Changes of nutrient contents in the log of *Quercus acutissima* by cutting period for *Lentinula edodes* log cultivation

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**ABSTRACT**

This study is to find out an appropriate log cutting period for saprotrophic *Lentinula edodes* log cultivation. Seasonal changes in nutrients in sapwood and cell activities in the inner bark from logs of *Quercus acutissima* cut during September to February were analyzed. Total carbon content in the sapwood was higher in September (76.5%) than in other months (67.8–69.2%). Total nitrogen content was higher in November and December (0.21–0.22%) than in other months (0.14–0.15%). Inorganic nutrients such as phosphate, potassium and magnesium, and heavy metals like cadmium were higher in November and December than in other months. Over 90% of axially vertical and radial parenchymatous cells contained starch granules in November and December while only 20–30% of these cells did in February. The cell activities in the inner bark of logs cut in October to November, measured with formazan absorbance values during mid March, were 0.013–0.018, which were lower than those cut in December (0.027–0.045). Therefore, our results support that it is better to cut logs for *L. edodes* cultivation in November when nutrients accumulate in the wood but wood cells are less active.

**Introduction**

*Lentinula edodes* is a popular mushroom which has been eaten for a long time around North-East Asia. It has unique taste and flavor (Mizuno 1995) and is useful because it can protect against adult diseases (Chang et al. 1993), reduce the risk of cancer, and has other various functions (Ohashi 1999). Since it was known to people in Europe and America, its industry has expanded throughout the world (Campbell and Racjan 1999). In Korea, *L. edodes* is a major short-term income forest product and about 4000 farmers produced 17,967 tonnes of fresh mushrooms and 1271 tonnes of dried mushrooms in 2014 (Korea Forest Service 2015).

Oak trees are usually used as logs for growing *L. edodes*. Among them, *Quercus acutissima* is the most favorite of *L. edodes* farmers. Because of its higher portion of sapwood, the tree log can produce good-quality *L. edodes* for a longer period than other oak species. However, due to its thin and smooth bark it is easy to dry, vulnerable to diseases and it is difficult for hyphae to colonize it. So it is more appropriate for expert growers than beginners (Ko et al. 2015).

*L. edodes* is saprophytic, so it can grow only when the wooden structure is dead. Therefore, the wood should be dead before its inoculation. It is also important to cut a tree at the right time when its wood has a high nutrient content for *L. edodes* growth (Park et al. 2015).

For a long time, the cutting period has been decided on the basis of the color change of the leaves or the cessation of sap flow. The time when 70% of the leaves turn to yellow or red is the most appropriate for cutting. Trees grow while they absorb moisture from the roots and their cells divide into new cells continuing active photosynthesis. At that time, sap flows very well and stems are full of moisture. However, in the fall, when the temperature drops, the carbohydrates in the leaves move to the roots and the moisture in the stems is reduced (Lee 2014). Thus, this study examined changes of nutrients, such as starch in sap wood and inorganic ones from aqueous extracts of *Q. acutissima*, by month in order to make a clear decision for the best cutting period, and also examined the activity of inner bark cells in the logs cut at different periods to test whether they are fully dead before inoculation.

**Materials and methods**

Changes of nutrients and aqueous extract in *Quercus acutissima* wood by cutting period

To understand the seasonal changes of nutrients in *Q. acutissima* logs for cultivating *L. edodes*, the amount of nutrients such as starch and inorganic minerals in aqueous extract from the sapwood was analyzed. About 5 g of sapwood in 5 cm depth was collected from four directions at 50 cm, 100 cm and 150 cm of *Q. acutissima* standing trees located at Chungbuk National University Experimental Forest in Cheongju on September 15, 2014 to February, 2015.

For the analysis of mineral nutrients, the collected samples were ground into powder and mixed well. The analysis items such as total carbon (T-C), total nitrogen (T-N), available phosphate (P), potassium (K), magnesium (Mg), manganese (Mn), calcium (Ca), iron (Fe), cadmium (Cd), and copper (Cu) were analyzed at the Environment and Resources Center of Chungbuk National University.

The starch accumulation in *Q. acutissima* wood was observed with Melzer’s solution under a microscope by dying...
cross-sections, longitudinal sections and tangential sections. The degree of accumulation was compared among samples collected monthly. Aqueous hot water and cold water extracts were analyzed. The samples that were ground and 2 g of the sample mixed with distilled water in a ratio of 1 : 50 (W : V) in a Erlenmeyer flask. For hot water extraction, the sample was warmed to 100°C in a water bath (WB-11, DAIHAN, Korea) for 3 hours, and cold water extraction was kept room temperature for 48 hours. After that, each extracts were moved in a weighing bottle (1G3) and then filtered out using a vacuum pump. The extracts were measured on an electronic scale.

Cell activity in the inner bark of Quercus acutissima logs

To understand cell activities in tissues of Q. acutissima logs, three trees were cut on each October 20, November 20, and December 20, 2015 in Seongbul-ri, Jangheung-eup, Jangheung-gun, Jeollanam-do Province and left with branches and leaves. On March 20, 2016, samples were collected from the inner bark and heartwood at the bottom end, middle part, and top end of Q. acutissima. The inner bark and the heartwood (0.5 g each) of Q. acutissima were put in a small test tube. Potassium di-phosphate (K2HPO4) (100 ml) was mixed with 2 ml of potassium di-hydrogen phosphate (KH2PO4) to make phosphate buffer (pH 8.5). Then, 3 ml of the phosphate buffer was put into the test tube. After shaking the test tube to mix the reaction solution well, it was left at 30°C for 24 hours. Then the samples were taken out of the reaction solution and their moisture was removed with a paper towel. The samples were mixed with 95% ethanol in a ratio of 1 : 100 (W : V). The samples were warmed in a water bath (WB-11, DAIHAN, Korea) at 60°C for 2 hours and formazan was extracted from them. Formazan was stirred and filtered, and the absorbance was measured with 5 µl of filtrate on 485-nm wavelength with a microspectrophotometer (NanoDrop 2000, Thermo Scientific, USA); 95% ethanol was used for comparison.

Results and discussion

Changes of nutrients in Quercus acutissima wood by cutting period

Total carbon content of the sapwood was higher in September (76.5%) than in December (67.8–69.2%) (Figure 1). Woody plants, conifers as well as deciduous trees, show a big difference in carbon content by season. Branches have the most concentrated carbohydrate when leaves are falling; in the winter, it is used as energy to increase cold resistance against low temperature and regrow next growing season (Pallard 2008). In spring, trees use carbohydrates to sprout new leaves and branches, so the carbon content decreases (Lee 2014). The major nutrients for wood-rotting fungi are high molecular cell wall substances of a log such as cellulose, hemicellulose, lignin, various carbohydrates and reserve substances in cells. The carbohydrates are glucose, mannnose, fructose, sucrose, maltose, and starch (Kim et al. 2004).

Total nitrogen content of the sapwood was higher in November and December (0.21–0.22%) than in next January and February (0.14–0.15%) (Figure 1). Total nitrogen content of the trees that grow freely more than once throughout the year decreases whenever new branches grow. Nitrogen content in the tree is higher in leaves than wood in summer, but the nitrogen moves into the branches in fall, and, it becomes the highest in winter (Pallard 2008). N content is considerably higher in the bark, which has phloem, than in the wood, and changes of nitrogen by season are bigger in phloem than in the woody part (Kozlovski and Pallardy, 1997). Proteins occur in barks and ray parenchyma cells of xylem in wood, and they are localized in protein bodies of storage vacuoles dispersed through the cytoplasm especially in winter, but not in summer (Wetzel et al. 1989).

C/N ratios in the sapwood were 328 in November and 322 in December, which were lower than 440–497 in other months (January, February, September, and October) (Figure 1). Generally, the best content ratio of carbon and nitrogen sources for mushrooms is considered 20 in the vegetative growth period and 30–40 in the reproductive growth period (Tasuku 1988). L. edodes and Agaricus subfunereus usually grow on the logs whose nitrogen content is 0.03–1.0%, so they prefer the logs with C/N ratio of 350–500 (Yu et al. 2010). In the sapwood of Q. acutissima, contents of phosphate, potassium, magnesium, manganese, and calcium were the largest in quantity in December. Heavy metals like cadmium and copper were higher in November and December than the other months (Figure 1). The amounts of inorganic substances like nitrogen, phosphate, etc., in one L. edodes fruit body of 6.1 g dry weight was the same as those in the sapwood of 86–152 g dry weight, and the necessary sapwood area for one L. edodes mushroom was about 56 ± 30.9 cm² (Matsumoto and Komatsu 1982). The log used for L. edodes cultivation for 5 years showed that the rate of utilization of microelements was higher in the bark, which has phloem, than in the woody part (Kozlovski and Pallardy, 1997). Spawns were inoculated into the sawdust media made of Qu. mongolica, Coprinus sp., Fraxinus mandshurica, Prunus sp., Castanea sp., Alnus hirsuta and Betula platyphylla. In three months the rate of utilization of microelements was higher in Qu. mongolica and Coprinus species than the others (Yoon and Cha 2003). Inorganic substances are also necessary nutrients for the growth and development of mushrooms. Magnesium and potassium are needed for osmotic regulation and buffering while calcium, copper, iron, manganese and zinc are needed for enzyme activity (Park et al. 2009).

In the process of L. edodes log cultivation, cutting period has a great influence on hyphal growth, resistance to contaminating fungi, and yield during the cultivation period (Park et al. 2009). Therefore, farmers are interested in nutrition and percentage of moisture content of Q. acutissima. This kind of tree grows while moisture is absorbed from the roots during spring and summer, photosynthesis is conducted in the leaves to make nutrients, and their cells divide into new cells. It is in the middle of November that the carbohydrates produced by photosynthesis accumulate the most in stems (Lee 2014). November is considered the best time for cutting because carbohydrates and inorganic substance including nitrogen in wood are higher than any other months.

During September–October in the sapwood of Q. acutissima, about 90% of axially vertical parenchymatous cells contained starch granules and about 40% of the radial parenchymatous cells contained starch (Figures 2 and 3). From November to December, over 90% of axially vertical and radial parenchymatous cells contained starch granules (Figures 4 and 5), whereas in February only 20–30% of them contained the starch granules (Figures 6 and 7).
Figure 1. Monthly changes of nutrients in *Quercus acutissima* logs. The bars are one standard deviation. Different letters show a significant difference among months at *p* < 0.05 and **p** < 0.01 by Duncan's multiple range test.
Figure 2. Starch (ST) granules in ray cells of sapwood of *Quercus acutissima* in September, 2014. Starch was not fully filled on radial parenchyma cell (RP) but almost fully filled on longitudinal parenchyma cells (LP), (radial section, left (×10), right (×40)).

Figure 3. Starch (ST) granules in ray cells of sapwood of *Quercus acutissima* in October, 2014. The amount of starch was more filled on radial parenchyma cell (RP) than that of starch in last September and fully filled on longitudinal parenchyma cells (LP) (radial section, left (×4), right (×40)).

Figure 4. Starch (ST) granules in ray cells of sapwood of *Quercus acutissima* in November, 2014. Starch was almost fully filled on radial parenchyma cell (RP) and longitudinal parenchyma cells (LP) (radial section, left (×10), right (×40)).
Figure 5. Starch (ST) granules in ray cells of wood of *Quercus acutissima* in December, 2014. Starch was fully filled on radial parenchyma cell (RP) and longitudinal parenchyma cells (LP) (tangential section, left (×10), right (×40)).

Figure 6. Starch (ST) granules in ray cells of sapwood of *Quercus acutissima* in January, 2015. Starch (ST) in sapwood of *Quercus acutissima*. Starch was less filled on radial parenchyma cell (RP) and longitudinal parenchyma cells (LP) in January than those of starch in previous months (radial section, left (×4), right (×20)).

Figure 7. Starch (ST) granules in ray cells of *Quercus acutissima* in February, 2015. Starch (ST) on sapwood of *Quercus acutissima*. Starch was much less filled on radial parenchyma cells (RP) and longitudinal parenchyma cells than the previous months (LP) (radial section, left (×10), right (×40)).
Axially vertical parenchyma cells take charge of storage and movement of nutrients. They consist of 2–8 closely bound parenchymatous strands in axial direction (Butterfield et al. 2000). While the size of a starch granules in a parenchyma cell of potato is about 5–100 μm (Dufresne et al. 2000), oval-shaped starch granules in Q. acutissima sapwood are about 3–6 μm.

Starch is the most basic reserve substance. It is made by photosynthesis using chlorophyll and it is reserved in roots, stems, and seeds. Many microorganisms produce amylase to decompose starch (Kim et al. 2004). In the case of Robinia pseudoacacia, its starch content decreases but reducing sugar content increases in winter, because starch changes into sugar and reducing sugar to increase cold resistance for tree
branches (Lee 2014). Starch is necessary for the synthesis of cell walls, nucleic acid, and reserve substances of mushrooms (Yu et al. 2010). Starch is not sufficiently stored when the trees get stressed. The cold season level of starch stored in drought-stressed sugar maple in early fall in the wood tissue was about one-third to one-fifth that in nondrought-stressed ones (Wong et al. 2009).

The amount of aqueous extracts in stem varied greatly depending on extraction methods but varied less by season (Figure 8). The extracts were significantly higher from cold extraction than hot extraction. However, the amounts of the extraction did not differ among months, although cold extracts were slightly higher during November and December than other months. The amount of extraction varied greatly depending on individuals trees.

Hot water extracts were $0.637 \pm 0.009$ g per 2 g of wood sample in November and $0.632 \pm 0.004$ g per 2 g of wood sample in February. Cold water extracts were $0.670 \pm 0.007$ g per 2 g of sample in November and $0.642 \pm 0.02$ g per 2 g of sample in September. Those extracts consist of low molecular weight and water-soluble carbohydrates, hydrolyzable tannin and glycoside. Low molecular weight and water-soluble carbohydrates, which can be used directly by wood-degrading fungi like *L. edodes*, varied greatly depending on climatic conditions (Cho 1994).

**Activity of cells in inner bark of Quercus acutissima logs**

Tetrazolium reacts on oxidoreductase enzymes in live cells of inner bark to form insoluble purple formazan. The intensity of purple color is proportional to the concentration and measured with absorbance value. The absorbance values of formazan in inner bark of the log cut in October was 0.01 at the bottom end and 0.015 at both the middle part and the top end (Figure 9). However, the absorbance value did not differ between logs cut in October and November. The absorbance value of the log cut in December, was 0.027 at the bottom end, 0.045 in the middle part and 0.044 at the top end. They were higher than those of October and November. The absorbance value of a live tree was 0.042 on average and zero in the heartwood. The absorbance value of a live tree was similar to that of the inner bark cut in December. The absorbance value of the bottom end was lower than that of the middle and the top end, because when a tree is cut and exposed to air, its moisture evaporated and cells died quickly. So, compared to the middle part which was intact for longer, the cell’s activity of the bottom end decreased quickly. Under a dissecting microscope, tetrazolium in the inner bark from a live tree or from the tree cut in December became red. However, the heartwood or the inner barks from the trees cut in October and November did not react with tetrazolium and remained brown (Figure 10). The tetrazolium chloride (TTC) colorimetric method is a way of distinguishing seed activity by examining changes in colors of embryos in a seed or the surface of a seed after reacting carbon dioxide (CO$_2$) emissions from breathing with TTC (Kim 1975). In most cases, healthy seeds can breathe actively so that their embryos or surfaces turn red (Lee et al. 2015).

In conclusion, because *L. edodes* is saprophytic, it grows much better when logs for cultivation have sufficient
nutrients such as carbohydrate and nitrogen, and cells are dead rather than when they are alive (Yu 2015). Therefore, *Q. acutissima* trees for cultivating *L. edode* should be cut around November or four months before inoculation.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

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