Comparative analysis of taste compounds in shiitake mushrooms processed by hot-air drying and freeze drying

Xiao Yang, Yuyu Zhang, Yan Kong, Jing Zhao, Ying Sun, and Mingquan Huang

Beijing Laboratory for Food Quality and Safety, Beijing Advanced Innovation Center for Food Nutrition and Human Health, Beijing Key Laboratory of Flavor Chemistry, Beijing Technology and Business University, Beijing China

ABSTRACT
Shiitake mushrooms were processed by hot-air drying (HD) and freeze-drying (FD) to investigate the taste compounds. The total free amino acids in HD samples treated at 60°C was the highest as compared to the other three dried samples. Although cysteine was not detected in fresh shiitake mushroom (FSM), it was released during the drying process and its content increased with the temperature increase from 50°C to 70°C. The content of total organic acids was the highest in the HD sample treated at 60°C (56579.6 µg/g DW). Based on the obtained moisture contents, all compounds were calculated to their contents in respective FSMs, the content of Glu, citric acid, and succinic acid increased during drying as temperature increase from 50°C to 70°C. It indicated that new taste compounds were produced during the HD process. The content of total nucleotides was the highest in HD samples treated at 50°C, and the lowest in FD sample. The HD samples treated at 70°C showed the highest equivalent umami concentrations (EUC) (25.2 g MSG/100 g), followed by at 60°C (19.7 g MSG/100 g), 50°C (14.7 g MSG/100 g) and FD samples (12.0 g MSG/100 g). Our results indicated that different drying treatments led to different physiochemical properties and nutritional contents in Shiitake mushrooms.

Introduction
Mushrooms have been enjoyed for their delicious taste. The shiitake mushroom (Lentinus edodes), an edible fungus, has been valued as both food and medicine since ancient times. As the second most cultivated edible mushroom in the world, the shiitake mushroom represents about 25% of worldwide mushroom production. Shiitake mushroom contains 18 types of free amino acids, providing ideal ratios of all the essential amino acids needed for human nutrition. Furthermore, shiitake mushroom provides important bioactive compounds with anti-tumor, anti-cancer and lowering blood pressure properties. Freshly harvested shiitake mushrooms deteriorated quickly due to higher moisture contents, while dried mushrooms are a significant-priced ingredient in various popular sauces, soups, and other dishes. Shiitake is often dried and rehydrated before use. Many people prefer dried Shiitake to fresh for the superior umami flavor (the flavor common to savory products such as meat, cheese, and mushrooms). Therefore, it is important to find a suitable method to prolong the shelf-life and maintain the quality, reduce the loss of nutrients during shiitake mushroom preservation.

The drying process does have the potential to inhibit some enzymes activities. Researchers have also reported that the physicochemical properties of the shiitake, such as chemical composition, molecular weight distribution, viscosity, and conformation were significantly affected by various
drying processes such as freeze-drying (FD), oven drying, spray drying, vacuum drying and microwave drying.\[7\] Hot-air drying (HD) and FD are the most common methods for drying mushrooms.\[8–10\] The solid state of water, low temperature and sublimation mode of moisture transport during FD treatment could protect the primary structure and shape of products. The resultant products have low bulk density, high porosity, and better rehydration characteristics.\[10–12\] The HD treatment is frequently used due to the lower costs. Among the different shiitake mushroom-drying techniques reported to date, HD treatment is the most popular owing to its simple operation and low-energy consumption.\[13\] The processing equipment is neither special nor expensive, the procedure is quite simple, and could be easily grasped by ordinary workers. The shelf life of dried mushroom could be extended to more than one year after HD treatment.\[14\]

Although drying indeed affected the preservation of shiitake mushrooms and prolonged their shelf life, the flavor was also significantly altered. Dried shiitake mushrooms have a superior umami flavor due to the breakdown of proteins into amino acids during the drying process.\[15\] The taste of edible mushrooms primarily derived from the presence of several water-soluble substances in their composition, including 5’-nucleotides, free amino acids, organic acids, soluble sugars, and polyols.\[16–18\] The mushroom flavor profiles have been extensively studied.\[19,20\] However, the relationship between the changes of taste components and texture of mushrooms remains unclear at different drying temperatures. The physico-chemical characteristics and taste compounds of shiitake mushrooms should be further studied.

The aims of this study were to investigate the effect of HD and FD on taste compounds and several other important quality parameters of shiitake mushrooms, such as moisture, pH, free amino acids, 5’-nucleotides, organic acids, and microstructure. The amino acids, 5’-nucleotides, and organic acids were determined by ultra performance liquid chromatography (UPLC). The equivalent umami concentration (EUC) of shiitake mushroom samples were calculated and compared.

### Materials and methods

#### Materials

Freshly harvested shiitake mushrooms were purchased in Yonghui market (Beijing, China) and stored at 4°C with 95% relative humidity prior to analysis. All samples were from the same batch. The average diameter of pileus was (52.7 ± 0.6) mm. The average length of the stipe was (32.3 ± 2.5) mm.

#### Drying methods

**FD treatment:** Each fresh shiitake mushroom (FSM) was cut into eight slices and frozen at −20°C for 24 h, then lyophilized in a freeze dryer (VFD-1000, Beijing Boyikang Laboratory Instrument Co. Ltd., Beijing, China) for 48 h (Figure 1). The condenser temperature was set at −45°C and the vacuum was maintained at 20 Pa.

**HD treatment:** The HD treatment was conducted by following a reported method with minor modification\[21\], the drying temperatures were set at 50°C, 60°C and 70°C, respectively (Figure 1). The drying process lasted until the shiitake mushrooms reached the constant weight. Hot-air dried and freeze-dried sliced shiitake mushroom samples were then ground into powder by a BJ-200 grinder (Baijie, Zhejiang, China), and subsequently stored at −20°C before use.

#### Moisture and pH measurements

The moisture content of the FSM and dried shiitake mushroom were measured using an electronic moisture analyzer (OHAUS MB35, Switzerland); each sample was repeated in triplicate. For pH analysis, the samples of dried powder (2 g) and fresh mushrooms (2 g) were added to 30 mL ultra-pure solution (Hangzhou Wahaha Group Co. Ltd., Hangzhou, China) and vortexed for 3 min. The mixture solutions were held at room temperature (25°C) for 1 h to separate solid and liquid phases, and then centrifuged (3922×g) in
50 mL centrifuge tube at 4°C for 10 min. The volume of supernatant was set to 100 mL, and its pH was measured using a Thermo Orion 868 pH meter (Thermo Fisher Scientific, Inc., Pittsburgh, PA).

**Browning index (BI)**

The BI value was determined by following a reported method with minor modification.[22] The samples of dried powder (4.0 g) and fresh mushrooms (4.0 g) were extracted with 20 mL of 80% ethanol and vortexed for 3 min. The mixture solution was held for 30 min at room temperature (25°C), and then centrifuged (3922×g) in 50 mL centrifuge tube at 4°C for 10 min. The ultra-violate (UV) absorbance of the supernatant was measured at 420 nm by an UV Spectrophotometer (T6, Persee, Beijing).
Scanning electron microscopy (SEM) analysis

The samples were identically prepared to the same size in order to observe the changes caused by the various drying temperatures to the cellular structure of the dried products. Thin slices, each approximately 1 mm thick, were cut from the dried samples and placed on a specimen holder using double-sided adhesive tape, sputtered with gold powder. The images of the cellular structure of the dried shiitake mushroom slices were observed using a VEGA/LSU SEM (Tescan, Czechoslovakia), at a magnification of 800.

Free amino acid analysis

Fresh mushroom was made into a muddy consistency and a dried mushroom sample was finely ground. The dried mushroom powder (2 g) and the fresh mushroom (5 g) were shaken with 50 mL 0.1 mol/L HCl at 25°C for 45 min. Then, it was centrifuged (3922×g) in 50 mL centrifuge tube at 4°C for 10 min. The supernatant was filtered through a 0.45 µm membrane filter and the volume of the filtrate was adjusted to 100 mL. The deproteinized supernatant was then lyophilized and redissolved by means of a sodium loading buffer (Biochrom Ltd, Cambridge, UK). After being filtered through a 0.45 µm nylon filter membrane, the solution was analyzed using an automatic amino acid analyzer (Biochrom 30+, Biochrom Ltd, Cambridge, UK) as reported by Li et al.[23] with some modifications. The chromatographic separations were carried out at 570 nm and 440 nm with an injection volume of 20 µL. The relatively quantitative analysis of the amino acids was calibrated using external standards. The standard mixture solution (the concentration of Cys-Cys was 1.25 µmol/mL and other amino acids were 2.50 µmol/mL) was sequentially diluted. The results were expressed as micrograms of amino acids per gram fresh weight (µg/g FW) and dry weight (µg/g DW), respectively.

Organic acid analysis

The dried powder sample (2 g) and fresh mushrooms (10 g) were added to 50 mL deionized water, respectively, and then treated with ultrasound (400 W, 30 min). The solutions were subsequently centrifuged (3922×g) at 4°C for 10 min. The supernatant was filtered through a 0.45 µm filter membrane and the volume of the filtrate was adjusted to 50 mL. The organic acids were analyzed by Thermo U3000 UPLC system (Thermo Fisher Scientific Inc., USA), following a reported method.[24] Each organic acid was quantified according to the calibration curve of the respective standard.

5′-nucleotides analysis

The dried sample powder (1 g) and fresh mushrooms (4 g) were extracted with 25 mL deionized water and boiled for 1 min, after cooling down to room temperature, the samples were centrifuged (3922×g) at 4°C for 10 min. The supernatant was filtered through a 0.45 µm filter membrane and the volume of the filtrate was adjusted to 50 mL. The ribonucleotide composition of the samples was analyzed using a Shimadzu model HPLC system (Shimadzu Corp., Kyoto, Japan), following the procedure of Kong et al.[24] Each 5′-nucleotide was quantified according to the calibration curve of the respective standards.

Equivalent umami concentrations (EUC) analysis

EUC is the concentration of MSG (g/100 g) equivalent to the umami intensity given by the mixture of umami amino acids (Glu: glutamic acid, Asp: aspartic acid) and 5′-nucleotides. It is calculated using the following equation[25]:

\[
\text{EUC} = \frac{\text{MSG concentration (g/100 g)}}{\text{MSG equivalent concentration (g/100 g)}}
\]
Y = \sum a_i b_i + 1218 \left( \sum a_i b_i \right) \left( \sum a_i b_i \right)

In this equation, \( Y \) is the EUC of the sample (g MSG/100 g); \( a_i \) is the concentration (g/100 g) of each umami amino acid (Asp or Glu); \( a_i \) is the concentration (g/100 g) of each umami 5’-nucleotide (IMP, GMP, XMP or AMP); \( b_i \) is the relative umami concentration (RUC) for each umami amino acid to MSG (Glu, 1 and Asp, 0.077); \( b_j \) is the RUC for each umami 5’-nucleotide to IMP (IMP, 1; GMP, 2.3; XMP, 0.61 and AMP, 0.18); and 1218 is a synergistic constant based on the concentration of g/100 g used.

**Statistical analysis**

All statistical analyses were conducted in triplicate, with the results expressed as means ± SD. Statistical analysis was performed using SPSS software (version 19.0, SPSS Inc., Chicago, IL, USA). Analysis of variance \((p < 0.05)\) was used to analyze the data and a Duncan’s multiple range test was used to separate the means.

**Results and discussion**

*Effects of different drying methods on moisture, BI value, and pH of shiitake mushrooms*

The effects of different drying processes on the moisture content, BI value and pH of shiitake mushrooms are summarized in Table 1. Generally, HD treatment removes water by evaporation, however, for FD treatment, the substrate was firstly frozen and then the water was removed by sublimation. According to Ratti’s finding\(^{[26]}\), the absence of lipid-water and the low temperature required for the FD process could mitigate deterioration and microbiological reactions, thus ensuring an excellent quality in the dried food. The moisture content of FD mushroom reduced from 89.61% to 3.57% after drying for 48 h, which is suitable for long time storage. The fresh mushrooms should be commonly dried to give a moisture content of ≤13% (GB 7096–2014, China Food Safety Regulations, 2014). For the HD process, the moisture contents kept stable at 50°C, 60°C and 70°C after drying for 14 h, 13 h, and 13 h, respectively. On the other hand, with the increase in drying temperature, the remained moisture contents decreased accordingly. The HD mushrooms treated at 70°C showed a significantly lower moisture content (2.50%, w/w) than that at 50°C (3.74%, w/w) and 60°C (3.31%, w/w). It is interesting to note that the moisture content of FD mushrooms (3.57%, w/w) was similar to that of HD mushrooms dried at 50°C and 60°C.

The FSM was found to have the highest pH of 6.55, which agreed with the finding (fresh mushroom, *Agaricus bisporus*, pH 6.58) of Derossi et al.\(^{[27]}\) The pH of three HD samples ranged from 5.95 (70 °C) to 6.36 (50 °C), indicating that the pH of the HD samples decreased with the increase of drying temperature; while the pH of the FD shiitake was 6.18, which is higher than that of HD samples treated at 70 °C but lower than that of at 60 °C.

A previous study reported that color retention was an important quality parameter of dried products.\(^{[11]}\) The BI values of the four dried samples were 0.29 (HD, 50°C), 0.60 (HD, 60°C), 0.75 (FD) and 0.92 (HD, 70°C), respectively. They were all significantly higher than the control group (0.05, FSM sample). For HD samples, it was clearly to note that the BI value increased significantly with the increase of drying temperature (Table 1). Our results corresponded to the

| Drying methods | Water content (%) | Browning index | pH       |
|----------------|------------------|----------------|----------|
| Hot-air drying 50 °C | 3.74 ± 0.07\(^b\) | 0.29 ± 0.01\(^d\) | 6.36 ± 0.00\(^b\) |
| 60 °C | 3.31 ± 0.06\(^bc\) | 0.60 ± 0.00\(^c\) | 6.25 ± 0.01\(^c\) |
| 70 °C | 2.50 ± 0.15\(^c\) | 0.92 ± 0.01\(^a\) | 5.95 ± 0.00\(^c\) |
| Freeze drying | 3.57 ± 0.20\(^a\) | 0.75 ± 0.00\(^b\) | 6.18 ± 0.02\(^d\) |
| Fresh | 89.61 ± 1.03\(^a\) | 0.05 ± 0.00\(^e\) | 6.55 ± 0.01\(^a\) |

Values represent means of triplicates ± standard deviations. Dissimilar small alphabets within the same row are significantly different \((p < 0.05)\).
findings of Xu et al.\textsuperscript{28} High temperature may destroy the cell structure of mushroom, resulted in the release of cellular chemicals including amino acids and reducing sugars, therefore enhancing the formation of brown substances caused by Maillard reaction.\textsuperscript{14,29} For FD sample, the BI value was between that of 60 °C and 70 °C of HD samples. The mushroom browning in FD treatment could be attributed to enzymatic browning.\textsuperscript{10,30,31}

**Effects of different drying methods on free amino acids**

The contents of taste compounds (including amino acids, organic acids, nucleotides, and EUC) in the five shiitake mushroom samples are listed in Table 2. The HPLC chromatogram of amino acids, organic acids and 5'-nucleotides are shown in Figure 2. It is interesting to note that the total free amino acids of HD samples treated at 60°C (13919.8 µg/g DW) was the highest, followed by 50°C (13498.6 µg/g DW), FD (12275.2 µg/g DW) and 70°C (11099.4 µg/g DW). Some previous studies reported that the increase of free amino acids at different drying temperatures could be due to different drying time.\textsuperscript{6,28} However, when converted the total amino acids of dry content to the fresh content, all the four dried samples were lower than that of the FSM sample (Table 2). This could be ascribed to the chemical reaction of active amino acids during drying treatment, in which the proteins could be degraded by related enzymes and/or Maillard reaction. On the other hand, FSMs are rich in Ser and Lys, however, their contents decreased significantly with the increase of drying temperature. This could be due to the Maillard reaction during heating. Xu et al.\textsuperscript{28} reported that enzymatic reaction could induce partial degradation of proteins to oligopeptides and amino acids, and therefore release more hydrophobic zones. They also hypothesized that the changes of free amino acids corresponded to the changes of proteins, indicating that high-temperature drying pretreatment could promote protein degradation better than FD treatment and enhance the taste of dried mushrooms.

Amino acids could be divided into different groups based on their taste characteristics. Glutamic acid (Glu) belongs to the group of umami-taste amino acids. As shown in Table 2, Glu content in dried samples increased with the drying temperature increased from 50°C to 70°C. When being converted the Glu content in dry weight to the content of fresh weight, the content of Glu in HD samples treated at 70°C (123.7 µg/g FW) was higher than that of the control sample (115.4 µg/g FW), indicating new Glu was produced during heating treatment.

Cysteine (Cys) has been regarded as one of the no-taste amino acids, but it could be degraded into flavor precursors. There is no Cys in the FSM. However, the Cys was produced during drying treatment and the content increased with the increase of drying temperature with the values of 646.2 µg/g DW (50°C), 1155.3 µg/g DW (60°C) and 1219.4 µg/g DW (70°C), respectively (Table 2). The Cys content in FD samples was 823.2 µg/g DW.

**Effects of different drying methods on organic acids**

The organic acids, including malic acid, lactic acid, citric acid, and succinic acid, were quantified in fresh and dried shiitake mushrooms (Table 2). The content of total organic acids was the highest in the HD sample treated at 60°C (56579.6 µg/g DW). Lactic acid was the dominant organic acid in FSM. In addition, the HD samples treated at 60°C showed significantly higher succinic acid content than other samples. A previous study reported that both lactic acid and succinic acid could contribute to sour and umami tastes.\textsuperscript{32}
### Table 2. Contents of amino acid, organic acid, and nucleotide, EUC in shiitake mushroom samples.

| Compounds       | Content (µg/g FW) | Content (µg/g DW) |
|-----------------|-------------------|-------------------|
|                 | 50 °C             | 60 °C             | 70 °C Freeze drying | Fresh |
| Amino acid      |                   |                   |                   |       |
| Asp             | 28.9 ± 0.3^e       | 20.6 ± 0.2^f      | 13.5 ± 0.2^gh     | 13.2 ± 0.3^h | 15.9 ± 0.1^i |
| Thr             | 76.5 ± 0.9^ef      | 692.1 ± 1.1^g      | 51.6 ± 1.4^h      | 59.5 ± 1.2^i | 86.3 ± 0.9^j |
| Ser             | 460.4 ± 4.8^e      | 398.1 ± 4.7^g      | 280.5 ± 6.3^h     | 388.0 ± 6.1^i | 4520 ± 3.6^ef |
| Glu             | 71.5 ± 2.1^g       | 113.3 ± 2.6^de     | 123.7 ± 1.4^e     | 94.2 ± 0.8^d | 1154 ± 4.6^ef |
| Gly             | 44.8 ± 0.5^g       | 514.0 ± 0.4^d      | 37.6 ± 0.5^h      | 35.0 ± 0.6^h | 59.8 ± 1.0^j |
| Ala             | 68.2 ± 1.1^h       | 124.7 ± 2.4^f      | 100.1 ± 1.7^g     | 71.3 ± 2.7^h | 1844 ± 6.3^d |
| Cys             | 69.7 ± 2.3^f       | 124.1 ± 3.9^e      | 129.9 ± 6.7^d     | 88.7 ± 3.7^e | ND             |
| Val             | 56.8 ± 1.1^f       | 51.6 ± 1.0^g       | 38.4 ± 0.4^h      | 43.3 ± 1.3^i | 779 ± 1.7^e   |
| Met             | 61.0 ± 0.0^f       | 46.0 ± 0.1^g       | 4.2 ± 0.1^h       | 3.4 ± 0.1^h | 11.9 ± 0.3^f |
| Ile             | 31.2 ± 0.1^f       | 302.0 ± 0.2^g      | 24.8 ± 0.1^h      | 18.4 ± 0.1^h | 455 ± 0.4^d   |
| Leu             | 41.7 ± 0.4^f       | 420.0 ± 0.4^g      | 34.6 ± 0.2^a      | 29.0 ± 0.3^g | 71.6 ± 0.2^e |
| Tyr             | 28.9 ± 8.0^de      | 254.0 ± 8.0^de     | 21.0 ± 1.0^e      | 20.6 ± 1.1^e | 31.8 ± 1.6^f |
| Phe             | 33.6 ± 0.6^f       | 282.1 ± 1.0^g      | 25.5 ± 0.1^h      | 21.0 ± 0.3^h | 464 ± 1.7^d   |
| His             | 25.1 ± 0.2^f       | 171.0 ± 0.0^g      | 7.4 ± 0.2^h       | 7.3 ± 0.3^g  | 290 ± 0.1^e   |
| Lys             | 331.0 ± 4.8^e      | 301.7 ± 11.8^g     | 201.6 ± 1.8^f     | 230.7 ± 2.7^e | 3413 ± 1.8^f |
| Arg             | 55.5 ± 0.3^f       | 562.0 ± 0.3^g      | 59.9 ± 1.3^h      | 75.3 ± 1.5^i | 1309 ± 1.8^d |
| Pro             | 28.8 ± 0.3^g       | 394.0 ± 0.5^h      | 26.5 ± 0.3^i      | 16.3 ± 0.0^h | 31.8 ± 1.2^f |
| Total           | 14565 ± 6.5^f      | 14953.5 ± 5.8^i    | 11825.3 ± 13.7^j  | 13222.9 ± 9.0^i | 17318 ± 8.5^g |
| Organic acid    |                   |                   |                   |       |
| Malic acid      | 728.0 ± 27.0^d     | 8148.2 ± 29.2^d    | 904.1 ± 21.3^d    | 837.9 ± 154.0^d | 13262 ± 102.2^d |
| Lactic acid     | 2795.9 ± 151.8^d   | 4660.6 ± 174.0^d   | 3730.1 ± 514.2^d  | 2914.8 ± 896.4^d | 53325 ± 3166.4^d |
| Citric acid     | 125.6 ± 11.9^f     | 2410.0 ± 16.8^f    | 483.5 ± 11.8^g    | 61.2 ± 120^f  | 491 ± 1.4^e   |
| Succinic acid   | 155.5 ± 15.4^f     | 3615.1 ± 5.4^g     | 171.9 ± 15.5^i    | 59.3 ± 4.2^g  | 1069 ± 42.8^d |
| Total           | 3805.0 ± 145.2^d   | 60779.7 ± 191.0^d  | 5289.6 ± 539.3^d  | 3873.2 ± 54.3^d | 68148 ± 277.6^d |
| Nucleotide      |                   |                   |                   |       |
| 5'-CMP          | 357.5 ± 11.1^f     | 2681.3 ± 1.3^gh    | 296.2 ± 1.1^g     | 231.8 ± 2.5^f | 493.9 ± 1.3^e |
| 5'-GMP          | 81.1 ± 0.3^f       | 687.0 ± 0.5^g      | 77.0 ± 1.3^g      | 48.1 ± 0.7^e | 1089 ± 2.6^d  |
| 5'-IMP          | 36.0 ± 0.0^f       | 36.0 ± 0.0^f       | 4.4 ± 0.0^e       | 2.9 ± 0.1^f  | 13.3 ± 2.1^d  |
| 5'-AMP          | 35.0 ± 0.9^e       | 175.0 ± 0.5^g      | 21.0 ± 0.8^h      | 28.9 ± 1.2^e | 35.3 ± 2.0^f  |
| Total           | 477.2 ± 10.8^e     | 3579.2 ± 2.0^h     | 3985.0 ± 0.9^g    | 311.6 ± 3.7^i | 651.5 ± 0.7^e |
| EUC (g MSG/100 g) | 0.4 ± 0.0^f      | 0.4 ± 0.0^f       | 0.4 ± 0.0^f      | 0.4 ± 0.0^f  | 0.4 ± 0.0^f   |

Different lowercase letters between columns represent significant differences between cultivars (p < 0.05).
Effects of different drying methods on 5'-nucleotide

5'-CMP, 5'-GMP, 5'-IMP, and 5'-AMP were identified and determined as shown in Table 2. The total nucleotides content was the highest in HD samples treated at 50°C and the lowest in FD.
samples. FSM was rich in 5’-CMP, which occupied over 75% of total nucleotides. A previous study reported that other mushrooms (e.g. pine mushroom) were also rich in 5’-CMP.\textsuperscript{[33]}

**EUC**

The EUC value related to the contents of Glu, Asp, 5’-IMP, 5’-AMP, and 5’-GMP. The HD samples treated at 70\(^{\circ}\)C (25.2 g MSG/100g) showed the highest EUC value, followed by 60\(^{\circ}\)C (19.7 g MSG/100 g DW), 50\(^{\circ}\)C (14.7 g MSG/100 g DW), FD sample (12.0 g MSG/100 g DW) and the lowest in FSM (0.4 g MSG/100 g FW). This indicated that the HD samples treated at 70\(^{\circ}\)C possessed a better umami tastes. In addition, it is clear to note that the EUC of FD sample was significantly lower than that of HD samples. Cho et al. investigated the umami-taste active components in pine-mushrooms.\textsuperscript{[16]} In their study, the EUC values and umami sensory intensities exhibited the same patterns in different grades pine-mushrooms. The EUC values of the pileus were higher than that of the stipe and ranged from 13.26 (in the stipe of first grade) to 204.26 g (in the pileus of second grade) per 100 g.

**Scanning electron microscopy results**

The drying process of mushroom could affect its microstructure. HD treatment could potentially undermine both the nutritional quality and texture, and could also cause discoloration during a long term drying process.\textsuperscript{[33]} FD treatment is especially suitable for heat sensitive products, such as fruits with high sugar content and certain high-value vegetables.\textsuperscript{[34]} The effects of different drying methods and conditions on the structure of dried shiitake samples were observed under an SEM.

All of the four dried samples possessed a large number of pores and honeycomb-like microstructure.\textsuperscript{[10]} The SEM micrographs clearly showed that the tissue structure was affected by heating temperature (Figure 3). In HD samples treated at 50\(^{\circ}\)C, it was obvious to find a fraction of the cell, in which the vacuoles are defined by cell walls (lighter areas to a pale white) as well as parts that are similar to pockets filled with cellular juice (darker areas). However, these vacuoles inside the cell could not be found in HD samples treated at 60\(^{\circ}\)C and 70\(^{\circ}\)C, this is possibly due to water evaporation and a merging or even breakage of the cell walls, thus indicating severe contraction of the tissue with the rise of temperature. It is clear that high temperature in drying process resulted in the tissue dehydration and heightened deformation of cellular texture. Wang et al. reported that the increase of temperature caused the collapse of the surface cellular structure but not the interior part, which could be attributed to much higher surface water evaporation or sublimation rate compared to the water migration flux through the interior to the surface.\textsuperscript{[10]} Moreover, the cell walls of the FD sample were also destroyed by vacuum adsorption and water loss. In four dry mushroom samples, the structure of the FD sample was destroyed most seriously.

**Conclusion**

The moisture content and pH decreased with the increase of temperature in HD treatment, the moisture content of the FD sample is similar to that of HD samples treated at 50\(^{\circ}\)C and 60\(^{\circ}\)C. The BI values increased with the increase of temperature in HD treatment, while the BI value of FD sample was significantly lower than that of HD samples treated at 70\(^{\circ}\)C but significantly higher than that of HD sample treated at 60\(^{\circ}\)C. The FSMs were rich in amino acids (e.g. Ser and Lys), organic acid (e.g. lactic acid) and nucleotide (e.g. 5’-CMP). After drying process, the HD samples treated at 70\(^{\circ}\)C remained the lowest total amino acids (11099.4 µg/g DW), while the HD samples treated at 50\(^{\circ}\)C and FD samples showed significantly lower levels of total organic acids than other samples. For total nucleotides, the highest content was found in HD samples treated at 50\(^{\circ}\)C, while FD samples showed the lowest level. The above results indicated that some amino acids, organic acids, and nucleotides underwent some chemical reactions during the drying process. The HD samples treated at 70\(^{\circ}\)C showed the highest EUC value,
followed by at 60°C, 50°C and FD samples. In brief, it seemed that the HD samples treated at 60°C improved a better taste quality of the shiitake mushroom than other treatments.

**Funding**

This work was supported by the the National Key R&D Program of China [2016YFD0400705]; the National Natural Science Foundation of China [31401604].

**ORCID**

Yuyu Zhang http://orcid.org/0000-0003-3095-3083

**References**

[1] Jiang, T.; Luo, Z.; Ying, T. Fumigation with Essential Oils Improves Sensory Quality and Enhanced Antioxidant Ability of Shiitake Mushroom (*Lentinus Edodes*). *Food Chem.* **2015**, *172*, 692–698. DOI: 10.1016/j.foodchem.2014.09.130.

[2] Turlo, J.; Gutkowska, B.; Herold, F.; Krzyczkowski, W.; Błażewicz, A.; Kocjan, R. Optimizing Vitamin B12 Biosynthesis by Mycelial Cultures of *Lentinula Edodes* (Berk.) Pegl. *Enzyme Microb. Technol.* **2008**, *43*, 369–374. DOI: 10.1016/j.enzmictec.2008.05.005.

[3] Ampere, A.; Delhaes, L.; Soots, J.; Bart, F.; Wallaert, B. Hypersensitivity Pneumonitis Induced by Shiitake Mushroom Spores. *Med. Mycol.* **2012**, *50*, 654–657. DOI: 10.3109/13693786.2012.658091.

[4] Giri, S. K.; Prasad, S. Drying Kinetics and Rehydration Characteristics of Microwave-Vacuum and Convective Hot-Air Dried Mushrooms. *J. Food Eng.* **2007**, *78*, 512–521. DOI: 10.1016/j.jfoodeng.2005.10.021.
[29] Das, I.; Alternate Microwave, A. A. Convective Hot Air Application for Rapid Mushroom Drying. *J. Food Eng.* 2018, 223, 208–219. DOI: 10.1016/j.jfoodeng.2017.10.018.

[30] Kumar, A.; Singh, M.; Singh, G. Effect of Different Pretreatments on the Quality of Mushrooms during Solar Drying. *J. Food Sci. Tech.* 2013, 50, 165–170. DOI: 10.1007/s13197-011-0320-5.

[31] Dutta, B.; Raghavan, G. S. V.; Dev, S. R. S.; Liplap, P.; Murugesan, R.; Anekella, K.; Kaushal, T. A Comparative Study on the Effects of Microwave and High Electric Field Pretreatments on Drying Kinetics and Quality of Mushrooms. *Dry. Technol.* 2012, 30, 891–897. DOI: 10.1080/07373937.2012.678957.

[32] Park, J. N.; Fukumoto, Y.; Fujiita, E.; Tanaka, T.; Washio, T.; Otsuka, S.; Shimizu, T.; Watanabe, K.; Abe, H. Chemical Composition of Fish Sauces Produced in Southeast and East Asian Countries. *J. Food Compos. Anal.* 2001, 14, 113–125. DOI: 10.1006/jfca.2000.0963.

[33] Alibas, I.; Determination of Drying Parameters, Ascorbic Acid Contents and Color Characteristics of Nettle Leaves during Microwave-, Air- and Combined Microwave-Air-Drying. *J. Food Process Eng.* 2010, 33, 213–233. DOI: 10.1111/j.1745-4530.2008.00268.x.

[34] Zhang, M.; Tang, J.; Mujumdar, A. S.; Wang, S. Trends in Microwave-Related Drying of Fruits and Vegetables. *Trends Food Sci. Tech.* 2006, 17, 524–534. DOI: 10.1016/j.tifs.2006.04.011.