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Effect of particle size on the physicochemical and morphological properties of *Hypsizygus marmoreus* mushroom powder and its hot-water extracts

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Abstract

Hypsizygus marmoreus, an edible mushroom with various functional properties, was prepared to obtain coarse and superfine powders. We evaluated the effect of particle size on mushroom powder quality. The water absorption index and total dietary fiber contents of superfine powders (average median diameter, 4.791 μm) were 5.79 g/g and 28.50 g/100 g, respectively, which were higher than the corresponding values of coarse powder (average median diameter, 168.085 μm). Similarly, extracts prepared from the superfine mushroom powders exhibited higher total polyphenolics contents (83.91 mgGAE/100 g), total sugar contents (5,138.50 mg/100 g), and total flavonoid contents (43.99 mgNE/100 g) compared with those prepared from coarse mushroom powders. The extraction yield and pH were higher for superfine powder concentrates than for coarse powder concentrates. According to the electronic tongue results, superfine powder extracts had high scores for umami, sourness, and sweetness, while coarse powder extracts scored high in bitterness. Furthermore, SEM images indicated that both powders differed in either the shape or size of the particles; coarse powder particles exhibited irregular shapes and high porosity, while superfine powder particles represented a uniform shape and lower porosity. Particle size reduction through superfine grinding improved the physicochemical and morphological properties of the mushroom powder samples and their hot-water extracts.

Key words : *Hypsizygus marmoreus*, particle size, superfine powder, physicochemical properties, morphological properties

Introduction

Hypsizygus marmoreus is an edible mushroom species that is widely grown in East Asian countries such as Japan, Taiwan, and South Korea. This mushroom has been reported to possess anti-inflammatory, antifungal, antitumor, and antioxidant properties (Lee et al., 2007; Suzuki et al., 2011); the use of *H. marmoreus* has there became increasingly popular in several countries, including Taiwan (Lee et al., 2008).

Superfine grinding is a modern food processing technology that has been applied to various food materials, including ginseng, fruits, cereals, and mushrooms (Seo et al., 2011; Shu et al., 2004). Pulverization of food materials using a jet mill (an air flow-type grinder) allows superfine grinding that yields a particle size of up to several micrometers. The micronization process in the jet mill helps to improve texture and digestion, maintain color and flavor, and control the rate of nutrient absorption. The use of superfine grinding technology in the production of red grape pomace powder and green tea powder can broaden the application of these natural materials (Hu et al., 2012; Zhao et al., 2015). Ming et al. (2014) and Choi et al. (2012) have reported the effect of particle size on the quality of *Lentinus edodes* mushroom

powder and *Angelica gigas* powder, respectively. Although previous studies focused on hot-water extraction of *H. marmoreus* mushroom powder and its properties, none have investigated the effect of particle size on the powder or extracts of this mushroom.

This study analyzed the physicochemical properties of extracts prepared from coarse and superfine powders of *H. marmoreus* mushroom using microparticle technology. Our results provide a basis for the development of high-value-added foods using superfine powders and their extracts.

Materials and methods

Sample preparation

Fresh and cold-air-dried *H. marmoreus* mushrooms were purchased from World Farm, a farming company in Yangyang, Gangwon-do, Korea and stored in a refrigerator. The air-dried mushrooms were pulverized using a blade grinder (Hanil Electronics Co. Ltd, Gyeonggi-do, South Korea) for 2 min to obtain a coarse powder. To obtain a superfine powder, we first coarsely ground air-dried mushrooms using a cutter mill and, subsequently, finely ground them using a blade mill, followed by jet milling (Jet mill, Korea Powder System Co., LTD, Incheon, Korea).

Analysis of particle sizes

The particle sizes of coarse and superfine mushroom powders were analyzed using the method described by Zhou and Kang (2018) with a particle size analyzer (Mastersizer 2000, Malvern Instruments Ltd., UK). Distilled water at room temperature was used as the dispersant, and the dispersant refractive index was 1.330. The particle refractive index and absorption set at 1.520 and 0.1, respectively, while the obscuration was set at 3.00% for coarse powder and 4.49% for superfine powder.

Hot-water extraction of *Hypsizygus marmoreus* mushroom powders

H. marmoreus mushroom extracts were obtained by extracting coarse powder or superfine mushroom powder (45 g) in boiling distilled water (1.5 L) for 1 h, followed by concentration of the hor-water extract to 100 mL under reduced pressure at 50℃ using a vacuum extractor (Rikakikai

Co., Ltd, Tokyo, Japan). The extracts were refrigerated until further use.

Determination of water absorption index and water solubility index

The water absorption index (WAI) and water solubility index (WSI) were determined using the method establsihed by Yousf et al. (2017). Briefly, coarse or superfine mushroom powder (0.5 g) was mixed with distilled water (10 mL) in a centrifuge tube, incubated at 30℃ in a constant temperature water bath for 30 min, and centrifuged at 3,000 ×*g* for 10 min. The sediments that remained after removal of the supernatant were used to ascertain the WAI.

For WSI determination, the supernatants were evaporated in an oven drier at 105℃ for 3 h on glass Petri dishes. After evaporation, the Petri dishes were cooled in a desiccator and weighed. The obtained weights were used to calculate WSI.

WAI (g/g) = Weight of sediment / Weight of sample
WSI (%) = (Weight of dry supernatant / Weight of sample)
　　　　× 100

Determination of beta glucan content

The β-glucan content of mushroom powders was determined following the method described by Bak et al. (2014) using a yeast-specific β-glucan kit provided by Megazyme International. The analysis protocol described by the kit manufacturer was followed. The MegaCalc Excel sheet obtained from the Megazyme website was used for calculations. β-Glucan content was calculated as the difference between total glucan content and α-glucan content.

Determination of total dietary fiber contents

Total dietary fiber contents of the mushroom powder samples was evaluated according to the general test method of the Korea Food Code (available at https://www. foodsafetykorea.go.kr/portal/safefoodlife/food/foodRvlv/food Rvlv.do). Briefly, in a beaker, mushroom powder (1+0.005 g) was mixed with MES/TRIS buffer solution (40 mL) and thermophile α-amylase (50 μL), and the mixture was incubated in a water bath at 95℃ for 40 min. Later, the water bath temperature was reduced to 60℃ and protease enzyme (100 μL) was added and incubated for 30 min. The pH of the mixture was adjusted to 4.0-4.7, using 0.56 N HCl

(5 mL) following which amyloglucosidase enzyme (300 μL) was added, and the mixture was stirred for 30 min at 60℃. Next, 95% ethanol (225 mL) heated at 60℃ was added to the digested samples at an ethanol-to-sample ratio of 4:1. The beaker was removed from the water bath and covered with aluminum foil; the mixture was allowed to settle at room temperature for 1 h. We dispersed the diatomaceous earth by adding 78% ethanol (15 mL) to a glass filter that had been weighed in advance with diatomaceous earth, followed by suction filtration to ensure that the diatomaceous earth layer was evenly formed. Next, the ethanol-treated enzyme digest was filtered through the prepared glass filter. The residue of the beaker was transferred to another glass filter using a washing bottle containing 78% ethanol and washed twice with 15 mL of 78% ethanol, 95% ethanol, and acetone, separately.

The glass filter with residual residue was dried at 105℃ overnight, cooled in a desiccator for 1 h to constant weight; the glass filter, including the dietary fiber residue and diatomaceous earth, was weighed. The weight of the dietary fiber residue was calculated by subtracting the weight of the glass filter, including the diatomaceous earth. One of the two samples was measured for protein content where 6.25 was used as the nitrogen coefficient. The ash content was analyzed by incinerating the sample at 525℃ for 5 h, cooling in the desiccator, and measuring the weight of the dried crucible containing incinerated samples. The weight of the ash was determined by subtracting the weight of the crucible containing diatomaceous earth. Total dietary fiber was calculated by subtracting the weight of ash and protein from the weight of the residues, and the results were expressed as a percentage.

Measurement of extraction yield

The extraction yield of mushroom powders was measured according to the method described by Kim and Kim (2015). For dry weight measurements, the extracts of coarse and superfine mushroom samples were evaporated at 105℃ for 3 h for the complete removal of distilled water, cooled in a desiccator, and subsequently weighed.

Measurement of pH and total soluble solids

The pH of mushroom concentrates was measured using a pH meter (pH 210, Hanna, Woonsocket, RI, USA) according to the method described by Lee et al. (2016) while total soluble solid content was measured using a hand refractometer (Atago., Ltd, Tokyo, Japan).

Determination of total sugar contents

Total sugar contents was determined according to the method described by Dai et al. (2010). Briefly, mushroom extract (0.5 mL), 5% phenol solution (0.5 mL), and 95% sulfuric acid (2.5 mL) were added to a test tube and mixed using a vortex mixer. The reaction mixture was allowed to stand for 5 min at room temperature and then incubated in a water bath at 25℃ for 15 min. The absorbance was read at 490 nm using a spectrophotometer (Cary8454 UV-Vis, Agilent Technologies) and glucose was used for standard-curve generation.

Determination of total polyphenol contents

The total polyphenol contents of mushroom extracts was determined following the method described by Pinto et al. (2007) with minor modifications. Briefly, Folin-Ciocalteu reagent (0.25 mL) was added to the sample (0.25 mL); distilled water (2 mL) was added to this mixture, which was mixed using a vortex mixer. The reaction mixture was allowed to stand at room temperature for 3 min, followed by the addition of 20% Na_2CO_3 (0.25 mL) and incubation in a water bath at 37℃ for 30 min. The absorbance was measured at 750 nm using a spectrophotometer (Cary8454 UV-Vis, Agilent Technologies) and gallic acid was used for creating a calibration curve.

Determination of total flavonoid contents

The total flavonoid contents of the mushroom extracts was determined as described by Kang et al. (2019). Briefly, diethylene glycol (10 mL) was added to the sample (1 mL), followed by vigorous mixing and addition of NaOH (1 N; 1 mL). This reaction mixture was vortexed and incubated in a water bath at 37℃ for 1 h. The absorbance was measured at 420 nm using a Cary8454 UV-Vis spectrophotometer (Agilent Technologies) and naringin used for calculating the standard curve.

Analysis of morphological properties of *Hypsizygus marmoreus* mushroom powders

The microstructures of the mushroom powders were

analyzed using a field-emission scanning electron microscope (SEM) (JSM-7500F; JEOL Ltd., Tokyo, Japan) using the method described by Zhou and Kang (2018). The coarse or superfine mushroom powder was coated on the SEM stub which had a double-sided, sticky tape on its surface. After the samples were placed on the stub surface, they were sputter-coated in vacuum using a gold sputter machine (LEICA EM SCD005, Austria). Images of the samples were recorded using a JSM-7500F field-emission SEM at 1,000× and 2,000× magnification for the coarse and superfine powders, respectively**.**

Analysis of taste and odor characteristics

The taste characteristics of the coarse and superfine mushroom extracts were analyzed using an ASTREE electronic tongue (E-tongue) (Alpha M.O.S, Toulouse, France) equipped with an auto-sampler and the following sensors, PKS and CPS (to detect complex taste) (Han et al., 2021), AHS (sourness), CTS (saltiness), NMS (umami), ANS (sweetness), and SCS (bitterness) sensors. We used the method described by Beullens et al. (2007) and Dong et al. (2019) to analyze the taste characteristics of the mushroom extracts. Briefly, the mushroom extracts prepared by extracting 45 g in distilled water (1.5 L) were concentrated to 200 mL and filtered through Whatman No. 2 filter paper. These extracts were used for the analysis of taste characteristics. Prior to the analysis, the electronic tongue sensors and system were calibrated and diagnosed using 0.01 M sodium-L-glutamate. For the analysis, filtered extracts (25 mL) were used, and all samples were analyzed in five replicates. The analysis parameters were set to 0 s for delay; 120 s, acquisition time; 1 s, interval; 1, stirring rate; and 1 s, acquisition period. After the measurement of each sample (5 replicates), the sensors were rinsed with double-distilled water (25 mL).

During the determination of odor characteristics of mushroom extracts, the samples were extracted in a manner similar to the preparation of electronic tongue samples. mushroom extracts were analyzed following a modification of the method by Zhou et al. (2017) and Koh et al. (2020) using Heracles II electronic nose (E-nose) (Alpha MOS, H2-0087; France). This electronic nose consists of an auto sampler, two capillary columns with different polarities (MXT-5, polar and MXT-1701, slightly polar), and two

flame ionization detectors (FID). The extracts (5 mL) were pipetted and sealed in 20 mL vials and analyzed according to the following analytical conditions: carrier gas, 99.995% hydrogen gas; oven, injector, trap, valve, and detector temperature were 40℃, 200℃, 40℃, 250℃ and 250℃, respectively. The injector pressure was set at 25 kPa, injector vent flow and split flow were both set at 10 mL/min, the FID temperature was 270℃, and the acquisition time was 120 s. For each sample, five replicates were analyzed. The ASTREE electronic tongue and Heracles II electronic nose are both equipped with statistical software, which was used for the determination of the function analysis for E-tongue and E-nose, respectively.

Statistical analysis

Experiments were conducted in triplicate, and the results are expressed as mean±SD except total dietary fiber and particle size distribution. The significance of the statistical difference of the obtained data was analyzed by an independent two-tailed t test using the SPSS software (Version 21 software, IBM, USA).

# Results and discussion

Particle size distribution

The particle size distribution of the mushroom samples were reported as d (0.1), d (0.5) and d (0.9) ; d (0.1) and d (0.9) represent, respectively, the particle size values below which 10% and 90% of the volume is distributed, d (0.5) is the average median diameter; 50% of the volume distribution is above this particle size volume and 50% is below. The results of particle size distributions of the coarse and superfine powders of *H. marmoreus* are shown in Table 1 and Fig. 1. For coarse and superfine powders, the particle size distributions were 25.392 μm and 2.102 μm, respectively, at d (0.1) and 542.109 μm and 10.428 μm, respectively, at d (0.9). The average median diameter at d (0.5) was 168.085 μm for the coarse powder and 4.791 μm for the superfine powder. These observations confirmed the effective reduction of the particle size of mushroom powder through the extensive milling process, as a result of superfine grinding technology. Superfine powder samples had the lowest span of 1.738 μm indicating that these samples had a narrow particle size distribution compared to coarse powder

Table 1. Particle size distribution of coarse and superfine *Hypsizygus marmoreus* mushroom powders

Sample	Particle size (μm)			
	d (0.1)[1]	d (0.5)	d (0.9)	Span[2]
Coarse powder	25.392	168.085	542.109	3.074
Superfine powder	2.102	4.791	10.428	1.738

[1] d (0.1), size when 10% in total particle size distribution; d (0.5), size when 50% in total particle size distribution; d (0.9), size when 90% in total particle size distribution.
[2] Span, distribution width of sample = [d (0.9) - d (0.1)] / d (0.5)
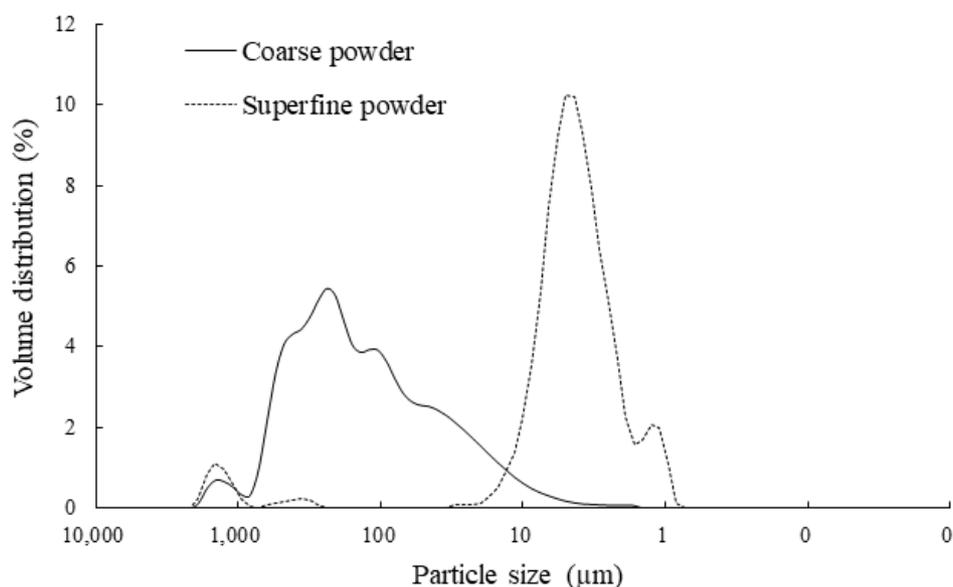
Fig. 1. Particle size distribution of *Hypsizygus marmoreus* mushroom powders.

samples. Similar results were obtained by Choi et al. (2012) and Lv et al. (2014) in their research on ultrafine *Angelica gigas* and *Agrocybe chaxingu* mushroom powders, respectively, which exhibited lower d (0.1), d (0.5), d (0.9), and span values for superfine-ground samples; and indicated that a lower span value corresponded to a narrow particle distribution yielding powder particles with a more uniform size.

Water absorption index, water solubility index, beta glucan content, and total dietary fiber contents

The WAI was calculated as the weight of the gel (obtained after discarding the supernatant) per unit of original dry solids and WSI as the weight of dry solids in the supernatant expressed as percentage of the original

weight of the sample. Table 2 represents the WAI, WSI, β-glucan content, and total dietary fiber contents of the coarse and superfine mushroom powders. Superfine grinding increased WAI and total dietary fiber contents, as the superfine mushroom powder exhibited higher values, 5.79 g/g and 28.5 g/100 g, respectively, than the coarse mushroom powder samples. Carvalho et al. (2010) and Zhao et al. (2008) reported that the WAI increased with a decrease in the particle size of corn starch and ginger powder, respectively, because smaller particles produced by superfine grinding had a greater contact area which in turn improved water absorption capacity. Additionally, size reduction of powders of the mushroom *Inonotus obliquus* by ball milling has been reported to improve the extractability of active ingredients in mushrooms (Wang et al., 2016). The

Table 2. Water solubility index, water absorption index, β-glucan content, and total dietary fiber contents of *Hypsizygus marmoreus* mushroom powders

Sample	WAI[1]	WSI(%)	β-Glucan (%)	Total dietary fiber (%)
Coarse powder	$2.91\pm0.19^{a[2]}$	51.33 ± 0.94^a	30.08 ± 0.46^a	27.30
Superfine powder	5.79 ± 0.36^b	42.00 ± 1.00^b	29.82 ± 1.36^a	28.50

[1]WAI, water absorption index; WSI, water solubility index.
[2]Values (Mean±SD, n=3) with different superscripts in the same column are significantly different at p<0.05.

reduction in particle size did not affect the β-glucan content of the mushroom samples, as the coarse powder contained 30.08% β-glucan and superfine powder contained 29.82%. Lv et al. (2014) reported that superfine mushroom powders exhibited higher total dietary fiber content and WAI (water holding capacity) compared to the control sample, which was coarsely ground. In contrast, the coarse powder had a higher solubility index (51.33%) than the superfine powder (42.00%) which contradicted previous reports that superfine grinding technology improved the WSI.

Extraction yield, pH, total soluble solids, total sugar contents, total polyphenol contents, and total flavonoid contents of *Hypsizigus marmoreus* hot water extracts

Table 3 presents the extraction yield, pH, total soluble solid contents, total sugar contents, total polyphenolics contents, and total flavonoid content of hot-water extracts prepared from coarse and superfine *H. marmoreus* powders. Extracts prepared from the superfine mushroom powders exhibited the highest values for all the aforementioned parameters except total soluble solid contents, which was similar for both samples. Total polyphenolics contents, total sugar contents, and total flavonoid contents of extracts prepared from superfine powders were 83.91 mg GAE/100 g, 5138.5 mg/100 g, and 43.99 mgNE/100 g, respectively. The

extraction yield was 3.53% for the coarse powder and 6.07% for the superfine powder. The pH of concentrates prepared from the coarse and superfine powders was 6.04 and 6.17, respectively. Park and Ahn (2019) reported a similar effect of particle size on total polyphenols. Similarly, Jiang et al. (2017) reported that the solubility of flavonoids increased with a decrease in the particle size of *Vaccinium bracteatum* Thunb. leaf powder extracted in ethanol, which is consistent with our results. Additionally, particle size reduction has also been reported to increase extraction yield because smaller particle sizes shorten the path that the solvent has to travel, which in turn reduces the time required for maximum extraction of functional constituents of the sample (Yeop et al., 2017). Moreover, Speroni et al. (2019) reported that the polyphenol content and antioxidant capacity of olive pomace were improved due by micronization and granulometric fraction processing technologies. Additionally, Zhang et al. (2020) determined that superfine grinding improved the accessibility of functional compounds in milled *Lycium ruthenicum* Murray which in turn increased their antioxidant abilities.

Morphological properties of *Hypsizygus marmoreus* mushroom powders

Scanning electron microscopy (SEM) images of coarse and superfine *H. marmoreus* powders are shown in Fig. 2.

Table 3. Extraction yield, pH, total soluble solid contents, total sugar contents, total polyphenol contents, and total flavonoid contents of hot-water extracts of *Hypsizygus marmoreus* mushroom powders

Sample	Extraction yield (%)	pH	TSS[1] (°Brix)	TS (mg/100 g)	TP (mgGAE/100 g)	TF (mgNE/100 g)
Coarse powder	$3.53\pm0.48^{a[2]}$	6.04 ± 0.01^a	5.00 ± 0.00^a	$4,402.24\pm294.47^a$	73.43 ± 1.51^a	38.38 ± 2.05^a
Superfine powder	6.07 ± 0.25^b	6.17 ± 0.01^b	5.00 ± 0.00^a	$5,138.50\pm334.90^a$	83.91 ± 0.98^b	43.99 ± 1.88^b

[1]TSS, total soluble solids; TS, total sugar; TP, total polyphenol; TF, total flavonoid.
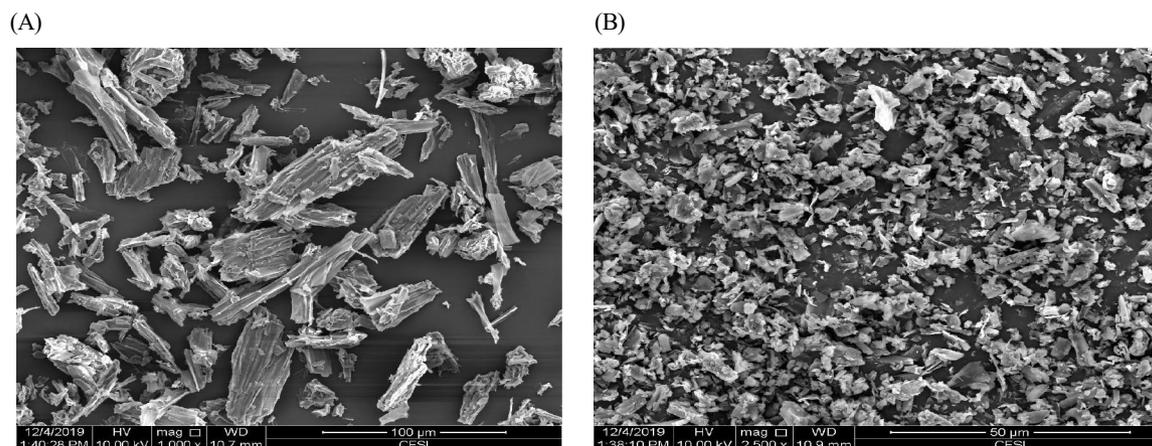[2]Values (Mean±SD, n=3) with different superscripts in the same column are significantly different at p<0.05.

(A)   (B)

Fig. 2. SEM analysis of coarse (A) and superfine (B) *Hypsizygus marmoreus* **mushroom powders (1,000× and 2,500× magnification, respectively).**

The superfine grinding technology resulted in considerably smaller powder particles that were only distinguishable under increased magnification; hence, the magnification was set at 2,500× instead of 1,000× which was used for coarse powder samples. The SEM images indicated that both powders differed in either the shape or size of the particles, with coarse powder particles exhibiting irregular shapes, mostly rectangular, polygonal, and other random shapes; rough surfaces; and high porosity. In contrast, the superfine powder particles exhibited more uniformity in shape (appearing in rectangular, circular, and needle-like shapes) and reduced porosity, displaying a denser structure. This size uniformity of superfine-ground mushroom powder particles was explained by the lower span values (presented in the particle size distribution analysis). Lv et al. (2014) reported that lower span values corresponded to a more uniform particle size; similarly, in their findings, superfine grinding resulted not only in the reduction of particle size but also in significant modification of the powder structure owing to the extensive milling process consisting of flattening, aggregation, and fracturing of mushroom particles (Zhao et al., 2013). These findings indicate that superfine grinding significantly modifies the surface structure of the powder particles, which may affect the physicochemical properties of the samples.

Taste and odor characteristics

Electronic noses and electronic tongues are devices equipped with gas and chemical sensors, respectively, that human sensory evaluation and electronic tongue system confirmed the ability of electronic tongue and nose to evaluate the taste and odor characteristics of various samples (Bleibaum et al., 2002). These devices were used for the determination of odor and taste characteristics of mushroom extracts (Fig. 3). Extracts prepared from coarse and superfine *H. marmoreus* mushroom powders were analyzed for five different taste characteristics, including sourness, saltiness, umami, sweetness, and bitterness, and the results are displayed in a spider plot (Fig. 3A). Mushroom extracts prepared from superfine powders had high scores for sourness, umami, and sweetness values, while those from coarse powders had high bitterness scores. Similar findings were reported by Phat et al. (2016), who indicated that mushrooms with low umami taste scores had high bitterness scores and those with high umami taste levels had high saltiness scores. Discriminant function analysis (DFA) was used to highlight the differences or similarities between the mushroom extracts. DFA for taste and odor characteristics is shown in Fig. 3B and C, respectively. Discriminant function 1 (DF1) and discriminant function 2 (DF2) were 100% and 0%, respectively, indicating that the taste and odor characteristics of the coarse powder and superfine powder extracts were significantly different and therefore classified into two significantly different clusters. The DFA results for odor and taste characteristics confirmed the taste score results of the mushroom extracts displayed in the spider plot. Furthermore, in a study conducted by Qiu et al. (2019), size reduction affected sourness, bitterness, and

(A)

(B)

(C)
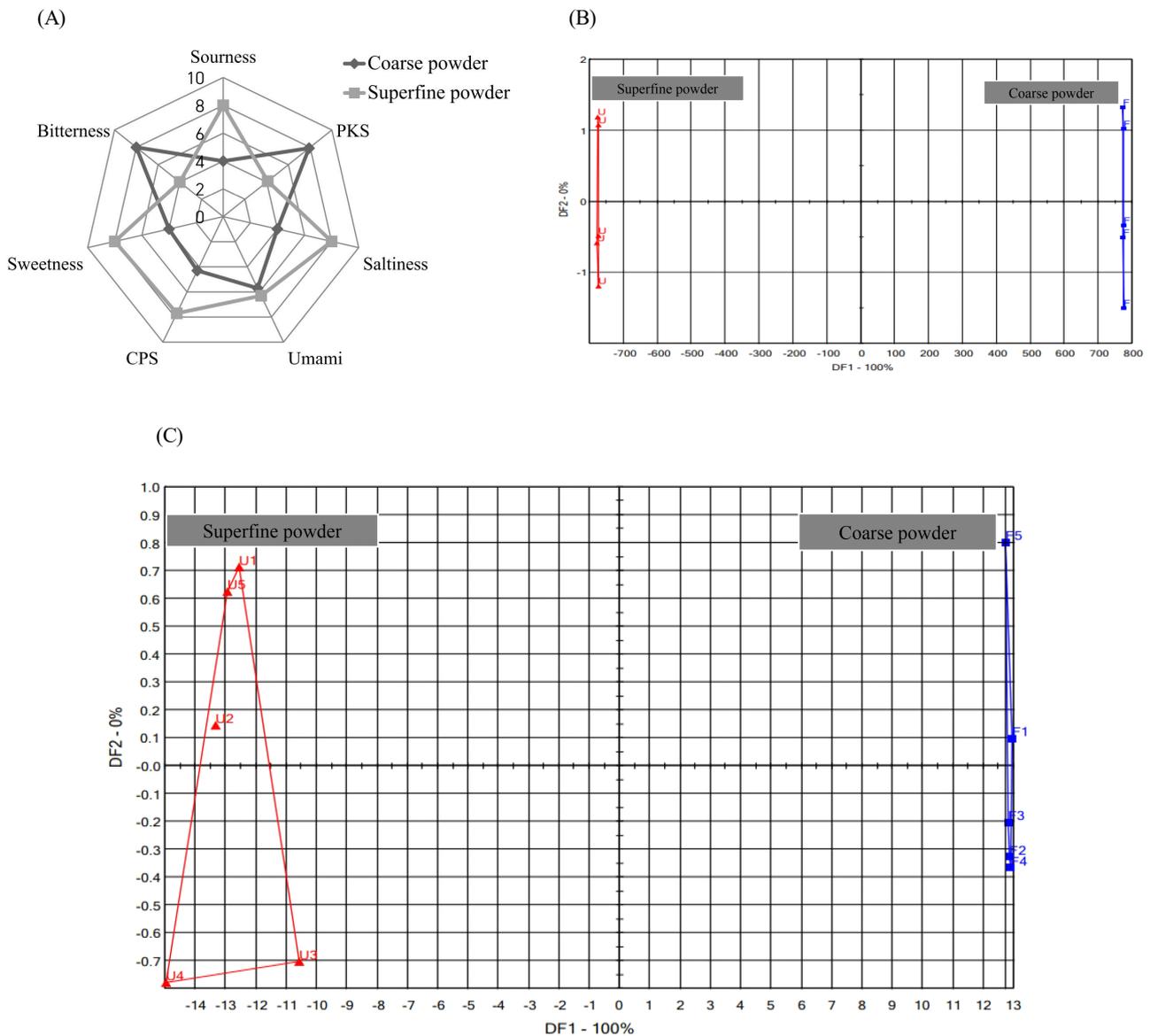
Fig. 3. Taste and odor characteristics of water extracts of *Hypsizygus marmoreus* mushroom powders.
A, radar representation of electronic tongue analysis; B, discriminant function analysis based on electronic tongue data; C, discriminant function analysis based on electronic nose data.

astringency of wasabi paste by increasing sourness while decreasing bitterness and astringency, which was possibly due to the grinding process that degraded taste substances.

In conclusion, our study found that particle size reduction through superfine grinding beneficially affected the physicochemical and morphological properties of mushroom powder samples and their water extracts. Therefore, the adoption of superfine milling in powder-processing industries could result in products with improved functional properties.

Acknowledgements

Conflict of interests

The authors declare no potential conflict of interest.

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