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ORIGINAL ARTICLE

Changes in the Levels of Ergosterol and Methionine as Indicators of *Lentinula edodes* Quality According to the Relative Humidity During the Storage Period

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Abstract

Lentinula edodes mushrooms cultivated under different relative humidities were wrapped at 4℃ and the results of storage characteristics were investigated. Changes in water content of fruiting bodies during the storage period showed the highest water content in fruit bodies harvested from the treatment with the highest relative humidity. The luminosity of the fresh fruiting bodies showed no significant change during the storage period, and the redness was higher in the relative humidity 95% treatment than in the other treatments. According to this study, the relative humidity of the pileus was 65%, and the content of Ergosterol was 0.67 ± 15 g / L at relative humidity of 65%, 80% and 95%. In addition, amino acid analysis and Principal Component Analysis (PCA) confirmed that methionine was the main cause of changes in storage time and relative humidity.

Key words : Ergosterol, Letinula edodes, Principal component analysis, Relative humidity, Storage characteristics

1. Introduction

Relative humidity is known to induce the formation of fruiting bodies during the growth of mushrooms, to play an important role in the growth of mushrooms, and to also affect the storage stability after harvest. Mushrooms are comprised of 90% water, and the relative humidity causes a large amount of water to be released. Thus, the released water is a major factor that causes mushrooms to become brown by bacteria, causing decay (Mahajan et al., 2008).

To control the bacterial diseases of cultivated mushrooms, it is important to control the relative humidity, temperature and ventilation in the cultivation house, in particular, to keep the mushroom pileus dry (Nair and Bradley, 1980). For the cultivation of *Lentinula edodes*, the maximum humidity was on average 100%, and the daily minimum humidity average was 74% in July-August(Kim et al., 2015). In the past decade, Jhune et al.(2010), Jang(2014), and Kim et al.(2013) studied the mushroom cultivation process and the effect of the relative humidity on the quality of the mushrooms.

Ergosterol is a major sterol component present in the

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cell membranes of higher fungi (cystic fungi and basidiomycetes) and is mainly distributed throughout the cells of living fungi. Ergosterol is a constituent of the plasma membrane in hyphae and spores (Pasanen et al., 1999). Ergosterol has a molecular weight of 396, which is different from cholesterol, because it has an additional double bond and a methyl group. It is converted into vitamin D in the body, which reduces blood cholesterol levels and prevents diseases such as arteriosclerosis and cerebrovascular disease (Yang et al., 1996; Cushman et al., 1980; Leaf et al., 1987). Typical fungi have an ergosterol content of approximately 0.1 to 0.5% in the tissues, which varies depending on the type of fungi, the period of incubation, the developmental stage, and the culture conditions (Gessner, 2005).

Lentinula edodes has a variety of flavors, which are largely determined by the amino acid composition (Maga, 1981). In particular, there is a large difference in the amino acid composition between mushrooms (Manzi et al., 1999). However, most studies have focused on the growth of mushrooms by the ratio of the nitrogen source to the carbon source (Buswell et al., 1995).

There have been few studies on the changes in individual amino acids over time, the pileus extension rate, and the storage characteristics. This study investigated the changes in the ergosterol content and structural amino acids in *Lentinula edodes* caused by the cultivation humidity and storage period.

2. Material and Methods

2.1. Mushroom materials and culture conditions

The *Lentinula edodes* (LE) cultivar Sanjo 701 was cultivated in accordance with standard culture methods. The medium to generate a mushroom fruit body was prepared in a sterile transplant plastic bag blended with oak sawdust and rice bran in a ratio of 8: 2 with 58% moisture content. After sterilization at 121℃ for 90 min, a spawn was inoculated onto the sawdust bag medium. For browning, the spawn was incubated at 21℃ in the dark for 50 days and then exposed to light for 30 days.

The relative humidity (RH) of the cultivation was 65% (RH65), 80% (RH80) and 95% (RH95). The growth temperature was 18 ± 1℃, and the carbon dioxide concentration was 800 ppm.

2.2. Storage conditions and sampling

The storage conditions was as follows: 200 g of LE were packed in a plastic container, wrapped and stored at 4 ± 1℃, and the amino acid changes and ergosterol contents were measured every 10 days with three repetitions. The treatment sample was used by freeze dry and stored at -20℃ until further analysis.

2.3. Ergosterol analysis

For the ergosterol analysis, 1 g of the sample was add to 50 mL of methanol. The mixture was sonicated for 10 min and centrifuged at 5,000 rpm for 10 min. After centrifugation, the supernatant was removed, and 20 mL of ethanol and 10 mL of 2M KOH were added, followed by saponification by shaking in a water bath at 80℃ for 1 h. Subsequently, 50 mL of distilled water was added to the saponified solution, which was then extracted twice with 50 mL of n-hexane using a separatory funnel. The extract was concentrated using a rotary evaporator at 40℃ for 10 min, dissolved in 1 mL of methanol and stored at -20℃. High performance liquid chromatography (HPLC) analysis was performed using a Waters Alliance system equipped with a C18 100A 5 μm column (4.6 mm × 150 mm). The mobile phase solvent was 99.9% methanol uniformly used at a flow rate of 1 mL/min at room temperature which was analyzed for ergosterol at 280 nm. The calibration curve for the quantitative analysis was an ergosterol standard (ACROS ORGANICS, 11781).

2.4. Content analysis of the constituent amino acids

First, 30 mL of distilled water was added to 1 g of the sample, heated at 100℃. for 10 min, adjusted to 50 mL with distilled water, and centrifuged at 3,000 rpm for 10

Fig. 1. Packing Form of Fruiting Body. Storage condition : 4℃ wrap packaging, Storage period: 30days A: RH65% under cultivation, B: RH80% under cultivation, C: RH95% under cultivation.

min. Following this, 1 mL of the supernatant and 1 mL of 5% trichloroacetic acid (TCA) were mixed together, and the mixture was centrifuged at 12,000 rpm for 15 min. n-hexane was filtered through a 0.2 μm membrane filter (Sartorius AG, Göttingen, Germany). The column was a HITACHI HPLC Packed Column # 2622PF Column (4.6 × 60) Ion Exchange column. The UV detectors used were VIS1 (570 nm) and VIS2 (440 nm). The injection volume was 20 μL, and the flow rate was 0.3 mL/min. The sample was analyzed with an automatic amino acid analyzer (Hitachi L-8900, Hitachi, Japan).

2.5. Statistical analysis

Principal Component Analysis (PCA) was performed by analyzing the mean values of the structural amino acid samples for the main analysis of the amino acid variation between the storage period and humidity. All statistical analyses were performed using SPSS for Windows 12.0 (SPSS Inc., Chicago, USA).

3. Results

3.1. Morphological Change of LE According to Relative Humidity

The morphological changes in LE due to the difference are relative humidity is shown in Fig. 1. At RH65, the brown color of the mushroom shade did not progress. At RH80, it was confirmed that the mushroom shade was brownish and at. RH95, the mushroom had a brown shade. The results show that with higher relative humidity while growing mushrooms, the color of the pileus was darker.

3.2. Storage characteristics of LE after post harvesting according to relative humidity

The storage characteristics of LE were investigated when the relative humidity was adjusted to 65%, 80% and 90% for 30 days under the same growth humidity conditions. Compared with RH80 and RH90, the fresh growth rate at RH65 tended to increase with storage time (Fig. 2.).

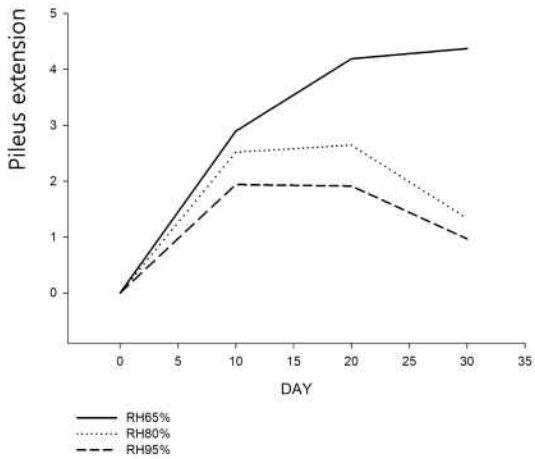

Fig. 2. Changes in pileus extension according to relative humidity during the storage period.

Table 1. Changes in ergosterol content based on the storage period and humidity

	RH65	RH80	RH95
Storage Period (day)	30	30	30
Increase ES (g/L)	0.26±0.015	0.18±0.021	0.33±0.018

ES: ergosterol contents

In the case of RH 65, the difference between RH80 and RH90 was approximately 0.5 cm after 10 days of storage, and more than 2 cm between 20 and 35 days after storage. In contrast, RH80 and RH90 decreased the freshness. Thus, we were able to confirm the difference in growth rate. These results suggest that the pileus extension due to the osmotic pressure in the mushroom tissue due to the relative humidity had not only increased but that there was also a difference in the pileus extension due to changes in the intracellular substances.

3.3. Changes in ergosterol content based on the storage period and humidity

The contents of ergosterol, a precursor of the cell wall constituents, were compared to confirm that the pileus extension was changed by the intracellular changes during storage. At RH65 and RH80, the ergosterol content had increased to 0.26 and 0.18 g/L, respectively, during the 30 day storage period. In addition, it was confirmed that at RH95, it had increased to 0.33 g/L. The rate of increase was the highest at RH95, but it was 0.67 ± 15 g/L at RH60. The result, confirmed that the RH65 condition had a lower elongation and a higher ES content during storage.

3.4. Changes in structural amino acids over the storage period

Analysis of 17 structural amino acids revealed that the percentage of amino acids increased in all treatments. These results could be attributed to the collapse of the cell material during the storage period. In particular, glutamine increased to 1.14154 g / 100 g after 30 days of storage at RH60, to 1.25679 g / 100 g at RH80 and to 2.50854 g / 100 g at RH95. Therefore, it was confirmed that glutamine was produced the most at RH90. In the case of proline used as food coloring, it increased to 0.22346 g / 100 g at RH65, to 0.16865 g / 100 g at RH80, and to 0.23604 g / 100 g at RH95. Thus, it could be deduced that these two amino acids were the main amino acids that caused morphological changes in LE.

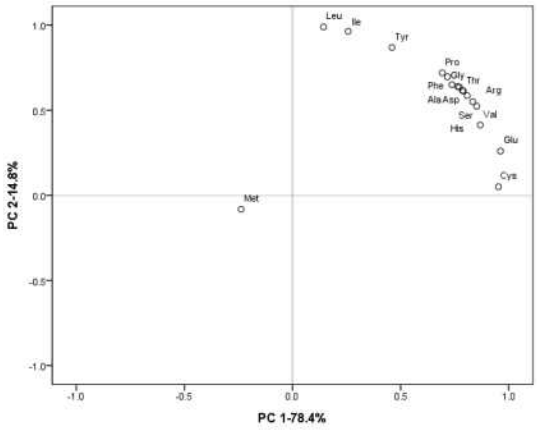

Fig. 3. Principal component analysis of the structural amino acids of LE over storage time.

From the above results, we analyzed the change in the LE amino acids by the growth time and growth humidity using Principal Component Analysis (PCA). The analysis revealed that the amino acids were positively correlated with the PC1 axis for all amino acids except for Met. As the storage time increased, the amino acids increased due to the collapse of proteins. As the humidity increased, protein collapse occurred faster. It

was also confirmed that the increase in amino acids according to the PC2 axis was positively correlated with all amino acids except for Met. Leu, Ile and Tyr were more affected by the storage time and humidity than the other amino acids, and Glu and Cys were less influenced by the humidity than the other amino acids.

At RH65 and RH80, Met decreased over time, whereas at RH95, it increased over time. This suggests that the relationship between cultivation humidity and the storage period is less in the case of Met.

4. Discussion and Conclusion

In this study, the morphology, ergosterol and amino acid changes of *Lentinula edodes* were investigated during storage. *Lentinula edodes* exhibited significant morphological differences depending on the growth and storage environment. In particular, relative humidity had a significant influence on the growth of the mushrooms, which was the main determinant of mushroom quality (Cağlarırmak, 2007). The above results show that the fresh growth rate was higher at a relative humidity of 80% and lower at a relative humidity of 95%. In particular, ergosterol is a precursor of vitamin D2 during the post-harvest period, and vitamin D2 was shown to increased in UV-B -irradiated *Agaricus bisporus* (Roberts et al., 2008). In this study, it was also found that the increase in ergosterol was the lowest at 80% relative humidity during the storage period. As the storage period increased, the respiration ratio decreased, and the 80% relative humidity showed the lowest change in ergosterol level. In addition, structural amino acids affect the flavor components of mushroom quality factors (Yu and Ho, 1995). Based on the above results, it was confirmed that all the amino acids except for methionine showed almost no change. The results of this study suggest that the quality of *Lentinula edodes* is directly influenced by the relative humidity and storage periods and that ergosterol and methionine may act as indicators of the quality of *Lentinula edodes*. Since both indicators were not affected by the storage period and humidity, it was considered that the point at which the above indicators decreased could be set as an indicator of quality deterioration for *Lentinula edodes*. In the near future, experiments will be conducted to determine the optimal production conditions and quality conditions based on other environmental conditions such as temperature and CO_2 concentration.

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