Influence of Cold Storage and Processing of Edible Mushroom on Ergothioneine Concentration

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***Abstract*—Edible and medicinal mushrooms are healthy foods relatively low in calories and fat but rich in proteins, vitamins, minerals and ergothioneine. After mushrooms are harvested, they continue to breathe and metabolize. Therefore, many physiologically active contents will change after harvest. Due to the different storage time and cooking ways, we studied a variety of ergothioneine content to determine the content of ergothioneine in *Flammulina velutipes, Pleurotus* citrinopileatus and *Pleurotus eryngii* after processing and cold storage. We compared the ergothioneine after different storage days in 4℃, dry way (freeze dry, cold air dry, hot air dry), radiation dose (pulsed light), cooking method (boil, stir-fry, gas fry) with high performance liquid chromatography to determine the process that can keep most ergothioneine. Ergothioneine content decreased while *P. citrinopileatus* storage days increased, and the highest content of ergothioneine was found to be 2241.6 μg/g on the third day. After irradiation of different dose of pulse light, the content of ergothioneine had no significant difference between 30 and 50 pulse form three types mushroom. The lowest content is to cook in boiled water for 3~10 minutes. Therefore, storage for 3 days, irradiation 50 pulse light, boiling for 3~5 minutes, gas-frying for 6 minutes, stir-frying for 10 minutes, and freeze drying was the best way to keep the ergothioneine in *P. citrinopileatus*. However, the processing treatment had little effect on *F. velutipes* and *P. eryngii*, except for boil treatment. Food processing and storage has the potential to slightly alter the stability of ergothioneine in mushrooms. Consumer guideline for estimating dietary intake of ergothioneine is to not boil mushrooms for too long, in order to retain more ergothioneine content.**

***Index Terms*—mushroom, ergothioneine, cold storage, pulsed light, cooking, drying**

I. Introduction

Ergothioneine is an imidazole-2-thione derivative of histidine betaine, food-derived, but not in animals and plants, hydrophilic amino acid [1]-[3]. Found in a plant-based diet, primarily from edible mushrooms, it is now recognized as important for human health and is generally considered an antioxidant [4]-[8]. Some researchers have documented that ergothioneine has powerful antioxidant and cytoprotective properties *in vivo*, and there is evidence that the body may concentrate it at sites of tissue injury by raising organic cation transporter, novel type-1 (OCTN1) levels [9]. Further, studies on various animal models, including protection against ischemia-reperfusion injury, damage caused by the pro-oxidants ferric-NTA , β-amyloid and d-galactose-induced dementia are predisposed to oxidative stress and inflammation [10]-[12].

Mushrooms have been analyzed to contain a significant amount of ergothioneine [13]. Chen *et al*. [14] studied the ergothioneine content of the fruiting bodies and mycelia of 20 edible and medicinal mushroom species and found that ergothioneine was detected in all samples. *P. citrinopileatus*, *P. ostreatus* (Korea), *P. ostreatus* (Taiwan), and *P. salmoneostramineus* contained the largest amounts of ergothioneine. The genus *Pleurotus* contained considerably large amounts of it. Aqueous extracts of rich ergothioneine mushroom have been applied in several storage studies as color stabilizers to inhibit lipid oxidation, metmyoglobin formation in beef and fish muscle and camellia oil oxidation [15]-[17].

Mushrooms are rarely eaten raw and they require processing before consumption [18], [19]. Most mushrooms are consumed after being cooked under different conditions, involving water or oil mediums and a wide range of temperatures [20]-[22]. Thus, heat treatment will become very important since it will affect directly the final composition of mushrooms [23]. Several cooking methods are widely used in mushrooms depending on the temperature, boiling, steaming, frying, or roasting etc. [24]. It is well known that processing of food may cause substantial changes in its chemical composition and thus affect nutritional and health properties. Dehydration is considered an effective preservation method to prolong the shelf life of mushrooms; a variety of drying methods, which include cold-air drying, hot-air drying and freeze-drying, are applied in processing mushrooms [25]. Pulsed light is a non-thermal technology, where sterilization and decontamination are achieved by impinging high-intensity light pulses of short durations on the surfaces of foods. Pulsed light is a rapid technology to convert ergosterol to vitamin D_2 in mushrooms [26].

There are not studies that determine ergothioneine into the mushrooms under thermal treatments, such as drying

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and subsequent boiling in water, and frying, at different times. Therefore, the main objective of this paper was to establish a relation among the types of processing (raw, boiled and fried) and time of processing (raw, usual time and well-done). This work was to determine the effect of these thermal processing, pulsed light irradiation and drying treatments of the *F. velutipes*, *P. citrinopileatus* and *P. eryngii* mushrooms on concentration of ergothioneine. This would provide valuable information about how culinary treatments and their degree of intensity can affect ergothioneine availability.

II. Materials and Methods

A. Mushroom Fruiting Bodies Collection

Three species of mushroom fruiting bodies that were used and categorized into edible mushrooms, including *F. velutipes*, *P. citrinopileatus* and *P. eryngii*, were obtained from Xi-Yyi Agricultural Farm, Wufeng, Taichung, Taiwan. After a harvest they were kept at 4 ℃ and were subjected to processing within 24 hrs. Fig. 1 shows the general description of the treatment assayed.

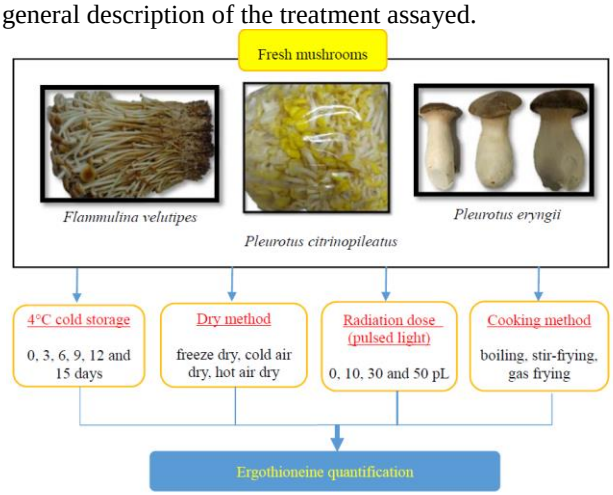

Figure 1. General scheme of all treatments assayed on edible mushrooms.

B. Mushroom Treatment

1) Cold storage treatments

Mushrooms from each species were randomly selected into nine samples and packaged by placing about 200 g in 1 kg PE bag. After packaging, three bags of mushrooms were randomly selected for day 0 analyses, and the remaining fifteen were stored in a 4 ℃ incubator. At days 3, 6, 9, 12 and 15, three trays were randomly selected for the study. All samples were freeze dried and ground into powder using Retsch Ultracentrifugal Mill and Sieving Machine (Haan, Germany) to obtain fine powder (60 mesh) for ergothioneine analysis.

2) Drying treatments

Fresh *P. eryngii* fruiting bodies were rinsed followed by removal of substrate and sliced to a thickness 3–4 cm. Fresh *F. velutipes* and *P. citrinopileatus* fruiting bodies were rinsed followed by removal of substrate and tear to a thickness 5 cm. The experiments were carried out using 200 g mushroom of cold-air, hot-air and freeze dried method, respectively. The cold-air dried using the food dehydrator (3926TB; Excalibur, California, USA) at 18 ℃ for 96 h. The hot-air dried using the oven dryer by hot air (DV453; Channel, New North City, Taiwan) at 50 ℃ for 72 h. The freeze dried using the lab-scale freeze dryer (FD8080; Hansor, Taichung, Taiwan) at -40 ℃ for 72 h. The drying process lasted until the moisture content of the mushrooms reached below 11% (wet basis). The samples were dried and ground into a fine powder (60 mesh) for ergothioneine analysis.

3) Pulsed light irradiation treatments

Samples were subjected to irradiation using a Xenon RC-801 pulsed light system (Xenon Corp.) employing an LH-840 lamp housing LH-840 and B-type 16″ linear lamp (280-700 nm). The apparatus generated three pulses per second at 169 J/pulse. An average dose was 11.50 kJ m^2/pulse [26], [27]. A 200 g sample mixture sample was placed on the iron plate and irradiated for with 0, 10, 30 and 50 pulses. After irradiation, the mushroom was freeze-dried and ground into powder to obtain a fine powder (60 mesh) for further analysis.

4) Processing treatments

Samples were treated by boiling (distilled water at 100 °C), stir-frying (hot plate with oil, 80 °C) and gas-frying (air flyer, 170 °C) process. Samples were subjected to boiling and stir-frying for 3, 5 and 10 min; then subjected to gas-frying for 3, 4 and 5 min. *Pleurotus citrinopileatus* were treated by gas-frying for 4, 6 and 8 min. The group three was not processed (control). After processing treatments, mushroom was freeze-dried and ground into powder to obtain fine powder (60 mesh) for further analysis.

C. Ergothioneine Assay

Ergothioneine was extracted and analyzed according to the method of Huang *et al*. [26] with slight modifications. Sample powder (1 g) was mixed with 20 mL of 10 mM 1,4-dithiothreitol (Sigma-Aldrich), 100 μM betaine (Sigma-Aldrich) and 100 μM 2-mercapt-1-methylimidazole (Sigma-Aldrich) in 70 % ethanol, and the resulting mixture was vortexed for 90 sec. Sodium dodecyl sulfate (1%, 4 mL, J. T. Baker) was added and the mixture was centrifuged at 3000 × *g* for 10 min. The combined filtrate was rotary evaporated at 40 ℃ to dryness, redissolved in 1 mL of methanol (LC grade, Merck), and filtered using a 0.45-μm polyvinylidene difluoride filter prior to injection onto high-performance liquid chromatograph (HPLC).

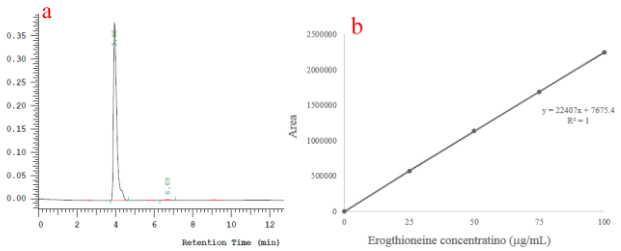

Figure 2. A map obtained by injecting ergothioneine into a standard by HPLC: Spike 1 is ergothioneine, retention time (RT) 3.96 min (a); Standard curve by different concentrations of ergothioneine due to the area of the standard (b).

HPLC with separation was performed on Kinetex PFP columns (4.6 × 250 mm, 5 µm, Phenomenex Inc., CA, USA). The mobile phase was 50 mM sodium phosphate in water with 3% acetonitrile (LC grade, Merck) and 0.1% triethylamine (Sigma-Aldrich) adjusted to a pH of 7.3, at a flow rate of 1.0 mL/min and UV detection was at 254 nm. The content of ergothioneine was calculated on the basis of the calibration curve of each authentic ergothioneine (Fig. 2).

D. Differential Scanning Calorimetry (Dsc) Assay

Temperature-programmed screening experiments were performed with a DSC (TA Q20-RCS90). For the DSC analyses on the samples sealed in 20 µL aluminium pans, the lid was pressed onto the crucible using heavy mechanic force, and the seal tightened the crucible [17]. The test cell was sealed manually using a special tool equipped with the TA's DSC. Approximately 0.3 mg of the ergothioneine standard (Sigma-Aldrich) was used to obtain the experimental data. The dynamic tests of the scanning rate selected for the programmed temperature ramp were 4 ℃/min. In the studies using DSC thermal analysis, high purity nitrogen was the purge gas, and the flow rate was 50 mL/min.

E. Statistical Analysis

All the measurements were determined in triplicate through all steps of extraction and analysis for each component. The experimental data were subjected to an analysis of variance (ANOVA) for a completely random design (CRD) using Statistical Analysis System (SAS Institute, Inc., Cary, USA,) to determine the least significant difference among means at the level of $P < 0.05$.

III. Results and Discussion

A. Stability of Ergothioneine Standard

The ergothioneine standard was obtained by DSC analysis, and the liquefaction temperature was 100 ℃ (Fig. 3). As the temperature increased, about 240 ℃ was gasified. It can be seen that in the presence of medium, ergothioneine can be liquefied in a soluble aqueous solution.

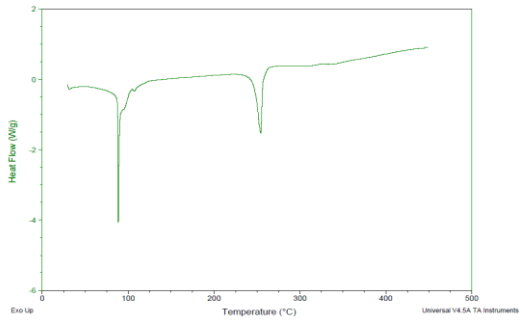

Figure 3. DSC analysis of ergothioneine standard.

B. Effect of Cold Storage

The ergothioneine of the *P. citrinopileatus* decreased with the increase of storage time (Fig. 4). The concentration of ergothioneine in the *P. eryngii* increased slowly with the storage time. The ergothioneine concentration of *F. velutipes* was unchanged with the increase of storage time. However, *P. citrinopileatus* had a high amount of ergothioneine; it had a significant loss with the increase of storage time. Nguyen et al. [24] reported that *F. velutipes* water extracts should be stored at low temperature and not more than 15 days to keep the ergothioneine content.

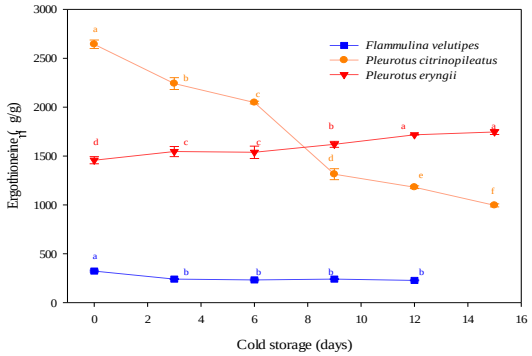

Figure 4. Effect of fresh mushrooms at cold storage on ergothioneine concentration.

C. Effect of Pulsed Light Treatment

The ergothioneine concentration in the pulsed light irradiation treatment of mushroom was similar to control (Fig. 5), which means that the pulsed light irradiation treatment did not affect the ergothioneine content in the mushrooms. Hausman [28] used pulsed light irradiation on *Agaricus bisporus*, *Lentinula edodes* and *P. ostreatus* and found that pulsed light irradiation did not change the ergothioneine content. It has been documented that ergothioneine is an *in vivo* antioxidant and a cellular protector against oxidative damage.

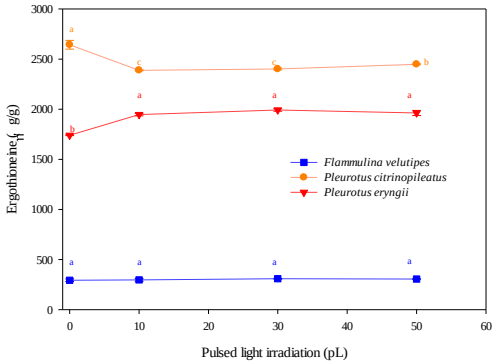

Figure 5. Effect of fresh mushrooms at pulsed light irradiation on ergothioneine concentration.

D. Effect of dry Treatment

TABLE I. Effect of Fresh Mushrooms at Drying Method on Ergothioneine Concentration

µg/g dw	*F. velutipes*	*P. citrinopileatus*	*P. eryngii*
Freeze drying	255.27 ±7.51A	2643.20 ±43.53A	1848.29 ±1.49A
Cold-air drying	220.71 ±8.12B	2380.82 ±95.83B	1787.08 ±2.47B
Hot-air drying	207.53 ±2.33C	2030.56 ±13.93C	1824.60 ±60.49A

One of the best mushroom preservation methods for long-term storage is drying. The results showed that freeze-drying resulted in higher ergothioneine content than cold-air drying and hot-air drying (Table I). Therefore, freeze-drying should be a potential method for obtaining high-quality dried mushrooms.

E. Effect of Boiling, Air-frying and Stir-frying Process

When the mushrooms were treated by temperature process, the boiling process of mushrooms had an adverse effect; at 3~10 min there was a decrease in ergothioneine content of 40.81~52.49% (*F. velutipes*), 71.38-88.44% (*P. citrinopileatus*) and 18.89-24.60% (*P. eryngii*), respectively (Fig. 6). This suggests that the boiling process with water may dissolve the ergothioneine compound, resulting as a decrement. It means that, above 100° C with water, there is destruction of ergothioneine in mushrooms. Nguyen *et al*. [24] reported a decrease in ergothioneine content of *F. velutipes* white stain during cooking. In our study the mushrooms were different mushroom stains and type may have affected the results obtained.

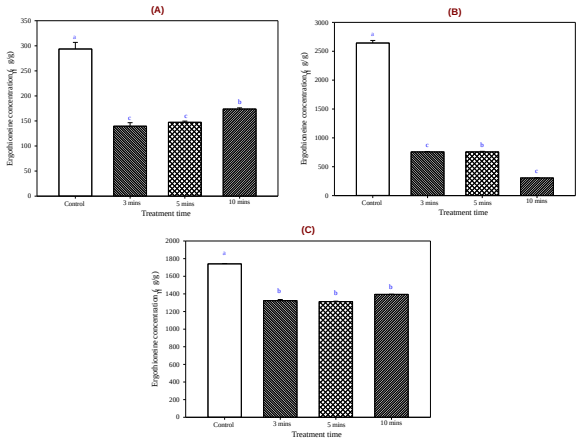

Figure 6. Effect of ergothioneine concentration by boiling on *F. velutipes (A), P. citrinopileatus* (B) and *P. eryngii* (C).

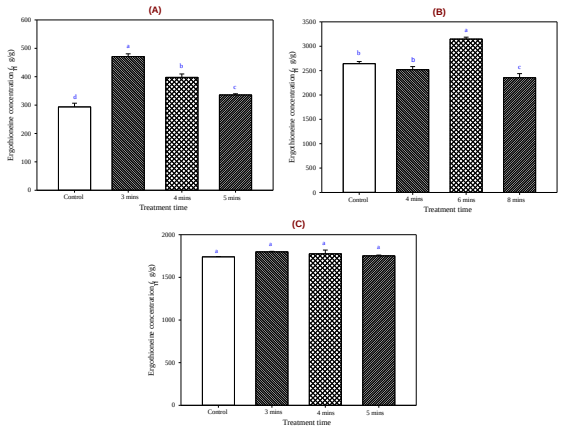

Figure 7. Effect of ergothioneine concentration by gas-frying on F. velutipes (A), P. citrinopileatus (B) and P. eryngii (C).

The ergothioneine concentration in the gas-frying was similar to control of *P. eryngii*. However, the ergothioneine concentration in the gas-frying was higher to control of *F. velutipes*. *P. citrinopileatus* were treated by gas-frying process; we observed an important decrease of ergothioneine (Fig. 7). The ergothioneine concentration in the stir-frying was decreased to control of *P. citrinopileatus*. However, *P. eryngii* and *F. velutipes* treated by stir-frying process was similar to control (Fig. 8). Overall, it was observed that the presence of water during treatments at high temperatures showed a positive effect on the ergothioneine. Among them, the loss rate of ergothioneine content in *P. citrinopileatus* was the highest, probably because it contained a high amount of ergothioneine, but the surface area of the factor entity was large, and it was easy to be lost through processing.

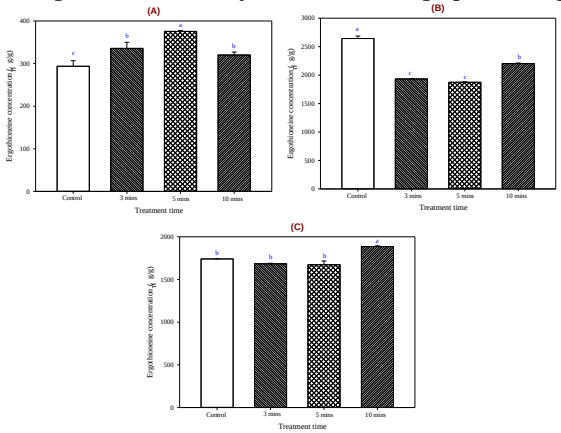

Figure 8. Effect of ergothioneine concentration by stir-frying on *F. velutipes* (A), *P. citrinopileatus* (B) and *P. eryngii* (C).

IV. Conclusions

Based on the results in this study, boiling may cause a significant loss of ergothioneine from mushrooms, mainly due to leaching into cooking water. Therefore, reduced boiling time is recommended for cooking mushrooms, especially in P. citrinopileatus. Regarding the effect of storage conditions, storage time affected the ergothioneine retention of P. citrinopileatus. Freeze-drying of mushroom is suggested to retain ergothioneine content. These results provide alternatives for the conservation of the mushroom and process options for preserving its properties. In addition, these observations may serve as a consumer guideline for estimating dietary intake of ergothioneine.

Acknowledgment

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