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Functional Cordyceps Coffee Containing Cordycepin and β-Glucan

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ABSTRACT: Cordyceps coffee, containing Cordycepin and β-glucan, was developed to improve quality and functionality of coffee. We evaluated its biological activities and quality characteristics. We treated green coffee beans with mixed extracts from three medicinal mushrooms, Cordyceps mushroom (*Cordyceps militaris*), Phellinus mushroom (*Phellinus linteus*), and Chaga mushroom (*Inonotus obliquus*). Both control [27.00 mg gallic acid equivalent (GAE)/g] and Cordyceps coffee (37.34 mg GAE/g) had higher contents of polyphenol than the Cordyceps mushroom raw materials (23.17 mg GAE/g). 1,1-Diphenyl-2-picrylhydrazyl scavenging activity was higher for both the control and Cordyceps coffee (80.56% and 82.21 %, respectively) compared with Cordyceps mushrooms (62.89%). β-Glucan content was determined by the enzyme method; the raw Cordyceps, Phellinus, and Chaga mushrooms contained 3.79%, 7.06%, and 8.57% β-glucan, respectively. However, β-glucan and total glucan contents were much lower in Cordyceps coffee (2.03% and 3.11%, respectively) than in the raw mushrooms. The amount of Cordycepin, as determined by high performance liquid chromatograph, was 2,274.70 mg/kg for the Cordyceps coffee and 11,533.22 mg/kg for the Cordyceps mushrooms. Through flavor pattern analysis using the electronic nose system, we showed Cordyceps coffee kept the original coffee aroma (similar as the control), and this was not obstructed by the off-flavor of the Cordyceps mushrooms. In conclusion, this study suggests that Cordyceps coffee may be a novel functional coffee containing the functional components Cordycepin and β-glucan.

Keywords: β-glucan, cordycepin, Cordyceps coffee, DPPH, Electronic Nose

INTRODUCTION

In Korea, coffee consumption per capita for individuals ≥20 years of age was approximately 353 cups/year in 2018, which is an increase of 21% from the 291 cups in 2015 (Park et al., 2019). However, the domestic coffee market is gradually becoming oversaturated and consumers' tastes are becoming more sophisticated. Therefore, customer needs are becoming more diversified and subdivided, and there is an increasing demand for specialty coffee and high-quality coffee. Specialty coffee refers to coffee with a rating of 80 or higher (out of 100), as determined by the International Specialty Coffee Association (SCA).

The increase in demand for high-quality coffee has led to various attempts to improve the biological activities and the preferences of coffee in Korea, such as through adding functional materials to coffee. Shin et al. (2013) analyzed the biological activity of coffee beans obtained by solid fermentation with fungal mycelium, whereas Kim et al. (2016) produced green beans by lactic acid bacteria fermentation to improve the antioxidant activity of coffee. Furthermore, Lim et al. (2015) soaked green coffee beans in mulberry extract, and Moon and Kim (2019) created wine germinated coffee to enhance coffee aroma.

To develop a new functional coffee, we chose to focus on medicinal mushrooms. Mushrooms are used as common food ingredients and are recognized as valuable medicines; therefore, there has been increasing interest in the role of their biological activities for preventing diseases (An et al., 2019b). Antioxidant activity, anti-inflammatory, antibacterial, and anti-cancer effects of mushrooms have been previously examined (Barros et al., 2007; Manzi et al., 2001). Typical medicinal mushrooms include Cordyceps mushrooms, Phellinus mushrooms (*Phellinus linteus*), Chaga mushrooms (*Inonotus obliquus*), Agaricus mushrooms (*Agaricus blazei*), *Ganoderma lucidum*, *Hericium erinaceus*, and *Coriolus versicolor*.

Cordyceps (Chinese caterpillar fungus; Dongchunghacho) is a genus of ascomycete fungi (sac fungi), and includes more than 400 species worldwide, notably in Asian countries, including Korea, China, Japan, Bhutan, Viet-

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nam, Thailand, and Nepal (Wang et al., 2008). Two species of Cordyceps, *Paecilomyces japonica* (or Silkworm Cordyceps, *Paecilomyces tenuipes*, and *Isaria japonica*) and *Cordyceps militaris*, are recognized as food ingredients by the Korea Food Standards [Ministry of Food and Drug Safety (MFDS), 2017]. As Cordyceps is used as a typical medicinal mushroom, Cordyceps is commercially cultivated in a variety of media using liquid culture, brown rice, or pupa (Sato and Shimazu, 2002). The functionality of Cordyceps was first studied in 1997 and 2002 (*C. militaris* and *Cordyceps sinensis*); these species are known to lower blood pressure and to suppress tuberculosis and asthma (Fukatsu et al., 1997; Jung and Park, 2002).

Of the physiologically active substances in Cordyceps, Cordycepin (3'-deoxyadenosine, Fig. 1) is currently recognized as the index component. Cordycepin was first isolated from *C. militaris* culture filtrates by Cunningham et al. (1950). The pharmacological effects of Cordycepin, such as antitumor, immunomodulation, anti-inflammatory, antiviral, anti-leukemia, anticancer, antidiabetic, and anti-obesity effects have been revealed, demonstrating the possibility of Cordycepin as a treatment or preventative material against various human diseases (Masuda et al., 2007; Wong et al., 2010; Wu et al., 2011a; Patel and Ingalhalli, 2013).

Phellinus mushrooms (*P. linteus*) are perennial mushrooms of basidiomycetes that grow in old trees in the alpine region and do not reproduce well. Phellinus is a valuable medicine known to be effective against gastric pain, gynecological diseases, and various tumors, with particular effects against digestive system cancer and for improving immunity after surgery (Song and Oh, 2002). Phellinus is rich in minerals such as potassium, calcium, and magnesium. Moreover, Phellinus contains a large amount of the polysaccharide β-glucan, which has roles in activating the immune function of normal cells. The MFDS has identified β-glucan in Phellinus mushroom extracts as an index component; therefore, when extracts contain 87～162 mg/g β-glucan, they are recognized as functional ingredients that can help improve immune function (Real Foods, 2016).

Chaga mushroom (*I. obliquus*) has been used as a traditional medicinal mushroom in Russia, Poland, and the Baltic States for 500 years, predominantly for treatment of pulmonary tuberculosis, liver disease, gastritis, gastrointestinal cancer, cardiovascular disease, and diabetes (Cui et al., 2005). Chaga contains a variety of polyphenols and flavonoids, thus acts as a powerful antioxidant (Kim et al., 2008; Liang et al., 2009). In addition, similar to other mushrooms, Chaga contains various polysaccharides and is rich in β-glucan.

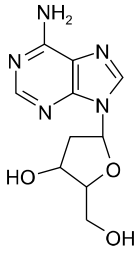

Fig. 1. Chemical structure of Cordycepin.

β-Glucan, one of the major bioactive substances present in several medicinal mushrooms like Phellinus and Chaga, strengthens the immune system and has antitumor effect (Chan et al., 2009; Goodridge et al., 2009). β-Glucan increases the number of white blood cells to improve the immunity of cellular tissues, and acts as an antidiabetic and a regulator of blood pressure (Chandrasekaran et al., 2011).

In this study, we developed a functional coffee from the medicinal mushrooms Cordyceps, Phellinus, and Chaga. We used Cordyceps as the main raw material; however, when Cordyceps raw materials are simply mixed with coffee powder or when the extracts are added to espressos, the coffee aroma is hindered by the unpleasant scent of Cordyceps, which decreases the desirability of the coffee to the consumer. During preliminary experiments, we selected Phellinus and Chaga mushrooms as ancillary ingredients, because these mushrooms could suppress the scent of Cordyceps and provided the functional component β-glucan to the coffee. Finally, we applied a new pretreatment process to the green coffee bean itself using the three mushrooms, which successively produced a functional coffee containing high levels of Cordycepin and β-glucan without an undesirable aroma. The process of development, quality characteristics and biological activities of our Cordyceps coffee are reported in this study.

MATERIALS AND METHODS

Materials

Green coffee beans of the Arabica variety (Kenya Bourbon SL28) were harvested from the Nyeri and regions in Kenya. Coffee beans were purchased from GSC International Co., Ltd. (Seoul, Korea) and washed. Cordyceps mushroom (*C. militaris*) was purchased from Gahwa F&B (Jincheon, Korea), and Phellinus (*P. linteus*) and Chaga mushrooms (*I. obliquus*) were purchased in an Oriental Pharmacist at Gyeongdong Market in Seoul. Folin-Ciocalteu reagent, gallic acid, 1,1-diphenyl-2-picrylhydrazyl (DPPH), and Cordycepin standard (Cordycepin from *C. militaris*) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). β-Glucan assay kits (yeast & mushroom) were purchased from Megazyme Inc. (Chicago, IL, USA), and Proteases M and A were purchased from Amano Enzyme Inc. (Nagoya, Japan). All other reagents were special grade.

Preparation of Cordyceps coffee

Cordyceps coffee beans were prepared and provided by Coffeesidae Co., Ltd. (Hanam, Korea) as detailed in Fig. 2. Phellinus mushroom powder diluted in 5 times (w/w) *d*-water at 80~90°C for 3 h and filtered. Chaga mushroom powder diluted in 5 times (w/w) *d*-water at 50~60°C for 12 h. Phellinus mushroom and Chaga mushroom extracts were mixed at the ratio of 6:4 (w/w). Cordyceps mushroom powder was mixed with 3 times (w/w) *d*-water, and the Phellinus and Chaga mushroom extract mixture were added at a ratio of 6:4. Protease mix (M/A=1:1) was then added [3% (w/w)] to the mixture, and reactions were incubated for 5 days at 50°C. The mixture was then extracted with 3 times (w/w) *d*-water for 6 h at 70~80°C, and filtered to obtain the final Cordyceps complex. The dried coffee beans were immersed in the above Cordyceps complex at a ratio of 2:1 (w/w) for 3 days at 25~33°C. Since the Cordyceps complex was almost completely absorbed into the green coffee beans without residuals, the beans were dried in a hot air dryer (60°C) without additional filtration. The beans were dried until they reached a moisture content of 12%, which was measured by checking every 30 min after the first 18 h of drying.

Roasting and extraction

Cordyceps coffee was roasted using a roaster (THCR-01, Taehwan, Bucheon, Korea) for 14 min at 180°C; these conditions were determined during preliminary experiments. Coffee extracts were finely ground using an automatic coffee grinder (K30ES, Mahlkönig Guatemala Lab, Hamburg, Germany) and 7 g of the ground coffee was extracted in 125 mL distilled water on a hot plate with stirring at 93°C for 4 min; extracts were then straightly filtered, based on the amount of one serving size of typical espresso coffee. These ground and liquid Cordyceps coffee samples were used for experimentation.

Determination of total polyphenol contents

Total polyphenol contents were analyzed using Folin-Ciocalteu reagent, which develops a molybdenum blue color when it is reduced by a polyphenolic substance in

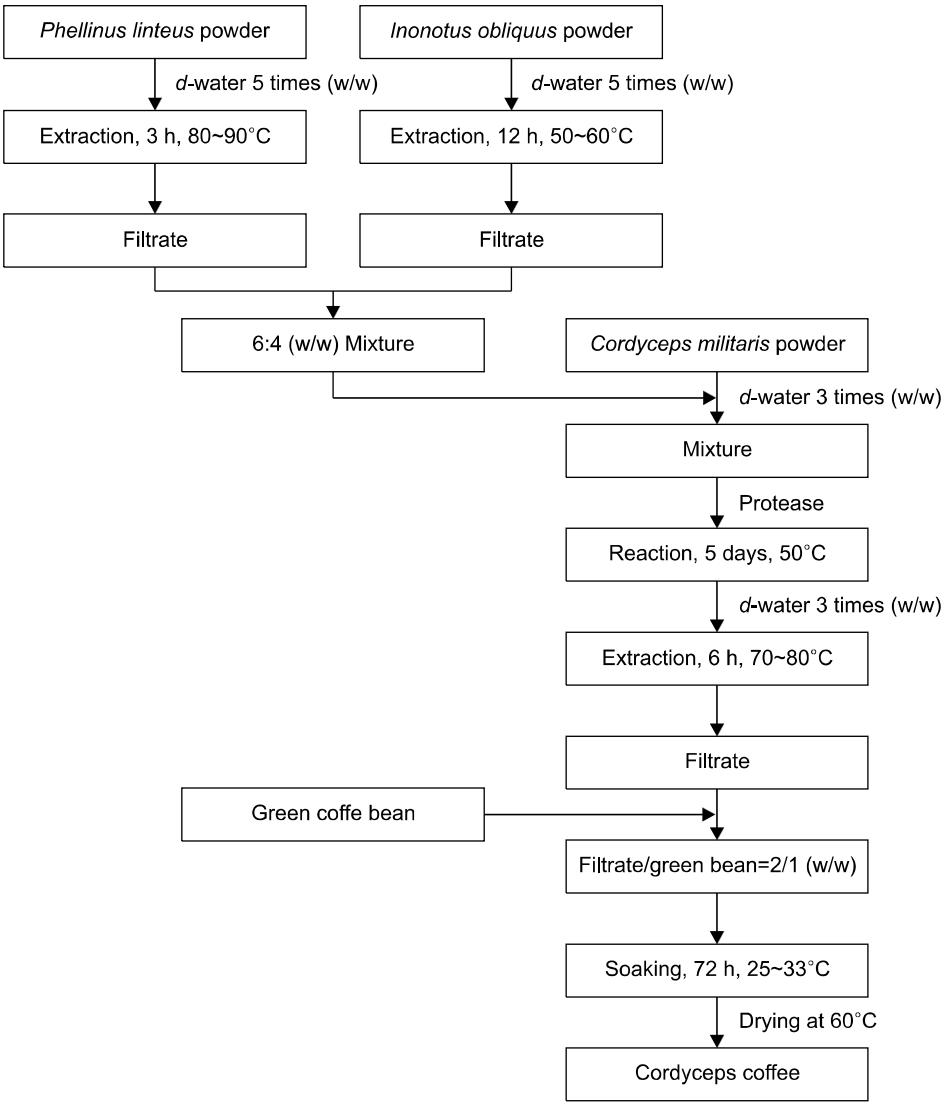

Fig. 2. Schematic diagram for the Cordyceps coffee preparation.

food. Twenty microliters of the coffee extracts were diluted in 1.4 mL *d*-water, then 0.1 mL Folin-Denis reagent was added, and the mixture was vortexed. After 5 min, a saturated solution of 0.3 mL sodium carbonate was added, and the mixture was filled to 2 mL and incubated at 40°C for 1 h. The absorbance was measured at 765 nm using an ultraviolet/visible spectrophotometer (DU 650, Beckman Coulter, Inc., Opa-locka, FL, USA). The standard curve was obtained using gallic acid and the total polyphenol content was expressed as mg gallic acid equivalent (GAE)/g (Song and Park, 2018).

DPPH radical scavenging activity

Antioxidant activity, expressed in electron donating ability [EDA (%)], was analyzed using the DPPH free radical scavenging method. Coffee extract (0.2 mL) was mixed with 0.8 mL of 0.2 mM DPPH solution and vortexed for 10 s. The reaction was incubated in the dark for 30 min and the absorbance was measured at 525 nm. The EDA (%) was calculated as follows (Song and Park, 2018):

$$\text{EDA (\%)} = 1 - \frac{\text{Sample absorbance}}{\text{Control absorbance}} \times 100$$

Determination of (1,3)(1,6)-β-glucan content

The content of β-glucan was determined by subtracting the content of α-glucan from the total glucan content, as measured using a Megazyme kit (yeast & mushroom) following the method of McCleary and Draga (2016).

***Measurement of total glucan content*:** To quantify total glucan, samples (0.1 g) and 1.5 mL of 37% v/v (12 M) hydrochloric acid were added to tubes. Tubes were capped, stirred on a vortex mixer, and placed in a water bath at 30°C for 45 min. Ten milliliters of *d*-water was then added to each tube, and the contents were vigorously stirred on a vortex mixer for 10 s. The tubes were incubated in a boiling-water bath (~100°C) for 2 h and then cooled to room temperature. Ten milliliters of 2 M KOH was then added and mixed well. The contents of each tube were quantitatively transferred into 100 mL volumetric flasks and the volume was adjusted to 100 mL with 200 mM sodium acetate buffer (pH 5). After centrifuging at 1,500 *g* for 10 min, 0.1 mL of the supernatant was incubated with 0.1 mL of a mixture of *exo*-1,3-β-glucanase (100 U/mL) plus β-glucosidase (4 U/mL) in 200 mM sodium acetate buffer at 40°C for 60 min. Glucose standards and reagent blanks were measured concurrently. For a reagent blank, 0.2 mL of acetate buffer was added and for the D-glucose standard, 0.1 mL of the D-glucose standard and 0.1 mL of the acetate buffer were added. Finally, 3 mL of glucose oxidase/peroxidase (GOPOD) reagent enzyme was added to the mixture, the mixture was incubated at 40°C for 20 min, and the absorbance was measured at 510 nm (all reagents and assay procedures used are available/described in the kit Cat. No. K-YBGL, Megazyme Inc.).

***Measurement of α-glucan*:** To quantify α-glucan, 0.1 mL and 2 mL ice-cold 2 M KOH were added to a tube and mixtures were stirred using a magnetic stirrer in an ice-water bath for 20 min to dissolve the starch/glycogen. Eight milliliters of 1.2 M sodium acetate buffer (pH 3.8) was then added and mixed using a vortex. A total of 0.2 mL of amyloglucosidase (1,630 U/mL) and invertase (500 U/mL) (Megazyme assay kit, Cat. No. K-YBGL, Megazyme Inc.) was immediately added and mixed, and the tubes were incubated at 40°C in a water bath for 30 min. After centrifuging the mixture at 11,000 *g* for 3 min, 0.1 mL of the supernatant was removed and added to 0.1 mL of 200 mM sodium acetate buffer and 3 mL of GOPOD and the mixture was incubated at 40°C for 20 min. The absorbance was measured at 510 nm.

***Calculation for β-glucan*:** Total glucan and α-glucan contents (%, w/w) were calculated from the absorbances obtained when glucose (1 mg/mL) reacted with GOPOD reagent. Finally, the β-glucan content was determined by calculating the difference between α-glucan and total glucan using the following Megazyme Mega-Calc™ equations (Megazyme Inc.):

Total glucan (%, w/w)
$= \Delta E \times F \times 100/0.1 \times 1/1{,}000 \times 100/W \times 162/180$
$= \Delta E \times F/W \times 90$

α-Glucan (%, w/w)
$= \Delta E \times F \times 1{,}000$ (or 103) $\times 1/1{,}000 \times 100/W \times 162/180$
$= \Delta E \times F/W \times 90$ (final volume 100 mL)
$= \Delta E \times F/W \times 9.27$ (final volume 10.3 mL)

β-Glucan = (Total glucan) − (α-glucan)

here,
ΔE = Sample absorbance − Blank absorbance
F = μg D-glucose conversion factor
= 100 / D-glucose standard absorbance
W = sample weight

Determination of Cordycepin by high performance liquid chromatograph (HPLC)

Analysis of Cordycepin was performed by following the methods of Oh et al. (2003) and Xie et al. (2010), with modifications. Ground coffee samples was mixed with deionized water, sonicated for 30 min, and then filtered with a 0.22 μm syringe filter. Instrumental conditions for HPLC were shown in Table 1. A calibration curve was obtained using Cordycepin standards at each concentrations.

Table 1. High performance liquid chromatograph (HPLC) conditions for Cordycepin analysis

HPLC system	Thermo Scientific Dionex UltiMate 30005 Standard Systems (Thermo Fisher Scientific, San Diego, CA, USA)
Column	VDSphere C18 column (4.6×250 mm, 5 μm; VDS Optilab, Berlin, Germany)
Detector	UV detector, 260 nm
Injection volume	10.0 μL
Flow rate	1.0 mL/min
Temperature	30°C
Mobile phase	Water (A), MeOH (B)
Gradient condition	0~0.5 min (A 10, B 90), 0.5~15 min (A 10→30, B 90→70), 15~20 min (A 30→90, B 70→10), 20~25 min (A 90, B 10), 25~26 min (A 90→10, B 10→90), 26~30 min (A 10, B 90)

Analysis of flavor patterns by Electronic Nose

The flavor pattern was analyzed with an Electronic Nose system (Model 7100 Fast GC Analyzer, Electronic Sensor Technology, Newbury Park, CA, USA) equipped with a surface acoustic wave (SAW) sensor based on the gas chromatograph (GC) system. Three different samples were prepared: control (non-treated coffee), Cordyceps coffee, and simple mixture of 30% Cordyceps powder in ground coffee. The ground coffees were directly analyzed, and the espresso were analyzed when freshly brewed in a semi-automatic coffee machine (EC 680M, De' Longhi Appliances, Treviso, Italy). Each sample was placed in a 40 mL vial (Supelco Inc., Bellefonte, PA, USA) and sealed with Teflon-coated septa (PTFE/silicone septa, Supelco Inc.). When samples were at equilibrium, the volatile components were injected through the sample injection port for 20 s. High purity helium was used as the carrier gas, and the DB-624 capillary column (0.33 μm, 0.25 mm ×1 m, J&W Scientific, Folsom, CA, USA) was heated from 30°C to 120°C at a rate of 3°C/s. The run time was set to 30 s, and the temperatures of the sensor, column, valve, and inlet were set to 30°C, 40°C, 50°C, and 50°C, respectively.

Statistics

Statistical analysis was performed using the SPSS statistical program (Statistical Package for the Social Science, ver. 12.0, SPSS Inc., Chicago, IL, USA). After performing ANOVA, the significance between each measurement was determined using Duncan's multiple range tests ($P<0.05$).

RESULTS AND DISCUSSION

Total polyphenol contents

The total polyphenol contents are shown in Table 2. Both the control (27.00 mg GAE/g) and Cordyceps coffee (37.34 mg GAE/g) had higher total polyphenol content than Cordyceps mushrooms (23.17 mg GAE/g). Coffee beans are rich in polyphenols, but the polyphenol content of Cordycepin coffee was further increased by Cordyceps mushroom. Suh et al. (2015) reported that the total phenolic contents of fresh green coffee beans from Brazil, Indonesia, and Kenya are 21.3, 20.6, and 23.1 mg GAE/g, respectively. These values are consistent with ours. In addition, when the coffee was slightly roasted, the polyphenol content in coffee from Brazil and Kenya increased to 36.2 and 26.1 mg GAE/g, respectively (Suh et al., 2015).

Polyphenol, characterized by the presence of two or more phenol groups per molecule, is a very abundant compounds in plants where it usually acts as a plant pigment and a functional component. Thousands of polyphenols exist; typical examples include catechins in green tea, resveratrol in grapes, and quercetin in onions. Extracts of medicinal mushrooms, such as Cordyceps, Chaga, Phellinus, and Youngji (*Ganoderma lucidum*) mushrooms, are reported to have a high content of polyphenols and flavonoids, which inhibit growth of human cancer cells and show antioxidant effects (Qi et al., 2013).

DPPH radical scavenging activity

The antioxidant activity of the control, Cordyceps coffee and Cordyceps mushrooms is shown in Table 2. DPPH is a relatively stable free radical. When DPPH reacts with a substance exhibiting antioxidant activity, it is reduced or

Table 2. Total polyphenol content, electron donating ability and Cordycepin content

Sample	Total polyphenol (mg GAE/g)	EDA (%)	Cordycepin (mg/kg)
Control	27.00±0.98^{b}	80.56±2.14^{b}	N.D.
Cordyceps coffee	37.34±1.54^{c}	82.21±1.95^{b}	2,274.70±9.87
Cordyceps mushroom	23.17±1.25^{a}	62.89±3.26^{a}	11,533.22±15.02

Data are presented as the mean±SD (n=3).
The different letters in the same column (a-c) indicate significant differences ($P<0.05$).
GAE, gallic acid equivalent; EDA, electron donating ability; N.D., not detected.

eliminated, resulting in a decrease of its dark purple color. Therefore, DPPH is widely used to measure antioxidant capacity (Gardner and Fridovich, 1991). The DPPH radical scavenging activity of the control and Cordyceps coffee (80.56% and 82.21%, respectively) was higher compared with that of Cordyceps mushrooms (62.89%); although the DPPH radical scavenging activity was slightly higher in Cordyceps coffee than the control, these did not significantly differ. In the study on several medicinal mushrooms, the DPPH radical scavenging activity was highest in Phellinus mushrooms (90%). In addition, DPPH radical scavenging activity was higher in Chaga mushrooms (78%) and Cordyceps mushrooms (48%) than *Lentinus edodes* (9%), which is widely used as a food ingredient (Qi et al., 2013).

Similarly, Lim et al. (2015) reported that the DPPH radical scavenging capacity of coffee immersed in mulberry extract was higher (50.67～55.25%) than the control (45.51～47.02%). The antioxidant activity of food is closely related to the polyphenol compounds present in the constituent plants, because it is thought that the DPPH radicals are scavenged by the phenolic hydroxyl groups present in a number of polyphenol compounds. Kim et al. (2007) isolated four major polyphenol compounds (e.g. caffeic acid) that contribute to the antioxidant activity of Chaga mushrooms.

β-Glucan contents

The β-glucan contents are shown in Table 3. The β-glucan content of the medicinal mushrooms were 3.79% (Cordyceps), 7.06% (Phellinus), and 8.57% (Chaga). Chaga mushrooms also had the highest total glucan content of

Table 3. Total glucan, α-glucan, and β-glucan contents (unit: %)

Sample	Total glucan	α-Glucan	β-Glucan
Control	N.D.	N.D.	N.D.
Cordyceps coffee	3.11±0.08[a]	1.08±0.05[c]	2.03±0.11[a]
Cordycpes mushroom	4.65±0.04[b]	0.86±0.06[b]	3.79±0.03[b]
Phellinus mushroom	7.48±0.07[c]	0.43±0.00[a]	7.06±0.07[c]
Chaga mushroom	9.70±0.10[d]	1.13±0.07[c]	8.57±0.05[d]

Data are presented as the mean±SD (n=3).
The different letters in the same column (a-d) indicate significant differences (P<0.05).
N.D., not detected.

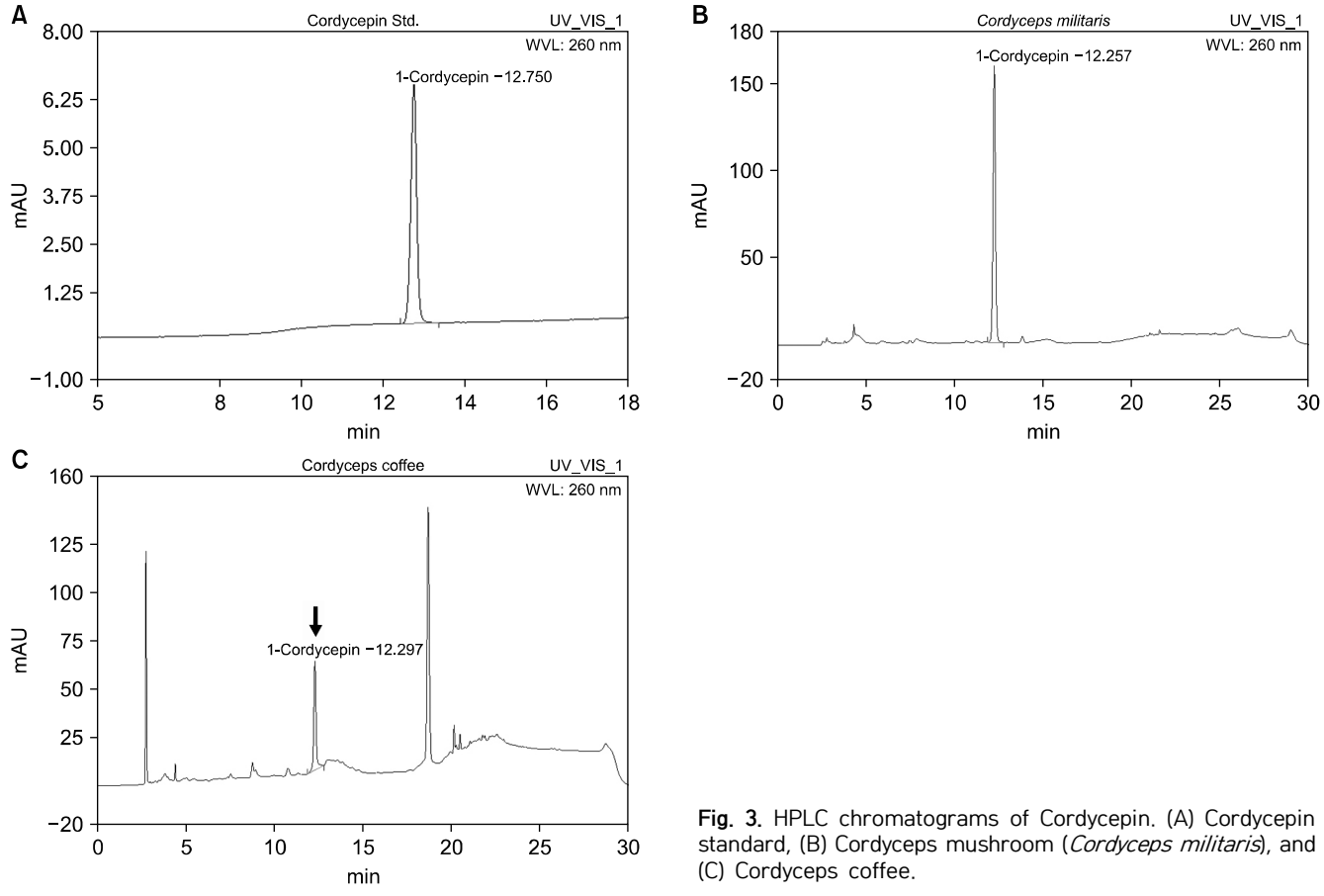

Fig. 3. HPLC chromatograms of Cordycepin. (A) Cordycepin standard, (B) Cordyceps mushroom (*Cordyceps militaris*), and (C) Cordyceps coffee.

9.70%. Cordyceps coffee contained β-glucan and total glucan contents of 2.03% and 3.11%, respectively, substantially lower than those for the raw mushrooms. However, the glucans from the mushrooms are thought to have been absorbed into the coffee beans. An et al. (2019b) reported the β-glucan content of result of ethanol extracts from various medicinal mushrooms in Korea and China. In this study, β-glucan contents of 45.11% and 24.7% were reported for Cordyceps mushrooms from Korea and China, respectively; these values significantly differ from

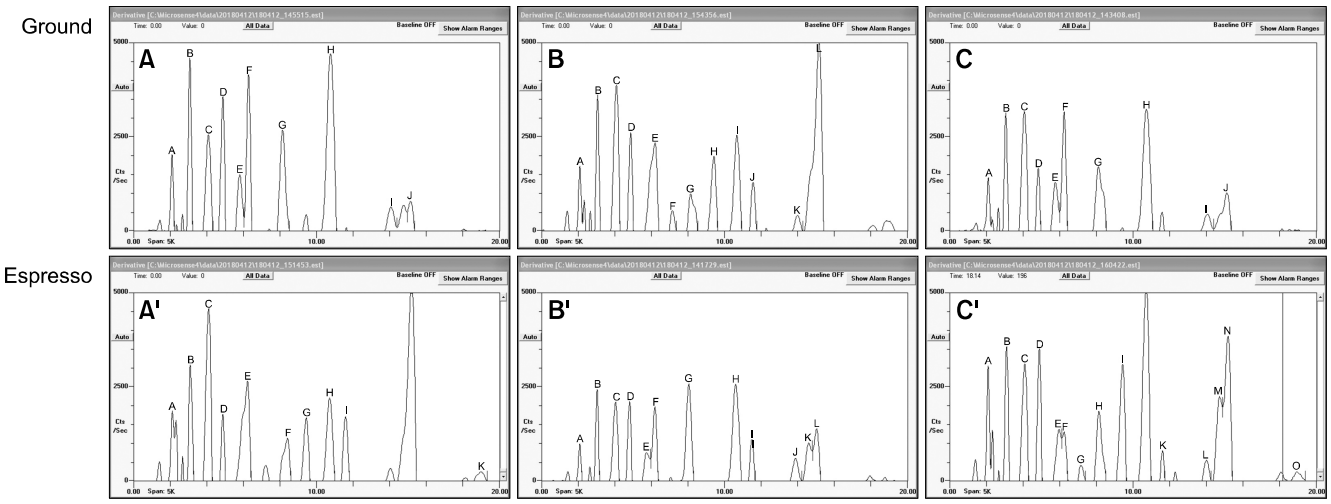

Fig. 4. Derivative chromatogram created using the gas chromatograph/surface acoustic wave (SAW) electronic nose system. (A) Control, (B) Cordyceps coffee, and (C) 30% mixture of Cordyceps mushroom (*Cordyceps militaris*). The X-axis stands for the retention time for 20 s and the Y-axis stands for the concentration expressed in counts (cts/s) of the frequency changes obtained by the SAW single sensor.

Table 4. Flavor compounds detected by gas chromatograph/surface acoustic wave (SAW) electronic nose system from ground coffees and their espressos

Peak ID	RT (s)	Amount (cts)[1)]					
		Control		Cordyceps coffee		Mixture	
		Ground	Espresso	Ground	Espresso	Ground	Espresso
1	2.06	316	256	228	157	142	453
2	3.06	819	658	549	624	393	658
3	4.06	780	1,167	957	1,396	590	921
4	4.84	752	506	297	312	404	805
5	5.72	357	—[2)]	284	—	114	—
6	5.94	—	—	—	—	—	207
7	6.20	882	1,123	652	1,240	433	124
8	7.12	—	148	—	—	—	—
9	8.12	897	—	732	—	777	645
10	8.38	—	332	—	462	—	—
11	9.42	—	538	—	469	—	935
12	10.50	—	—	—	—	978	—
13	10.68	2,142	811	1,405	786	—	1,886
14	11.46	—	—	—	—	215	—
15	11.56	—	376	—	450	—	174
16	13.86	—	—	—	—	162	—
17	14.05	237	—	154	—	—	177
18	14.70	—	—	—	—	110	271
19	15.02	—	—	—	—	239	—
20	15.12	128	—	459	—	—	—
21	15.16	—	2,425	—	2,440	—	863
22	18.98	—	119	—	120	—	104

[1)]The concentration expressed in counts of the frequency changes obtained by the SAW single sensor.
[2)]Not detected.
RT, retention time; cts, counts.

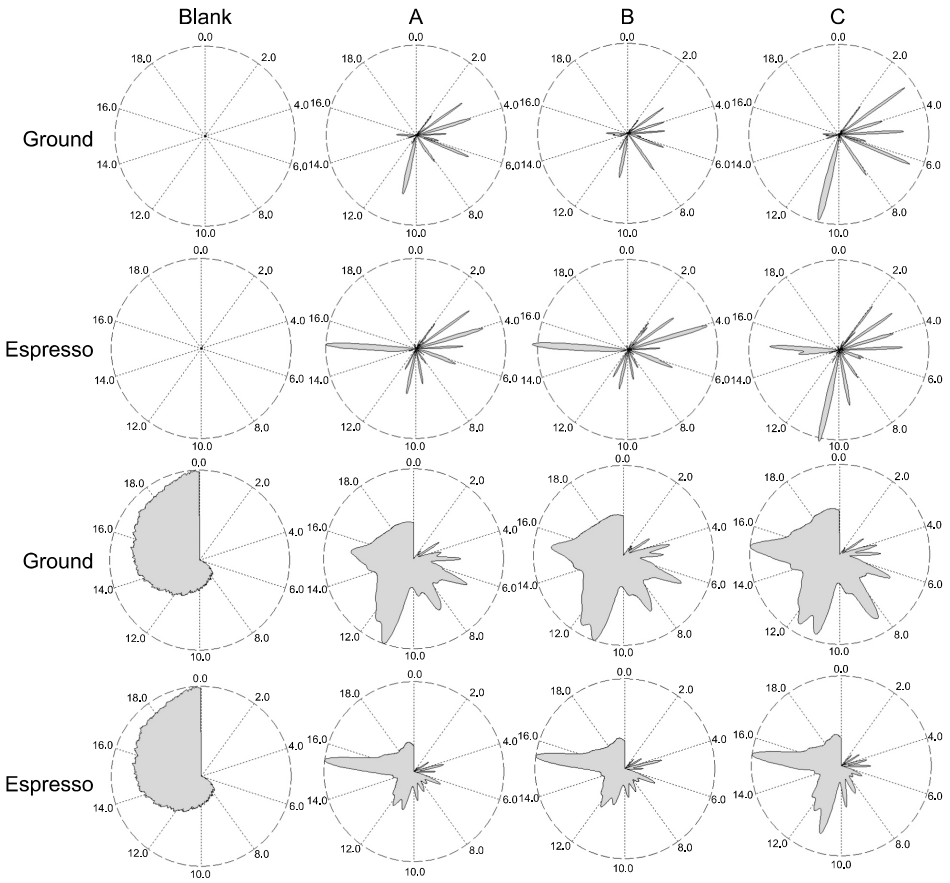

Fig. 5. Polar derivative and polar frequency pattern created by Vapor Print™ analysis using the gas chromatograph/surface acoustic wave electronic nose system. (A) Control, (B) Cordyceps coffee, and (C) 30% mixture of Cordyceps mushroom (*Cordyceps militaris*).

our results.

In addition to enhancing immunity and exhibiting anti-tumor effects (Hazama et al., 2009), β-glucan has been reported to exhibit antioxidant activity (An et al., 2019a; Li et al., 2019). Wu et al. (2011b) explained that molecular interactions with β-glucan polyphenols can increase antioxidant activity. Mebrek et al. (2018) proposed that β-glucan can provide electrons to reactive free radicals, converting them into more stable non-reactive molecules. Wang et al. (2014) reported that this scavenging effect is more effective when the hydroxyl groups in polysaccharides are substituted by carboxymethyl or sulfate groups. This implies that sulfate and carboxymethyl groups of β-glucan can enhance its antioxidant capability compared with compounds with only a single sulfate or carboxymethyl group.

Cordycepin contents

The Cordycepin contents are shown in Table 2 and Fig. 3. The Cordycepin contents of Cordyceps mushroom and Cordyceps coffee were 11,533.22 mg/kg and 2,274.70 mg/kg, respectively. The Cordycepin content was lower in Cordyceps coffee than in Cordyceps mushrooms, however Cordycepin is considered to have been relatively well implemented into the coffee beans, similar to β-glucan. Oh et al. (2003) reported that the Cordycepin content of Cordyceps mushrooms differs depending on the variety and the part of the plant; the fruiting body contains 448 mg/100 g Cordycepin whereas the host part contains 238 mg/100 g Cordycepin, 8～23 times higher than the content in other varieties.

Flavor analysis by electronic nose

Coffee is one of the most complex foods, containing over 800 flavor compounds. Therefore, it is very difficult to isolate and identify all individual flavor components and this may not be meaningful for qualitatively evaluating the coffee. However, it is evident that the differences in taste and aroma of coffee can be influenced by minor changes to the flavor components; therefore, changes to the flavor pattern can give useful information about the quality of the coffee. Electronic nose can be a useful tool by analyze flavor patterns to distinguish the differences objectively and reproducibly.

The flavor patterns of three samples (control, Cordyceps coffee, and the mixture of 30% Cordyceps mushroom powder with ground coffee) were analyzed by an electronic nose equipped with a GC-based SAW sensor; the derivative is shown in Fig. 4. Derivative chromatograms are obtained by differentiating changes in the frequencies detected from the sensor on the retention time (RT). The chromatograms for the control (A) and Cordyceps coffee (B) were almost identical. However, there were distinct differences in the chromatograms between

the mixture (C) and A/B, despite a similar overall peak pattern (Table 4). In the ground state, some flavor components (e.g. the peaks of 12, 14, 16, 18, and 19) were only detected in C, which implies that these flavors are from the Cordyceps mushrooms. However, the compounds at peaks 10 and 20 were only detected in A and B. Furthermore, the peaks at 7, 13, and 21, which were considered to be major peaks in A and B, were significantly reduced in C.

These differences can be more clearly distinguished in the Vapor Print graph (Fig. 5). Vapor Print is a 360° circular image based on from the initial RT to the last compound detection time, which uses two variables, the RT as an angular variable and the response level as a radial variable. The polar derivative pattern refers to a pattern image obtained by differentiating the frequency, and the polar frequency pattern is a pattern image representing a change in frequency obtained from a sensor according to the RT in Herz. As shown in Fig. 5, A and B showed a very similar pattern, whereas C showed a different overall pattern, including unusually strong peaks or almost disappeared peaks.

These results are supported by studies showing that coffee aroma changes depending on the processing method of green coffee beans (Lyman et al., 2011), and that the coffee flavor pattern varies according to the variety of coffee beans (Kim et al., 2014). In conclusion, Electronic Nose analysis, which can clearly distinguish between different coffee aromas within a short period of time, is considered to be a very effective method for evaluating the quality of coffee.

Overall, we show that our newly developed method for making Cordyceps coffee successfully maintains the original aroma in the control coffee without incorporating the off-flavors from the Cordyceps mushroom. These results show that Cordyceps coffee may be a new functional coffee containing Cordycepin and β-glucan.

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AUTHOR DISCLOSURE STATEMENT

The author declares no conflict of interest.

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