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Volatile Flavor and Nonvolatile Taste Components in the Wild Mushroom *Sarcodon aspratus* (Berk.) S. Ito

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능이버섯의 맛 성분과 향기성분

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Abstract

Sarcodon aspratus* (Berk.) S. Ito is a wild mushroom commonly consumed in Korea due to its beneficial effects on health. However, only limited information on the volatile and nonvolatile constituents of *S. aspratus* is available. In the present study, the total concentration of mushroom soluble sugars, including glucose, trehalose, sucrose, and xylose, was found to be 202.5 mg/kg. The total contents of free and essential amino acids were 2,592.1 mg/kg and 1,249.5 mg/kg, respectively; arginine, lysine, methionine, and valine were the major amino acids present. The contents of total 5′-nucleotides and flavor 5′-nucleotides in *S. aspratus* were 2,510.7 mg/kg and 773.4 mg/kg, respectively. The volatile components of *S. aspratus* were collected by simultaneous distillation-extraction (SDE) and analyzed by gas chromatography-massspectrometry (GC-MS). A total of 27 volatile compounds were isolated and identified. The most abundant was 1-octen-3-ol, which accounted for more than 68% of total volatiles; other important compounds were 2-octen-1-ol, 1-octen-3-one, and 2-octenol. Our results provide preliminary data for the development of *S. aspratus* as a food material.

Key words : *Sarcodon aspratus*, mushroom flavor, volatile composition, taste component, 5′-nucleotide

Introduction

Mushrooms are rich in proteins, vitamin B-complexes, and minerals. They have been used in oriental cultures for a long time as nutritional foods and food flavoring materials because of their unique and subtle flavors (1). Some nontoxic edible mushrooms have been used as flavor additives as well as traditional medicines against cancers, aging, and viral infections (2-4).

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Such beneficial mushrooms are now being grown for nutraceutical or functional food purposes. When it is used as food ingredients, the composition of their non-volatile taste components is highly correlated with their acceptability (5). The flavor components in mushrooms are generally divided into nonvolatile and volatile compounds (6). And the unique taste of mushrooms is caused by certain water-soluble substances such as free amino acids and soluble carbohydrates (1,7,8). A group of eight-carbon (C8) compounds, particularly 1-octen-3-ol, is responsible for mushroom flavor (9).

Sarcodon aspratus (Berk.) S. Ito is a medicinal mushroom

and mainly grows in Korea, China, and East Japan. It is also called 'Neung-i' or 'Hyang (fragrant)' mushroom; it is famous for its flavor and for its use as a traditional remedy for indigestion caused by meat. This fungus has an ectomycorrhizal relationship with oak trees. It grows naturally in fertile and moist soil and very often in valleys with oak trees. The consumption of wild edible mushrooms such as *S. aspratus, Calvatia gigantea*, and *Cantharellus cibarius* has increased, even in the developed world, due to their many beneficial physiological effects and high levels of minerals (10). Although flavor is one of the most important qualities responsible for the wide spread consumption of edible mushrooms, there is still a paucity of information regarding some mushroom species such as *S. aspratus*. This research was performed to identify the volatile flavor and non-volatile taste components in *S. aspratus*, including soluble sugars, free amino acids, and 5′-nucleotides.

Materials and Methods

Samples

The *S. aspratus* mushrooms were picked from forests in Yecheon County, South Korea. Select samples were cut in to small cubes and stored at -75℃ for nonvolatile component analysis, while fresh mushroom samples were used for volatile flavor analysis.

Analysis of soluble sugars

The soluble sugars were extracted and analyzed as described by Kim *et al.* (11). About 10 g of frozen mushroom was extracted with 60 mL of 80% ethanol. This suspension was shaken for 45 min at room temperature and centrifuged for 10 min at 15,000 x g. The supernatant was concentrated at 60℃ under reduced pressure and defatted three times with 10 mL of ethyl ether successively. After being concentrated by a rotary evaporator at 40℃, the solid residues were dissolved in de-ionized water to 10 mL of the final volume. An aliquot of the aqueous extract was filtered using a 0.45 μm membrane filter and was passed through a Sep-pak C_{18} filter prior to injection on to a HPLC (Model 600, Waters, USA) under the following conditions: a carbohydrate analysis (3.9x300 mm, Waters, USA) column with a Waters Associates Differential Refractometer RI 410; column temperature, 85℃; mobile phase, acetonitrile : deionezed water (75:25); and flow rate, 0.4 mL/mL. Commercialized soluble sugars were used as standards (Sigma Chemical Co., USA).

Analysis of free amino acids

The free amino acids were extracted and analyzed as described by Kim *et al.* (11). In order to determine the composition of free amino acids, 5 g samples of fresh mushrooms were homogenized using a homogenizer with 20 mL of ice-cold distilled water in a 50 mL centrifuge tube for 2 min on ice. The homogenized samples were then incubated for 30 min on ice before centrifugation for 15 min at 2000 x g. This step was repeated twice. The supernatants from the first and second extractions were combined and filtered through a Whatman filter paper (No. 4). Finally, the samples were analyzed using an amino acid analyzer (Biochrom 20, Phamarcia, Sweden) with a Lithium High Resolution PEEK column under the following conditions: detection type, the ninhydrin method; ninhydrin flow rate, 0.3 mL/min; retention time, 45 min; buffer, 5 lithium citrate buffer+hydroxide solution; buffer flow rate, 0.35 mL/min; column temperature range, 20-99℃; reaction coil temperature range, 40-145℃; detector, 550 nm.

The free amino acids were divided into several classes according to their taste characteristics, as described by Mau *et al.* (5). The taste characteristics were presented based on the contents of amino acids, which included tastes of bitter, MSG-like, sweet and tasteless, respectively.

Analysis of 5′-nucleotides

The 5′-nucleotides in *S. aspratus* were extracted and analyzed according to the method of Lee *et al.* (12) with some modification. A frozen mushroom (40 g) sample was extracted with 400 mL of deionized water. This suspension was heated to boiling for 5 min, cooled and then centrifuged at 11,800 x g for 15 min. The extraction was repeated once with 300 mL of deionized water. The combined filtrate was then evaporated and filtered prior to HPLC injection in the same manner as for the soluble sugar determination.

HPLC analysis was performed using a Shimadzu LC-20A apparatus with a -Pack ODS A-302 (4.6 x 150 mm, Co. Ltd., Japan). The mobile phase was 0.5 M KH_2PO_4/H_3PO_4 (pH 4.3) at a flow rate of 0.8 mL/min and UV detection at 254 nm. Each 5′-nucleotide was identified using an authentic 5′-nucleotide (Sigma Chemical Co., USA), and quantified by the calibration curve of the authentic compound.

Volatile compound extraction

The volatile components of *S. aspratus* were isolated by the simultaneous distillation and extraction (SDE) method in a Likens-Nickerson apparatus (13). Fresh mushrooms (200

g) were cut into pieces and homogenized with 1,000 mL of distilled water. A 100 mL amount of ether-pentane (1:1 v/v) was added as an extraction solvent and left for 2 h at room temperature. The samples were subjected to SDE under reduced pressure in the Likens-Nickerson apparatus. The flavor extract was dried over anhydrous sodium sulfate and concentrated to 0.1 mL at room temperature.

GC and GC-MS analysis of flavor components

The flavor components were analyzed and identified by GC and GC-MS according to the method of Selli *et al.* (14) with some modification. GC analysis of the volatile components was performed using a Hewlett Packard 5890 Series II chromatograph equipped with a HP-Innowax (60 m x 0.32 mm x 0.52 μm, Hewlett Packard, USA). Two μL of a sample was injected into the column, and the temperature of the injector and detector was 260℃. The oven temperature was programmed to 40-240℃ and ramped up at a rate of 2℃/min for 80 min per run. Nitrogen gas was used as a carrier with a flow rate of 1.8 mL/min. The relative percentage of the constituents was computed from the GC peak areas without using correction factors.

The flavor components were identified by a GC-MS equipped with the column already specified above. The flow rate of the helium carrier gas was 1.3 mL/min. The injection volume and oven temperature were the same as for GC analysis. The on-column injector temperature was programmed to rise from 40-240℃ at a rate of 2℃/min, and was held at 240℃ for 80 min. The mass spectra were obtained using an electron multiplier voltage and ionization energy of 1500 V and 70 eV, respectively. The volatile compounds were identified by comparing the mass spectral data with those spectra available from the Wiley 138 data libraries along with the GC retention times of authentic compounds.

Results and Discussion

Soluble sugars

The content of total soluble sugars in *S. aspratus* was 202.5 mg/kg, while the levels of glucose, trehalose, sucrose and xylose were found to be 82.0, 109.6, 8.9, and 2.0 mg/kg, respectively (Table1). The total soluble sugar and polyol content of different edible mushroom species varies; for example, based on dry weight (DW), levels have been determined as 9.37 mg/g in *P. djamor*, 20.2 mg/g in *P. ferulae*, 23.7 mg/g in *P. nebrodensis*, 31.9 mg/g in *P. sapidus*, 83.89 mg/g in *G. frodosa* and 74.83 mg/g in *M. esculenta* (15,16). Trehalose and glucose were found to be major soluble sugars in *S. aspratus*. 'Yun Chih' (*Coriolus versicolor*), another medicinal mushroom, contained high amounts of trehalose and glucose (5), similar to *S. aspratus*. Hammond and Nichols (17) reported that mannitol and trehalose were two major sugar in edible mushrooms including straw mushrooms (*Volvariella volvacea*) (9), 'Ling Chin' (*Ganoderma lucidum*)-paddy straw mushrooms (5), and other oyster mushrooms (*P. ostteauts* and *Pleurotus flabellatus*) (18). Chen (19) found that mannitol was the major taste-active element in common mushrooms. However, Mau *et al.* (9) reported that total soluble sugars, including trehalose, accumulated in the fruiting bodies of straw mushrooms and then decreased quickly due to stripe elongation and cap expansion. In addition, it was reported that a high trehalose and low mannitol content was the most likely factor contributing to the sweet taste of straw mushrooms. Litchfield (1) reported that soluble sugars usually account for the sweet taste of common mushrooms. However, in the present study, *S. aspratus* contained only 0.02% soluble sugars, while mannitol was not detectable. Therefore, it is plausible that the fruit bodies in *S. aspratus* offer a slightly sweet perception.

Table 1. Contents of soluble sugars from *Sarcodon aspratus*

Soluble sugars	Content (mg/kg sample fresh weight)
Glucose	82.0 ± 2.2
Mannitol	Nd[a]
Sucrose	8.9 ± 2.5
Trehalose	109.6 ± 6.8
Xylose	2.0 ± 0.3
Total	202.5 ± 8.9

[a]Nd, not detected.

Free amino acids

The total content of free amino acids in *S. aspratus* was 2,592.1 mg/kg. In addition, the total contents of essential and nonessential amino acids were 1,249.5 and 1,342.6 mg/kg, respectively (Table 2). Four major free amino acids, arginine, lysine, methionine, and valine, were found; whereas alanine, glycine, isoleucine, leucine, threonine and tryptophan were not found. Table 3 shows several classes of free amino acids based on their taste characteristics described by Mau *et al.* (5). The concentration of bitter components was higher than those of MSG-like and sweet components. The levels of

MSG-like, sweet, and bitter components in *S. aspratus* were 309.0, 66.3, and 1,570.3 mg/kg, respectively. However, in other studies, contents of total free amino acids and bitter components were found to be 60.18 and 15.82 mg/g DW in *Volvariella volvacea* (9), 2.5 and 0.84 mg/g DW in *Ganoderma tsugae* (20), and 13.78 and 6.58 mg/g DW in *Coriolus versicolor* (5), respectively. The bitterness from bitter components in common mushrooms can be shielded by sweetness from a high amount of soluble sugars (9,15). However, the bitterness of *S. aspratus* is probably more intense compared to common mushrooms due to its higher concentrations of bitter components. Since these compounds are predominant, this might be the contributing factor in the unique taste of *S. aspratus*. Chen (19) found that alanine, glycine, threonine, and MSG-like components, including aspartic and glutamic acids, were taste-active amino acids contributing to the sweetness of common mushrooms. However, none of the bitter components were found to be taste-active. Yamaguchi (21) reported that the most typical mushroom flavor, the umami taste or palatable taste, was produced by monosodium glutamate-like (MSG-like) components such as aspartic acid and glutamic acid.

Table 2. Contents of free amino acids from *Sarcodon aspratus*

Free amino acids	Content (mg/kg sample fresh weight)
L-Alanine	Nd[a]
L-Arginine	685.4 ± 15.2
L-Aspartic acid	162.8 ± 8.5
L-Glutamic acid	146.2 ± 2.1
Glycine	Nd
L-Histidine	90.1 ± 2.3
L-Isoleucine[b]	Nd
L-Leucine[b]	Nd
L-Lysine[b]	454.7 ± 3.1
L-Methionine[b]	461.2 ± 12.9
L-Phenylalanine[b]	6.1 ± 0.1
L-Serine	66.3 ± 4.9
L-Threonine[b]	Nd
L-Tryptophan[b]	Nd
L-Tyrosine	191.8 ± 9.8
L-Valine[b]	327.5 ± 12.6
Essential amino acid	1,249.5 ± 2.7
Total	2,592.1 ± 4.4

[a]Nd, not detected.
[b]Essential amino acid.

Table 3. Taste characteristics by free amino acid contents in *Sarcodon aspratus*

Taste characteristics[a]	Content (mg/kg sample fresh weight)
MSG-likea	309.0 ± 1.2
Sweet	66.3 ± 2.6
Bitter	1,570.3 ± 12.5
Tasteless	646.5 ± 2.1
Total	2,592.1 ± 12.4

[a]MSG like, monosodium glutamate-like, Asp+Glu;
sweet, Ala+Gly+Ser+Thr;
bitter, Arg+His+Ile+Leu+Met+Phe+Trp+Val;
tasteless, Lys+Tyr.

5′-Nucleotides

The contents of total 5′-nucleotides and flavor 5′-nucleotide in *S. aspratus* were 251.7 mg/kg and 773.4 mg/kg, respectively (Table 4). The flavour 5′-nucleotides, which give the umami or palatable taste, were determined as 5′-guanosine monophosphate (5′-GMP), 5′-inosine monophosphate (5′-IMP), and 5′-xanthosine monophosphate (5′-XMP) (23). 5′-GMP gives a meaty flavor, and is a much stronger flavor enhancer than MSG (1). The umami taste of mushrooms is attributable to flavor 5′-nucleotides, including 5′-GMP, and might be greatly increased by MSG-like components (15). The contents of 5′-GMP and 5′-XMP in *S. aspratus* were 50.2 mg/kg and 723.2 mg/kg, respectively, and 5′-IMP was found as a trace amount. Flavor 5′-nucleotide content is multifarious by species and growth regions of mushrooms. In an earlier study, levels of flavor 5′-nucleotide were found to be 66.5% in *F. velutipes* (white), 47.9% in *F. velutipes* (yellow) and 47.9% in *L. edodes* (22). In addition, Chiang *et al.* (15) found that flavor 5′-nucleotide contents were 59.9% and 49.5% in the fruit body and broth of *A. bisporus* and 68.8% and 73.3% in the fruit body and broth of *V. volvacea*, respectively. In *S. aspratus*, umami taste derived from 5′-nucleotides may be weak since its percentage (30.77%) of flavor 5′-nucleotides to 5′-nucleotides is low compared to that of other mushrooms.

Volatile components

Twenty-nine volatile compounds were identified in *S. aspratus* (Table 5). Alcohols (6), ketones (4), aldehydes (8), esters (3) and sulfur-containing compounds (3) were predominant, while only one kind of furan, acid and phenol, respectively, was detected. Alcohols and ketones were the most abundant compounds and consisted of a series of eight

Table 4. Contents of 5′-nucleotides in *Sarcodon aspratus*

5′-Nucleotide[a]	Content (mg/kg sample fresh weight)
5′-AMP	1,382.9±4.5
5′-CMP	139.8±9.8
5′-GMP	50.2±8.5
5′-IMP	Tr[c]
5′-UMP	214.6±3.4
5′-XMP	723.2±5.7
Flavor 5′-nucleotide[b]	773.4±8.1
Total	2,510.7±5.8

[a]5′-AMP, 5′-adenosine monophosphate; 5′-CMP, 5′-xytosine monophosphate; 5′-GMP, 5′-guanosine monophosphate; 5′-IMP, 5′-inosine monophosphate; 5′-UMP, 5′-uridine monophosphate; 5′-XMP, 5′-xanthosine monophosphate.
[b]Flavor 5′-nucleotide, 5′-GMP + 5′-IMP + 5′-XMP.
[c]Tr, trace.

carbons (C8), including 1-octanol, 3-octanol, 3-octanone, 1-octen-3-ol, 2-octen-1-ol and 1-octen-3-one. These are the primary volatiles contributing to mushroom flavor (6). And the main flavor role is ascribed to "mushroom alcohol", 1-octen-3-ol (23). The C8 compounds are derived from unsaturated fatty acids by the activation of lipoxygenase (24). Both 1-octen-3-one (19.69%) and 1-octen-3-ol (68.72%) were detected at the highest concentrations in *S. aspratus*. This result confirmed the fact that C8 compounds are the primary volatiles in many edible mushrooms. For example, 58.32% of the volatile fraction isolated from fresh milky mushrooms (*Calocybeindica*) was C8 compounds (25). They were also present in *Pleurotus florida* at 68%, in *Agaricu sbisporus* at 56.7%, and in *Calocybe indica* at 48%. The fragrance of each mushroom is very different and unique even though 1-octen-3-ol is the main aroma component in most mushrooms (26). Picardi and Issenerg (27) compared the volatiles present in raw *A. bisporus* with induced volatiles after cooking for up to 3 h. They found that 1-octen-3-one was not detected in the raw product but appeared after boiling for 15 min, and reached its maximum concentration after 30 min of boiling. Thus, they suggested that the basic difference in flavor between raw and cooked mushrooms may be correlated with the concentration of 1-octen-3-one. Other C8 compounds such as 3-octanol, 2-octanol, 2-octen-1-ol, 3-octanone or 1-octanol contribute significantly to the typical odor of mushroom sporophores, but were are not found in significant quantities in *S. aspratus*. The relative percentages of the present C8 compounds, with the exceptions of 2-octen-1-ol and 1-octen-3-one, were quite low. Aliphatic aldehydes like pentanal, octanal, decanal, acetaldehyde, undecanal and

Table 5. Volatile flavor components of *Sarcodon aspratus*

Component[a]	Retention time (min)	Relative percentage[b](%)
Alcohols		72.1
Pentanol	10.65	0.09
3-Octanol	15.86	0.14
2-Octenol	17.89	1.17
1-Octen-3-ol	18.11	68.72
1-Octanol	22.56	0.22
2-Octen-1-ol	24.96	1.76
Ketones		20.46
2-Heptanone	8.95	0.19
3-Octanone	11.16	0.42
1-Octen-3-one	12.80	19.69
3-Decen-2-one	25.42	0.16
Aldehydes		3.0
Pentanal	4.86	0.11
Octanal	12.35	0.10
Decanal	20.28	0.03
Acetaldehyde	21.25	0.37
Benzaldehyde	21.91	0.21
Phenyl acetaldehyde	26.73	1.61
Undecanal	30.85	0.37
(E,E)-2,4-Decadienal	33.24	0.20
Sulfur containing compounds		0.48
Dimethyl disulfide	6.53	0.32
Dimethyl trisulfide	16.10	0.10
Dimethyl sulfide	23.80	0.06
Esters		4.37
Ethyl acetate	3.87	2.15
2,3-Dimethyl butane dinitrite	6.71	2.13
Dioctyl adipate	62.77	0.09
Other compounds		
2-Amyl furan	10.26	0.12
Acetic acid	18.38	0.25
Octyl phenol	43.34	0.21

[a]Identification based on coincidence of GC retention indices and MS data with those of authentic standards from the Wiley 138 database.
[b]Relative percentage of the identified volatiles based on GC peak area.

2,4-decadienal were also found in *S. aspratus*. The relative percentages of acetaldehyde (0.37%) and undecanal (0.37%) were relatively high compared to other compounds. The aromatic compounds benzaldehyde and phenyl acetaldehyde were also detected. The concentration of phenyl acetaldehyde was highest (1.61%) among the aldehyde components in *S.*

aspratus, which arises from the oxidation of n-9 MUFAs (28). Three volatile compounds, dimethyl sulfide, dimethyl disulfide and dimethyl trisulfide, were identified and accounted for 0.48% of the total volatile compounds present. Dimethyl disulfide comes from methanethiol oxidation and is produced by the bacterial degradation of methionine (29). In addition, three ester components were identified, ethyl acetate, 2,3-dimethyl butane dinitrite, and dioctyl adipate. The concentration of esters (4.37%) in *S. aspratus* was quite high.

From this study, it is assumed that *S. aspratus* has a much more bitter taste than other mushrooms, since it presented high concentrations of bitter compounds with a low mannitol concentration; although its major volatile compounds were similar to those of common mushrooms. In order to increase the acceptance of *S. aspratus* as a functional food among consumers, further studies should focus on the interactions between taste-active components, which aid the reduction of bitter taste or enhance the umami taste. In conclusion, this experiment will provide foundational data related to the acceptability of *S. aspratus* for its development as a functional food material.

요 약

본 연구는 능이버섯의 식품학적 이용성 증진을 위한 기초적인 연구로써 능이버섯의 휘발성 및 비휘발성 기호성분을 분석하였다. 단맛에 관여하는 유리당은 glucose, trehalose, sucrose, xylose가 검출되었고 총 함량은 202.5 mg/kg이었다. 단맛, 쓴맛 및 감칠맛에 관여하는 아미노산의 함량을 분석한 결과, 필수아미노산과 총 유리아미노산의 함량은 각각 1,249.5 mg/kg과 2,592.1 mg/kg으로 나타났다. 버섯의 5'-nucleotide 함량을 분석한 결과, 감칠맛에 관여하는 5'-nucleotide의 함량은 773.4 mg/kg으로 측정되어 총 5'-nucleotide의 30.8%를 차지하였다. 능이버섯의 향기성분을 분석한 결과, 6종의 alcohol류, 4종의 ketone류, 8종의 aldehyde류 및 3종의 함황화합물을 비롯하여 총 27종의 향기성분이 확인되었다. 이 중 1-octen-3-ol이 전체 향기성분의 68%를 차지하였고, 2-octen-1-ol, 1-octen-3-one 그리고 2-octenol 순으로 나타났다. 이러한 결과는 능이버섯을 이용한 기능성 식품 및 식품재료로서의 이용성 증진을 위한 기초 자료로 제공될 수 있을 것으로 판단된다.

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