

## Effect of Different Drying Method on Volatile Flavor Compounds of *Lactarius deliciosus*

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### Abstract

The effect of different drying methods on volatile flavor compounds of *Lactarius deliciosus* was investigated, such as hot-air drying, vacuum freeze drying and sunshine drying. By adopting the solid-phase micro-extraction method, volatile flavor compounds were extracted from *Lactarius deliciosus*, then analyzed and identified through the application of gas chromatography-mass spectrometer (GC-MS). Results indicated that different drying methods could lead to large differences in volatile flavor compounds. Main volatile flavor compounds of fresh *Lactarius deliciosus* involved acids and aldehydes, which accounted for 86.31%; Hot-air dried *Lactarius deliciosus* mainly included acids and alkene, which accounted for 87.16%; *Lactarius deliciosus* dried with vacuum freeze dried *Lactarius deliciosus* was mostly composed of acids, esters and aromatic substance, which accounted for 94.74%; *Lactarius deliciosus* dried with sunshine was constituted by acid and aldehydes, which was as high as 90.67%.

**Keywords:** *Lactarius deliciosus*; Volatile flavor compounds; Drying method; Gas chromatography-mass spectrometer (GC-MS)

### Introduction

The wild edible mushroom market is concentrated within a small group of species belonging to genera such as *Lactarius*, *Boletus*, *Cantharellus*, *Amanita*, *Pleurotus*, and *Calocybe* [1]. Among them, *Lactarius deliciosus* (i.e., *Agaricus deliciosus*), belonging to Russulaceae, is the most commercially important wild edible mushroom because of their high consumption by the rural population and their economic value in the markets of Southeast Asia [2].

As a popular natural and rare biological resource, *Lactarius deliciosus* have been used as food and food-flavoring material in soups and sauces for centuries, due to their unique and subtle flavour. Recently, they have become attractive as functional foods and as a source of physiologically beneficial medicines, while being devoid of undesirable side-effects. *Lactarius deliciosus* were also found to be medically active in several therapies, such as anti-tumor, antibacterial, antiviral and immune-modulating treatments [3,4]. However, like all wild edible mushrooms, *Lactarius deliciosus* are highly perishable with a short shelf-life of 1-3 days at room temperature, and tend to lose quality immediately after harvest due to high respiration rate and the lack of physical protection of cuticle [5,6]. Furthermore, *Lactarius deliciosus* lose marketability early during the storage period due to enzymatic activity, weight loss, shrinkage, browning, spore formation, bacteria and yeasts and moulds [7,8]. Because short shelf-life is an impediment to the distribution and marketing of the fresh product, its exploitation is mainly carried out by amateur personal, and the use of collected production is related to self-consumption or restaurants, intermediate links within the value chain being unregulated. Consumer interest for health and wellbeing has been reflected in a significant rise in the marketing and distribution of edible mushrooms in the latest years [9]. Thus, it's so very urgent to process and utilize this high quality wild resource in order to increase its added value.

It's a more perfect method to dry fresh *Lactarius deliciosus* for the sake of storage and development, but a series of biochemical reaction will react resulting in product quality deterioration through the change of colour, flavor and nutrition. To the best of our knowledge, there is no research focused on the effect of processing method on product quality of *Lactarius deliciosus*. For the first time, the present research investigated the effect of different drying methods, such as hot air, vacuum freeze and sunlight, on volatile flavor compounds of *Lactarius*

*deliciosus* by extracting with head space solid phase micro extraction (SPME) technique and analyzing with gas chromatography-mass spectrometry (GC-MS). In addition, the change mechanism of volatile flavor was discussed in order to lay theoretical basis for the choice of drying methods and to provide reference data for production quality control.

### Materials and Methods

#### Sample treatment

Fresh *Lactarius deliciosus* was purchased from Farmers. After washing raw materials, 4 samples of fresh *Lactarius deliciosus* were treated with different drying methods. Sample A was cut into cubes of 0.5 cm using knife and no longer processed, which served as the blank control, Sample B was dried with hot air, sample C was processed with vacuum freeze drying, sample D was treated with sunshine drying.

**Hot air drying:** 500 g of fresh *Lactarius deliciosus* was put in electric oven and dried at 45°C for 3-4 h. In the next step, the temperature rising was controlled at a rate of 1°C/h. *Lactarius deliciosus* was dried for 4-5 h when the temperature reached 50°C. And then, the temperature increased to 55°C at a rate of 1°C/h, and drying was performed for 8-9 h. Finally, the temperature rose to 65°C at the same rate, and drying was continued until the moisture content was about 13.0%.

**Vacuum freeze drying:** Fresh *Lactarius deliciosus* was frozen at -40°C for 9 h, and then dried at 65 Pa and -65°C for 8-9 h.

**Sunshine drying:** Fresh *Lactarius deliciosus* was exposed to the sunlight until the water mass fraction was less than 13.0%.

**Volatile compounds extraction:** Volatile flavor compounds

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were extracted using solid-phase microextraction (SPME). A SPME device (Supelco, Bellefonte, PA, USA) containing a fused-silica fibre (10 mm length) coated with a 50/30  $\mu\text{m}$  layer of DVD/CAR/PDMS was used. The fibre was conditioned prior to analysis by heating it in a gas chromatograph injection port at 270°C for 60 min. Extraction was performed at 35°C for 30 min. Before extraction, samples were equilibrated for 15 min at the temperature used for extraction. Once sampling was finished, the fibre was withdrawn into the needle and transferred to the injection port of the gas chromatograph-mass spectrometer (GC-MS) system.

**Gas chromatographic conditions:** Agilent 19091s-433 (30 m  $\times$  250  $\mu\text{m}$   $\times$  125  $\mu\text{m}$ ) quartz capillary column was adopted. Temperature programming was following as: the temperature was maintained at 50°C for 3 minutes and increased to 80°C at a rate of 3°C/min. And then temperature was risen to 150°C at a rate of 5°C/min and maintained at 150°C for 4 minutes. Next, the temperature was speeded up to 240°C at a rate of 3°C/min and maintained at 240°C for 5 minutes. The carrier gas was high purity He, the inlet temperature was 270°C, the auxiliary heater temperature was 230°C, automatic injection volume was 1  $\mu\text{L}$  and the split ratio was 1:1.

**Mass spectrometric conditions:** Advance sample temperature was 280°C, and EI ionization was employed. Ion source temperature was 230°C, and quadropole temperature was 150°C. Electron energy was 70 eV, and filament current was 34.6  $\mu\text{A}$ . The scanning mass range was 50.0-550.0 aum, and the solvent delay time was 4 minutes.

**Qualitative and quantitative analysis:** Artificial analysis and computer retrieval (NIST) were performed on mass spectra, so as to

determine chemical compositions corresponding to various spectral peaks. By adopting peak area normalization method, relative content (%) of each component was calculated. Assuming that the correction factor was 1 and using internal standard (phenyl ethyl acetate) method, the content of components to be determined was calculated as the equation below.

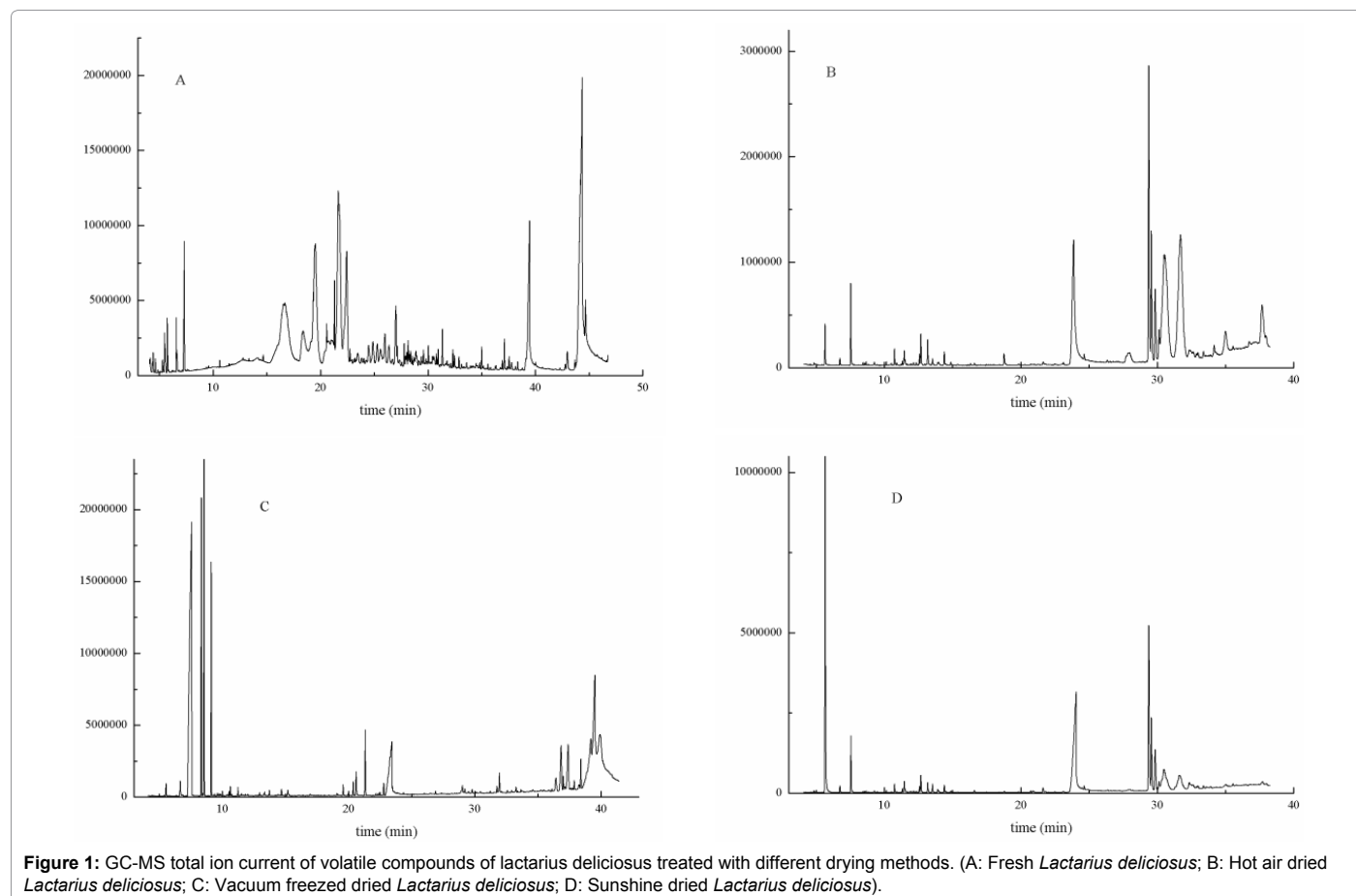
## Results and Discussion

### Total ion chromatogram and analysis of volatile flavor compounds

According to Figure 1, it could be known that volatile compounds of *Lactarius deliciosus* treated with different drying methods were quite different. Tables 1-3 revealed that totally 79 volatile compounds were identified in *Lactarius deliciosus* treated with different drying methods, including alcohols (5), alkenes (18), aldehydes (19), ketones (4), phenols (11), acids (5) and esters (17). The majority of these volatile substances were found naturally in *Lactarius deliciosus*, while some were resulted from a series of chemical reactions during the drying process, such as Maillard reaction between reducing sugars and amino compounds, oxidation reaction of unsaturated fatty acid, decomposition reaction of ester oxide, which could generate new volatile flavor compounds [6,10].

### Volatile flavor compounds of fresh *Lactarius deliciosus*

24 volatile flavor compounds were detected in fresh *Lactarius deliciosus*, including 1 alcohol (0.91%), 2 alkenes (1.16%), 9 aldehydes (13.50%), 1 ketone (0.87%), 1 phenol (0.65%), 3 acids (72.81%) and 7 esters (10.10%). Components with higher content mainly involved



S.no.	Volatile compounds	Relative amount/%			
		A	B	C	D
1	Trimethyl butanol	-	0.26	-	-
2	N-butanol	-	-	-	0.29
3	Hexanal	1.31	0.57	0.79	0.88
4	Methoxy hexanal	-	6.77	-	-
5	Ethylbenzene	-	-	9.37	0.15
6	Para xylene	-	-	11.97	-
7	Benzene	-	0.26	-	-
8	O-xylene	-	-	-	0.21
9	1,3-dimethyl-benzene	-	-	6.13	-
10	Cyclohexanone	-	0.43	-	0.33
11	Heptanal	-	-	0.06	-
12	(1-methyl ethyl)-benzene	-	-	0.05	-
13	α-song pinene	-	0.28	-	-
14	1R-2,6,6-trimethoxy	-	-	-	0.22
15	Propyl benzene	-	-	0.14	-
16	Anti-2-heptenal	-	-	0.30	-
17	D-limonene	-	1.37	-	2.82
18	Limonene	-	3.29	-	1.18
19	Benzaldehyde	-	0.26	-	-
20	1-octene-3-alcohol	-	-	0.26	1.61
21	1-octene	-	1.36	-	-
22	Caprylic aldehyde	-	-	-	0.17
23	3-carene	-	0.25	-	0.21
24	2-carene	-	0.23	-	0.17
25	O- cymene	-	0.99	-	-
26	Isopropyl ortho toluene	-	-	-	0.87
27	Benzoic hexanal	-	2.78	0.15	1.73
28	γ-terpinene	-	0.80	-	-
29	Formic acid octyl ester	-	-	0.20	-
30	Anti-2-octene 1-alcohol	-	-	-	0.63
31	(+) -4- carene	-	1.48	-	-
32	Terpinolene	-	-	-	1.19
33	2-nonanone	-	-	-	0.25
34	Pelargonic aldehyde	-	0.44	0.23	0.34
35	Anti-2-nonene aldehyde	-	-	-	0.40
36	Anti-2-decene aldehyde	-	-	0.42	-
37	Anethole	-	-	0.55	0.35
38	E,E-2,2-dodecadienal	-	-	-	0.36
39	E,E-2,4-decadienal	4.44	0.43	1.09	1.10
40	2-undecenal	0.74	-	-	-
41	Tetradecanoic acid ethyl ester	-	-	5.13	-
42	Decanoic acid	-	59.14	-	69.73
43	9-oxo methyl nonanoate	3.46	-	-	-
44	Butylated hydroxytoluene	-	0.63	-	-
45	10-carbonyl methyl decanoate	2.03	-	-	-
46	Methoxy phenol	0.65	-	-	-
47	Anti-1,9-dodecadiene	0.74	-	-	-
48	Tetramethyl-1H-cyclopropene	-	17.97	-	14.81
49	Undecalactone	1.51	-	-	-
50	1,11-Dodecadiene	-	-	0.29	-
51	8-heptadecene	-	-	0.86	-
52	Triethylene cyclooctene	0.42	-	-	-
53	Cis-9-hexadecenal	1.28	-	-	-
54	2-pentadecanone	0.87	-	-	-
55	Pentadecanal	0.89	-	-	-
56	Methyl-12-methyl tridecanoate	-	-	0.28	-
57	3,3-dimethyl benzidine	-	-	0.09	-
58	Anti -9- eicosene	-	-	0.15	-
59	7-cis-10-cis-hexadecadienoic acid aldehyde	0.61	-	-	-

60	Trans-11-hexedecanal	1.69	-	-	-
61	Anti-2-tetradecene-1-alcohol acetate	0.43	-	-	-
62	Trans-9,12-Octadecadienoic acid methyl ester	-	-	1.32	-
63	Methyl cis-9-octadecenoate	-	-	4.36	-
64	9,12-Tetradecadien-1-acetate	0.70	-	-	-
65	Diisobutyl phthalate	-	-	0.63	-
66	13-tetradecenal	1.03	-	-	-
67	2-hydroxy-cyclopentadecanone	-	-	0.11	-
68	14-methyl-8-hexyne-1-alcohol	0.91	-	-	-
69	Methyl cis-9-hexadecenoate	-	-	0.35	-
70	Methyl stearate	-	-	0.40	-
71	Methyl 2-methyl-hexadecanoate	0.70	-	-	-
72	Palmitic acid methyl ester	-	-	1.23	-
73	Oleic acid	-	-	11.54	-
74	Tetradecanoic acid	31.41	-	-	-
75	Palmitic acid	1.48	-	18.95	-
76	Glyceryl monooleate	-	-	22.62	-
77	Methyl linoleate	0.44	-	-	-
78	Anti-methyl oleate	2.35	-	-	-
79	Cis-9, cis-12-octodecane dienoic acid	39.92	-	-	-

Note: Means not detected.

**Table 1:** GC-MS analysis of volatile flavor compounds of *Lactarius deliciosus* dried with different methods.

Compound	A	B	C	D
Alcohols	1	1	1	3
Alkenes	2	10	4	8
Aldehydes	9	6	7	7
Ketones	1	1	1	2
Phenols	1	2	6	3
Acids	3	1	2	1
Esters	7	0	10	0
Total	24	21	31	24

**Table 2:** Volatile flavor compound species of *Lactarius deliciosus* dried with different methods.

Compound	A	B	C	D
Alcohols	0.91	0.26	0.26	2.53
Alkenes	1.16	28.02	1.85	20.94
Aldehydes	13.5	11.26	3.04	4.99
Ketones	0.87	0.43	0.11	0.58
Phenols	0.65	0.89	27.74	1.23
Acids	72.81	59.14	30.48	69.73
Esters	10.1	0	36.52	0
Total	100	100	100	100

**Table 3:** Content of volatile flavor compounds of *Lactarius deliciosus* dried with different methods (%).

C-9,C-12-octodecane dienoic acid (39.92%), tetradecanoic acid (31.41%), E,E-2,4-Sebacic olefine aldehyde (4.44%), 9-oxo-methyl nonanoate (3.46%) and anti-methyl oleate (2.35%). The volatile composition of fresh *Lactarius deliciosus* was characterized by fresh fruity and slight green grass, dominating by acids and esters with the highest aldehydes content. Moreover, its acids and esters were dominated by unsaturated compounds, which were easy to produce peroxides under the action of enzymes during the drying process, then peroxide decomposition could generate aldehydes, ketones and alcohols compounds which play a harmonic, synergistic or complementary role in flavor resulting in different flavors [7].

#### Volatile flavor compounds of *Lactarius deliciosus* dried with hot air

21 volatile flavor compounds were detected in *Lactarius deliciosus*

sample dried with hot air, including 1 alcohol (0.26%), 10 alkenes (28.02%), 6 aldehydes (11.26%), 1 ketone (0.43%), 2 phenols (0.89%) and 1 acid (59.14%). Components with higher content mainly involved decanoic acid (59.14%), methoxy hexanal (6.77%), limonene (3.29%), Benzene hexanal (2.78%). The volatile composition of *Lactarius deliciosus* dried with hot air was characterized by dominating by acids with higher content of aldehydes and alkenes, but the content of other volatile flavor compound was low. Compared with other drying methods, there were more unique volatile flavor compounds, such as  $\gamma$ -gamma terpinene (0.80%), 4-carene (0.99%),  $\alpha$ -sobrerone (0.28%), O-cymene (1.48%) and 1-octene (1.36%). During the process of hot air drying, many small molecule volatile components were produced, and olefinic terpene was the most prominent which might be the outcome of decomposition reaction of unsaturated fatty acid and ester. As a result of these reactions and compounds formation, *Lactarius deliciosus* dried with hot air eventually formed the smell of bitter almonds, fried flavor and unique flavor of lactarius [5].

#### Volatile flavor compounds of *Lactarius deliciosus* dried with vacuum freeze

31 volatile flavor compounds were detected in *Lactarius deliciosus* dried with vacuum freeze, including 1 alcohol (0.26%), 4 alkenes (1.85%), 7 aldehydes (3.04%), 1 ketone (0.11%), 6 phenols (27.74%), 2 acids (30.48%) and 10 esters (36.52%). Components with higher content mainly involved glyceryl monooleate (22.62%), oleic acid (11.54%), limonene (3.29%), E,E-2,4-Decedienal (1.91%). The characteristic of volatile flavor compound in *Lactarius deliciosus* dried with vacuum freeze was more content of acids, esters and aromatic substances, but lower other compound. Compared with other drying methods, its unique volatile flavor compounds mainly included 8-Heptadecene (0.86%), n-Ethyl Myristate (5.13%), methyl cis-9-octadecenoate (4.36%), paraxylene (11.97%) and ethylbenzene (9.37%). Vacuum freeze is a desired method formed maximum volatile flavor compounds, it's deduced that the temperature was maintained at extremely low levels so as to original volatile constituents could be preserved perfectly.

## Volatile flavor compounds of *Lactarius deliciosus* dried with sunshine

24 volatile flavor compounds were detected in *Lactarius deliciosus* dried with sunshine, including 3 alcohols (2.53%), 8 alkenes (20.94%), 7 aldehydes (4.99%), 2 ketones (0.58%), 3 phenols (1.23%) and 1 acid (69.73%). The mainly ingredients with higher content involved decanoic acid (69.73%), d-limonene (2.82%), benzene hexanal (1.73%), meaning that volatile flavor compounds of *Lactarius deliciosus* dried with sunshine were dominated by acids but lower content of other compound. Compared with other drying methods, its unique volatile substances mainly included Anti-2-octene-1-alcohol (0.63%), terpinolene (1.19%), isopropyl ortho toluene (0.87%), o-xylene (0.21%), (2E)-2-Nonenal (0.40%). Though the principle of sunshine drying was basically the same as hot air drying, its volatile composition is rather changeable and very different because of a circulation environment with a greatly fluctuated temperature. For example, sunshine drying produced more flavor substances of eight carbon alcohol compound (2.24%) which did not exist air drying.

## Comparative analysis of major volatile flavor compounds

**Alcohols:** Alcohols is produced by fat oxidation or activity of part microorganism. The main alcohol in *Lactarius deliciosus* was 1-octene-3-alcohol, commonly known as mushroom essence, which respectively accounted for 0.26% and 1.61% of volatile flavor compounds of *Lactarius deliciosus* dried with vacuum freeze and sunshine. Alcohol is present richly in full mushroom flavour owing to its lower flavor threshold and existing in almost all kinds of mushrooms. It could be concluded that the main source of alcohols was the oxidation and decomposition products of unsaturated fatty acid because the precursor was mainly 16-22 carbon unsaturated fatty acid [11].

**Carbonyl compounds:** Carbonyl compounds mainly referred to some aldehydes and ketones, and the major contributor to flavor formation were micromolecular aldehydes and ketones and their precursors because of the low volatility of macromolecular carbonyl compounds. There are 2 kinds of carbonyl compounds in fresh *Lactarius deliciosus*, 3 in hot air drying, 7 in vacuum freeze drying and 5 in sunshine drying respectively. It could be known that carbonyl compound might be the intermediate product of fat oxidation and Maillard reaction [12,13].

**Acid compounds:** Acid compound, with fresh and sweet fruit flavor, is the leading volatile flavor compound in *Lactarius deliciosus*, and it mainly included decanoic acid, myristic acid, oleic acid and palmitic acid. Decanoic acid, a important ingredient in acid compounds, accounted for 59.14% and 69.13% of volatile flavor compounds in hot air drying and sunshine drying respectively, but didn't exist in wet and vacuum freeze-dried *Lactarius*, therefore it is the reaction product of during the heating process. Decanoic acid is allowed to be a food flavouring agent which is mainly used in the preparation of dairy, rum, brandy and coconut flavor for offering a special grease smell, thus the present experiment could provide a basis for further development research of *Lactarius deliciosus* flavor [14,15].

**Ester compounds:** Some mainly ester compounds, such as glyceryl monooleate, tetradecanoic acid ethyl ester, anti-methyl oleate and

methyl cis-9-octadecenoate, were detected in the sample. The kinds of ester compounds of vacuum freeze-dried *Lactarius* were 10 with the highest content of 36.52%, and wet *Lactarius* 7 with 10.10%, but there is no ester detected in hot air dried and sunshine dried *Lactarius*. Thus it can be seen that the esters of *Lactarius* were unstable at relatively high temperature [15,16].

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