by the addition of *C. militaris* mushrooms. The shear force value, which reflects the degree of meat tenderness, seemed to be significantly affected by the addition of *C. militaris* mushrooms at a level of more than 2%. Both breast and thigh meat with the addition of *C. militaris* mushrooms at levels of 2% and 3% in fresh and dried conditions contributed to a lower shear force value compared to the control and 1%

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

Sample	Variables	Condition	Treatments[1)]				SEM
			C	1%	2%	3%	
Breast	Shear force (kg)	Fresh	1.97[a]	1.98[a]	1.55[b]	1.57[b]	0.11
		Dried	1.92[a]	1.91[a]	1.52[b]	1.54[b]	0.18
Thigh	Shear force (kg)	Fresh	1.17[a]	1.08[a]	0.80[b]	0.79[b]	0.10
		Dried	1.13[a]	1.11[a]	0.89[b]	0.82[b]	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

[1)] C, control; 1%, addition of 1% (w/v) of either fresh or dried *C. militaris* mushroom; 2%, addition of 2% (w/v) of either fresh or dried *C. militaris* mushroom; 3%, addition of 3% (w/v) of either fresh or dried *C. militaris* mushroom.
[a–c] Means within each row are significantly different ($p<0.05$).

treatments. However, no significant difference was found ($p>0.05$) between fresh and dried *C. militaris* mushroom addition. The increment in tenderness level was seemed only affected by the level of *Cordceps militaris* mushroom addition as reflected by no significant interaction effect ($p>0.05$) between different condition and addition level of *Cordceps militaris* mushroom. The tenderization effect on both breast and thigh meat might be due to the existence of adenosine monophosphate within the *C. militaris* mushroom, which in turn leads to the increased activation of protease enzymes (Wang et al., 2016). The addition of adenosine monophosphate is often used to improve meat tenderness (Wang et al., 2013).

Lipid oxidation

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

Antioxidant activity

DPPH is known as a stable radical at room temperature that accepts electrons or hydrogen radicals to become a stable diamagnetic molecule (Islam et al., 2016). It has been used to determine the antioxidant activity of various neutral products. The effect of antioxidants on DPPH has been thought to be due to their hydrogen donating ability (Adebayo et al., 2012). Antioxidant content within a product is an important variable for consumers because higher antioxidant levels positively

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

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

[1)] C, control; 1%, addition of 1% (w/v) of either fresh or dried *C. militaris* mushroom; 2%, addition of 2% (w/v) of either fresh or dried *C. militaris* mushroom; 3%, addition of 3% (w/v) of either fresh or dried *C. militaris* mushroom.
[a–c] Means within each row are significantly different ($p<0.05$).
[x–y] Means within each column are significantly different ($p<0.05$).
TBARS, thiobarbituric acid reactive substances; DPPH, 1,1-diphenyl-2-picrylhydrazyl.

correlate with health-promoting functions after consumption. The effect of *C. militaris* mushroom addition, either fresh or dried, at different concentrations on the antioxidant activity of retorted samgyetang is shown in Table 3. The highest scavenging activities were observed with the addition of dried *C. militaris* mushrooms at a level of 3% in both breast and thigh samples, with values of 59.96% and 50.16%, respectively. The lowest scavenging activities were recorded for the control sample, with values of 32.92% and 34.92% for thigh and breast meat, respectively. The addition of *C. militaris* mushrooms at a level of more than 2% significantly improved the antioxidant activities of retorted samgyetang, as noted by a significant difference from the control and 1% addition treatments ($p<0.05$). *C. militaris* mushrooms under dried conditions contributed to higher antioxidant activities than those under fresh conditions. Furthermore, this study did not found significant interaction between different condition and addition level of *C. militaris* mushroom ($p>0.05$) on DPPH scavenging activity. Drying might lead to important changes in several pharmacological-related compounds (Akinmoladun et al., 2010) through a cell destruction mechanism that inactivates peroxidation enzymes, releases more short bioactive chains and improves their activities (Yu et al., 2009). However, no significant difference was found between the 2% and 3% treatments.

Cordycepin and adenosine content

For centuries, *C. militaris* mushrooms have been widely used as versatile medicines for curing diseases, including liver disease, renal dysfunction, hyperlipidemia, and hyperglycemia (Zhu et al., 2013). Moreover, bioactive compounds, mainly cordycepin, adenosine, adenine, polysaccharide and cordyheptapeptide, as well as D-mannitol, are essential for immunostimulation, a mechanism of improving body defense in elderly people as well as in cancer patients (Katsube et al., 2009). This study investigated the functional effect of samgyetang products after the addition of either fresh or dried *C. militaris* mushroom, especially the existence of major bioactive compounds, mainly cordycepin and adenosine, after cooking. As displayed in Table 4, the higher concentration of *C. militaris* mushroom addition significantly improved the contents of bioactive compounds within the broth sample. For the cordycepin content, the highest level was observed in the treatment with the addition of dried *C. militaris* mushrooms at 3% with 0.66 mg/g db. The dried *C. militaris* mushroom addition had a higher level of cordycepin than the fresh mushroom addition ($p<0.05$). However, insignificant interaction between different

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

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

[1)] C, control; 1%, addition of 1% (w/v) of either fresh or dried *C. militaris* mushroom; 2%, addition of 2% (w/v) of either fresh or dried *C. militaris* mushroom; 3%, addition of 3% (w/v) of either fresh or dried *C. militaris* mushroom.
[a–c] Means within each row are significantly different ($p<0.05$).
[x–y] Means within each column are significantly different ($p<0.05$).
ND, not detected.

conditions and an additional level of *C. militaris* mushroom ($p>0.05$) was documented, which implies that each treatment variable had a certain effect on samgyetang. High temperature promoted cell wall destruction. This destruction of cells contributed to the exposure of the substrates and the enzymes, while the decrease in moisture content also increased their concentrations. The autocatalytic oxidation triggered by the oxygen contact and decrease in moisture content promoted the increase in cordycepin content (Ke et al., 2011). It also revealed that cordycepin is apparently thermally stable. In addition, a similar trend was found for adenosine, where the highest addition level of *C. militaris* mushrooms had the highest adenosine content among the samples. However, for the adenosine content, no difference was found between fresh and dried additions in the samgyetang broth sample.

Taste-related compounds

Flavor plays an important role in eating satisfaction and eventually promotes more consumption (Chiang and Yen, 2007). In samgyetang, flavor variations are mainly affected by 5'-nucleotides, free amino acids, soluble sugars and volatile compounds (Jayasena et al., 2015). However, even after being subjected to the processing stage, other essential nutrients, such as carbohydrates, lipids, micronutrients and soluble proteins, are less dissolved into broth (Beluhan and Ranogajec, 2011; Takakura et al., 2014; Zhang et al., 2017). Therefore, this study aimed to enrich the samgyetang flavor through the addition of *C. militaris* mushroom. Since, aside from its nutrient contents, mushrooms are consumed because of their unique taste and aroma (Mau et al., 1998; Tsai et al., 2008). The effect of *C. militaris* mushrooms on taste-related compounds in this study is shown in Table 5. Adenosine monophosphate (5'-AMP) was strongly affected by the addition of *C. militaris* mushrooms with no significant difference between fresh and dried mushroom addition. In this study, the AMP in 2% and 3% was significantly higher compared to that of control and 1% group, while regardless the condition and addition level of *C. militaris* mushrooms, inosine monophosphate was not significantly affected. It might indicate that high content of AMP within the *C. militaris* mushroom could contribute to a higher generation of meat AMP. The expected mechanism is that exogenous AMP, which penetrates into the meat muscle was not completely converted into IMP and remain unchanged from a state of AMP residue. AMP is one of the major bioactive compounds within the *C. militaris* mushroom with a broad range of pharmacological functions (Gamage et al., 2018). Through adenosine monophosphate deaminase (AMPD) pathway, AMP could be converted into ammonia and IMP with the rate of convertion is depend on various factors including heat (Wang et al., 2016). In addition, guanosine monophosphate was not affected regardless the concentration of *C. militaris* mushroom

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

Sample	Variables	Condition	Treatments[1)]				SEM
			C	1%	2%	3%	
Broth	5'-AMP (mg/100 g dry weight)	Fresh	0.35[b]	0.39[b]	0.81[a]	0.92[a]	0.07
		Dried	0.39[b]	0.39[b]	0.92[a]	0.93[a]	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

[1)] C, control; 1%, addition of 1% (w/v) of either fresh or dried *C. militaris* mushroom; 2%, addition of 2% (w/v) of either fresh or dried *C. militaris* mushroom; 3%, addition of 3% (w/v) of either fresh or dried *C. militaris* mushroom.
[a,b] Means within each row are significantly different ($p<0.05$).

added ($p>0.05$). There was no significant interaction effect between different conditions and an additional level of *C. militaris* mushroom ($p>0.05$) on taste-related 5'-nucleotide compounds in this study.

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

Conclusion

This study aimed to investigate the taste-related compounds and antioxidative profile of retorted samgyetang made with the

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

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

[1)] C, control; 1%, addition of 1% (w/v) of either fresh or dried *C. militaris* mushroom; 2%, addition of 2% (w/v) of either fresh or dried *C. militaris* mushroom; 3%, addition of 3% (w/v) of either fresh or dried *C. militaris* mushroom.
[a,b] Means within each row are significantly different ($p<0.05$).

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

Conflicts of Interest

The authors declare no potential conflicts of interest.

Acknowledgements

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).

Author Contributions

Conceptualization: Lee SK. Data curation: Barido FH, Kim DY. Formal analysis: Barido FH. Methodology: Barido FH, Jang A, Pak JI, Lee SK. Software: Barido FH, Kim DY. Validation: Jang A, Pak JI, Lee SK. Investigation: Jang A, Pak JI, Lee SK. Writing - original draft: Barido FH. Writing - review & editing: Barido FH, Jang A, Pak JI, Kim DY, Lee SK.

Ethics Approval

This article does not require IRB/IACUC approval because there are no human and animal participants.

References

Adebayo EA, Oloke JK, Ayandele AA, Adegunlola CO. 2012. Phytochemical, antioxidant and antimicrobial assay of mushroom metabolite from *Pleurotus pulmonariuse* LAU 09 (JF736658). J Microbiol Biotechnol Res 2:366-374.

Akinmoladun AC, Obuotor EM, Farombi EO. 2010. Evaluation of antioxidant and free radical scavenging capacities of some Nigerian indigenous medicinal plants. J Med Food 13:444-451.

Ali MS, Kang GH, Yang HS, Jeong JY, Hwang YH, Park GB, Joo ST. 2007. A comparison of meat characteristics between duck and chicken breast. Asian-Australas J Anim Sci 20:1002-1006.

AOAC. 2002. Official methods of analysis. 17th ed. AOAC International, Gaithersburg, MD, USA.

Barido FH, Utama DT, Jong HS, Kim J, Lee CW, Park YS, Lee SK. 2020. The effect of finishing diet supplemented with methionine/lysine and methionine/α-tocopherol on performance, carcass traits and meat quality of Hanwoo steers. Asian-Australas J Anim Sci 33:69-78.

Beluhan S, Ranogajec A. 2011. Chemical composition and non-volatile components of Croatian wild edible mushrooms. Food Chem 124:1076-1082.

Chen B, Jia XB. 2012. Research progress and strategy on material base of fresh herbal medicine.Chin Trad Herbal Drugs 43:592-596.

Chen RY, Wu JJ,Tsai MJ, Liu MS. 2000. Effect of storage and thermal treatments on the antioxidant activity of tomato fruits. J Chin Chem Soc-Taip 38:353-360.

Chiang PD, Yen CT, Mau JL. 2007. Non-volatile taste components of various broth cubes. Food Chem 101:932-937.

Das SK, Masuda M, Sakurai A, Sakakibara M. 2010. Medicinal uses of the mushroom *Cordyceps militaris*: Current state and prospects. Fitoterapia 81:961-968.

Dermiki M, Phanphensophon N, Mottram DS, Methven L. 2013. Contributions of non-volatile and volatile compounds to the umami taste and overall flavour of shiitake mushroom extracts and their application as flavour enhancers in cooked minced meat. Food Chem 141:77-83.

Díaz MMC, Pérez CMS,Cabezudo MD. 2002. Effect of drying method on the volatiles in bay leaf (*Laurus nobilis* L.). J Agric Food Chem 50:4520-4524.

Dong JZ, Ding J, Yu PZ, Lei C, Zheng XJ, Wang Y. 2013. Composition and distribution of the main active components in selenium-enriched fruit bodies of *Cordyceps militaris* link. Food Chem 137:164-167.

Elhamirad AH, Zamanipoor MH. 2012. Thermal stability of some flavonoids and phenolic acids in sheep tallow olein. Eur J Lipid Sci Technol 114:602-606.

Gamage S, Jiro N, Yusuke F, Ohga S. 2018. The effect of the hot water extracts of the *Paecilomyces hepiali* and *Cordyceps militaris* mycelia on the growth of gastrointestinal bacteria. Adv Microbiol 8:490-505.

He J, Fang D, Daodong P, Xiaoqun Z. 2019. Investigation of the relationships between different enzymes and postmortem duck muscle tenderization. Poult Sci 98:6125-6130.

Islam T, Xiaoming Y, Baojun X. 2016. Phenolic profiles, antioxidant capacities and metal chelating ability of edible mushrooms commonly consumed in China. LWT-Food Sci Technol 72:423-431.

Jayasena DD, Jung S, Alahakoon AU, Nam KC, Lee JH, Jo C. 2015. Bioactive and taste-related compounds in defatted freeze-dried chicken soup made from two different chicken breeds obtained at retail. J Poult Sci 52:156-165.

Jayasena DD, Jung S, Kim HJ, Alahakoon AU, Nam KC, Jo C. 2014. Effect of sex on flavor-related and functional compounds in freeze-dried broth made from Korean native chicken. Korean J Food Sci Anim Resour 34:448-456.

Jeong DY, Su JH, Lee SH, Eun JB. 2012. Effect of the dried-medicinal-herbs mixing ratio on the sensory and quality characteristics of Samgyetang for ginseng chicken soup. Korean J Food Preserv 19:696-702.

Jeong HS, Utama DT, Kim J, Barido FH, Lee SK. 2020. Quality comparison of retorted Samgyetang made from white semi-broilers, commercial broilers, Korean native chickens, and old laying hens. Asian-Australas J Anim Sci 33:139-147.

Jing Y, Zhu J, Liu T, Bi S, Hu X, Chen Z, Song L, Lv W, Yu R. 2015. Structural characterization and biological activities of a novel polysaccharide from cultured *Cordyceps militaris* and its sulfated derivative. J Agric Food Chem 63:3464-3471.

Katsube T, Tsurunaga Y, Sugiyama M, Furuno T, Yamasaki Y. 2009. Effect of air drying temperature on antioxidant capacity and stability of polyphenolic compounds in mulberry (*Morus alba* L.) leaves. Food Chem 113:964-969.

Ke L, Zhou J, Lu W, Gao G, Rao P. 2011. The power of soups: Super-hero or team-work? Trends Food Sci Technol 22:492-497.

Kemp CM, Wheeler TL. 2011. Effects of manipulation of the caspase system on myofibrillar protein degradation *in vitro*. J

Anim Sci 89:3262-3271.

Kong Y, Yang X, Ding Q, Zhang YY, Sun BG, Chen HT, Sun Y. 2017. Comparison of non-volatile umami components in chicken soup and chicken enzymatic hydrolysate. Food Res Int 102:559-566.

Mau JL, Lin YP, Chen PT, Wu YH, Peng JT. 1998. Flavor compounds in King oyster mushrooms *Pleurotus eryngii*. J Agric Food Chem 46:4587-4591.

McMillin KW. 2008. Where is MAP Going? A review and future potential of modified atmosphere packaging for meat. Meat Sci 80:43-65.

Meng J, Zong XP, Dong XP. 2008. Study on phalanacological effects of fresh and dry *Houttuynia cordata* Thunb. Lishizhen Med Mater Med Res 19:1315-1316.

Takakura Y, Mizushima M, Hayashi K, Masuzawa T, Nishimura T. 2014. Characterization of the key aroma compounds in chicken soup stock using aroma extract dilution analysis. Food Sci Technol Res 20:109-113.

Triyannanto E, Lee JH, Lee KT. 2014. Effects of sucrose stearate addition on the quality improvement of ready-to-eat Samgyetang during storage at 25℃. Korean J Food Sci Anim Resour 34:683-691.

Tsai SY, Tsai HL, Mau JL. 2008. Non-volatile taste components of *Agaricus blazei*, *Agrocybe cylindracea* and *Boletus edulis*. Food Chem 107:977-983.

Venskutonis PR. 1997. Effect of drying on the volatile constituents of thyme (*Thymus vulgaris* L.) and sage (*Salvia officinalis* L.). Food Chem 59:219-227.

Wang D, Dong H, Zhang M, Liu F, Bian H, Zhu Y, Xu W. 2013. Changes in actomyosin dissociation and endogenous enzyme activities during heating and their relationship with duck meat tenderness. Food Chem 141:675-679.

Wang D, Shaoying D, Zhang M, Geng Z, Sun C, Bian H, Xu W, Zhu Y, Liu F, Wu H. 2016. The effect of adenosine 5'-monophosphate (AMP) on tenderness, microstructure and chemical-physical index of duck breast meat. J Sci Food Agric 96:1467-1473.

Won SY, Park EH. 2005. Anti-inflammatory and related pharmacological activities of cultured mycelia and fruiting bodies of *Cordyceps militaris*. J Ethnopharmacol 96:555-561.

Wu X, Zhang M, Li Z. 2019. Influence of infrared drying on the drying kinetics, bioactive compounds and flavor of *Cordyceps militaris*. LWT-Food Sci Technol 111:790-798.

Yang JH, Lin HC, Mau JL. 2001. Non-volatile taste components of several commercial mushrooms. Food Chem 72:465-471.

Yu R, Yin Y, Yang W, Ma W, Yang L, Chen X, Zhang Z, Ye B, Song L. 2009. Structural elucidation and biological activity of a novel polysaccharide by alkaline extraction from cultured *Cordyceps militaris*. Carbohydr Polym 75:166-171.

Zhang J, Yang Z, Yang Y, Han L, Yu Q, Cao H, Zhang W. 2017. Development of a flavor fingerprint by GC-MS with chemometric method for volatile compounds of yak and yellow cattle bone soup. Food Anal Methods 10:943-954.

Zhang Y, Venkitasamy C, Pan Z, Wang W. 2013. Recent developments on umami ingredients of edible mushrooms: A review. Trends Food Sci Technol 33:78-92.

Zhu SJ, Pan J, Zhao B, Liang J, Wu Z, Yang J. 2013. Comparisons on enhancing the immunity of fresh and dry *Cordyceps militaris in vivo* and *in vitro*. J Ethnopharmacol 149:713-719.