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An Investigation into the Cause of Change in the Antioxidant Activity of Half-dried Mushrooms

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半乾燥キノコにおける抗酸化能変化の原因についての研究

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Introduction

Lifestyle-related diseases have increased in Japan[1].

"Appropriate eating habits" include increasing the amount of vegetables in the daily diet and should be a goal for many people. However, many people fail to achieve this goal. The purpose of this study was to develop methods for increasing the amount of vegetables consumed by people.

The water content of vegetables is approximately 80–90%[2]. When the water content of the vegetables is lower, the volume could be reduced, more vegetables can be consumed.

People in Japan consume numerous dried foods, some of which need to be subjected to some pre-cooking processes such as water absorption and reduction of the bitter taste. These food items acquire different properties when they are dried. In this study, we examined half-dried food items. Our primary goal was to examine whether these food items can be used for cooking after drying or being subjected to any type of processing.

We focused on the health functionality of mushrooms. We hypothesized that the use of the half-drying method would aid in retaining the antioxidant properties of mushrooms. The effect of preparation conditions on the quality and antioxidant activity of half-dried mushrooms was examined previously[3]. Of the many cooking methods, "blanching" is predominantly used in Japan[5]. Therefore, in the present study, we determined the antioxidant activity of blanched fresh and half-dried mushrooms. In our previous study, we compared the antioxidant activity of blanched fresh mushrooms and half-dried mushrooms, and found that the antioxidant activity of half-dried mushrooms was higher.

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However, the mechanisms underlying high antioxidant activity in blanched half-dry mushrooms remain unknown. Antioxidant activity of blanched fresh vegetables and mushrooms decreased the most when the antioxidant components were extracted into the boiled soup[6, 7, 8]. It is assumed that the half-drying method suppresses the elution of antioxidant components of mushrooms during the blanched process; thus, it can be proposed as a cooking method for enhancing the health functionality of mushrooms.

In this study, we examined the effect of preparation conditions on the antioxidant activity of half-dried mushrooms and subsequently blanched the mushrooms in boiling water to determine the components that had been extracted into the boiled soup.

Materials and methods

Materials

Mushroom samples were purchased from a supermarket from March through June of 2014. The mushrooms used in the present study included Shiitake mushroom grown in Hokkaido, Shimeji mushroom grown in Nagano, and Maitake mushroom grown in Niigata.

Preparation of half-dried mushrooms

The ferrules of mushroom samples (Shiitake, Shimeji, and Maitake) were removed and 30.0 ± 0.1 g of each mushroom sample was used. In order to reduce the raw weight of the samples by 30% and 50%, two drying methods were used: sun drying (the time required to reduce the sample weight by 30%: Shiitake 4.6 h, Shimeji 2.9 h, and Maitake 2.7 h; and by 50%: Shiitake 9.1 h, Shimeji 4.8 h, and Maitake 4.3 h) and machine

drying (the time required to reduce the sample weight by 30%: Shiitake 1.8 h, Shimeji 0.5 h, and Maitake 0.5 h; and by 50%: Shiitake 3.5 h, Shimeji 1.2 h, and Maitake 1.2 h), respectively. Half-dried mushroom samples were used for all measurements. To prepare half-dried mushrooms, the samples were placed in boiling water (200 mL) until the center of the mushroom sample reached a temperature of 98℃. After straining the mushrooms, the boiled soup and the mushrooms were subjected to the appropriate measurements. Non-dried mushrooms were included in the measurements as controls.

Measurement of lightness, hue, and chroma

A colorimeter (Nippon Denshoku Industries, Tokyo, Japan) was used to measure various boiled soup samples. In the L* a* b* color system, lightness is expressed in L* (0–100, dark to bright), while chromaticity, which indicates the hue, and chroma were expressed as a* (+:a* shows a red direction, -:a* shows a green direction) and b* (+:b* shows a yellow direction, -:b* shows a blue direction) values, respectively. The ΔE* value (color difference) was calculated based on these numerical values ($(\Delta a^{*2} + \Delta b^{*2} + \Delta L^{*2})^{1/2}$). The color difference (ΔE*) represents the "distance" between the two sample colors.

Chemiluminescence experimental procedure

This method has been described previously[8]. Briefly, 40 mM 2,2′-azo*bis* (2-amidinopropane dihydrochloride) (AAPH) (Wako Pure Chemical Industries, Ltd., Osaka, Japan) was dissolved in phosphate buffer. Boiled soup samples prepared from each mushroom were diluted to the desired concentrations using the same buffer. AAPH solution was heated at 37°C for 2 min to generate peroxyl radicals. Next, AAPH solution (0.2 mL) was mixed with 0.2 mL phosphate buffer (control) or mixed with 0.2 mL of each diluted sample and then heated at 37°C for 2 min. Immediately after heating, 0.2 mL of luminol solution was added to each mixture for chemiluminescence measurements. Chemiluminescence intensity was measured using the photon counter Lumitester C-100 (Kikkoman Co., Tokyo, Japan). One relative light unit (RLU) represents 43.48 photons/sec.

Calculation of IC_{50} value for peroxyl radical scavenging activity

As an indicator of antioxidative activity, the inhibition of chemiluminescence intensity was measured by determining the change in the RLU value. A lower RLU value indicates greater inhibition of chemiluminescence intensity (peroxyl radical generation). The IC_{50} value was defined as the concentration of boiled soup samples (prepared from each mushroom) that reduced the RLU value of phosphate buffer (control) to half of the maximum value. First, the antioxidative activity value was calculated using the following formula: (log Io/I) × 100; Io = RLU value or peak height ratio of the control, where I = RLU value or peak height ratio of each concentration of the sample solution. When the value was 30.103, the "I" value corresponded to half-inhibition. Next, based on the relationship between the antioxidative activity value and the sample concentration, IC_{50} values were calculated.

Determination of total polyphenol using Folin-Denis method[10]

Total polyphenol content was measured using the Folin-Denis method. Briefly, 5.0 mL of boiled soup sample for each mushroom and Folin-Denis reagent (Kishida Chemical Co., Ltd., Osaka, Japan) were mixed in a centrifuge tube. After 3 min, 5.0 mL of 10 wt% Na_2CO_3 (Kanto Chemical Co., Inc., Tokyo, Japan) was added. The contents in the tubes were mixed and then incubated for 60 min at room temperature. After centrifugation at 1450 ×*g* for 5 min, the supernatant was collected as the sample solution for the Folin-Denis method. Each sample solution was measured at an absorbance of 700 nm.

To prepare the standard curve, 2.0 mg/dL solution of tannic acid (Nacalai Tesque Co., Ltd., Kyoto, Japan) was serially diluted to prepare tannic acid standard solutions of 0.5, 1.0, 1.5, and 2.0 mg/dL. These samples were then analyzed using the Folin-Denis method to generate the standard curve. The correlation coefficient of the standard curve was $r^2 = 0.9912$.

Measuring changes in shape

We measured the change in size before drying, before boiling, and after boiling for a Shiitake sample. The diameter and the height of the mushroom were measured. The diameter of the cap was the average of the major axis diameter and the minor axis diameter.

Statistics

The data are represented as the mean ± S.D. of three samples of mushroom and boiled soup. Statistical significance was evaluated using R (Ver.3.2.0). Differences in the mean were determined based on multiple comparison (Tukey way). *p* values < 0.05 were considered significant.

Results and Discussion

Measurement of lightness, hue, and chroma

During boiling in the sample preparation step, the color of the boiled soup prepared from dried mushrooms differed between samples. The lightness, hue, and chroma of boiled soup samples were measured using a colorimeter. Figure 1 depicts the comparison (ΔE* value) of boiled soup prepared using half-dried samples with that using standard samples (the soup boiled with raw mushroom). The measurement of ΔE* revealed that 50% of the weight of dried Shiitake and Maitake samples was quite different from that of the standard sample.

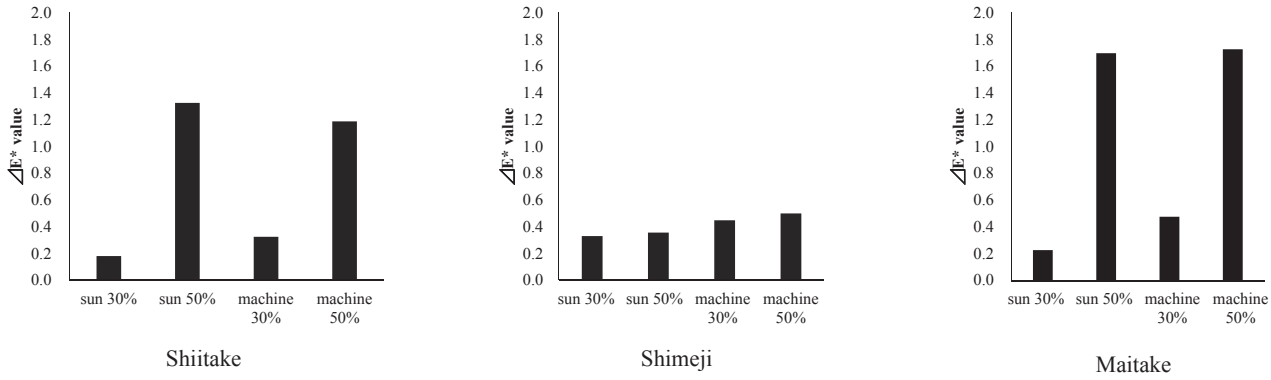

Fig. 1 Comparing the color change of boiled mushrooms subjected to different drying processes
Values shown in the figure represent the difference in color of each sample with that of the non-dry (0%) sample.
(n = 6)

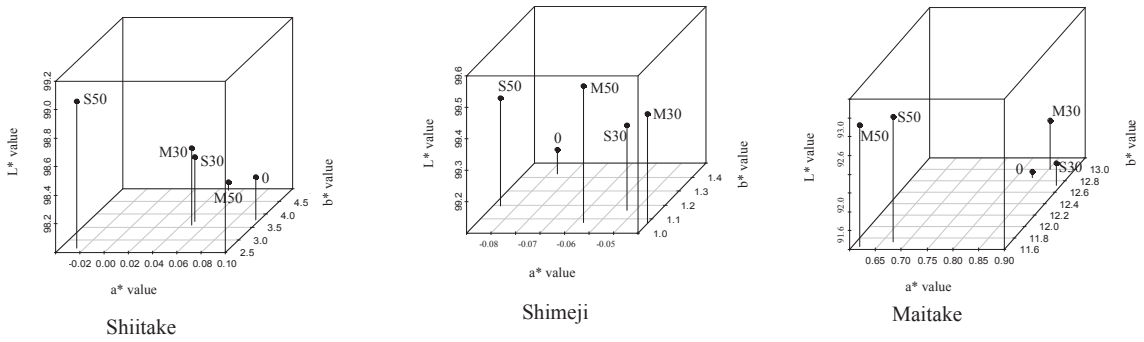

Fig. 2 Comparison of change in the lightness, the hue, and the chroma of boiled mushrooms subjected to different drying process
0…0%, S30…sun 30%, S50…sun 50%,
M30…machine 30%, M50…machine 50%
(n = 6)

Figure 2 shows a three-dimensional view, based on the Munsell table, of the method used to determine the details regarding color changes. The Shiitake and the Maitake mushrooms showed differences in color during the drying process; in these samples, lightness increased and chroma decreased. The boiled soup containing half-dried samples became clearer. This may have been because the surface texture of the half-dried mushroom was contracted, while the water-soluble components extracted from the mushroom were suppressed[11].

Suppression of dye component extraction in the mushroom soup was not expected to suppress functional component extraction from the mushroom.

Measurement of antioxidant activity by the chemiluminescence method

Peroxyl radical scavenging activity was measured as antioxidant activity of the boiled soup prepared from each mushroom sample. As previously reported[3], for the boiled samples of Shiitake and Maitake, the weights of half-dried mushroom samples were reduced by 50%, while relatively high antioxidant activity was maintained.

When dissolution of the water-soluble antioxidant component of the mushrooms in the boiled soup was suppressed, the antioxidant content of the boiled soup was expected to be low.

The antioxidant activities of the boiled soup prepared from each mushroom are shown in Figure 3. However, no such changes were observed when using boiled soup of Shiitake. The antioxidant activity of Maitake decreased when the weight was 50% of the original sample. Extraction of antioxidant components into the boiled soup may have limited.

Measurement of the total polyphenol content using the Folin-Denis method

We measured the content of polyphenol, a water-soluble ingredient with peroxyl radical scavenging activity. Polyphenol levels in the boiled soup of each mushroom are shown in Figure 4.

There was no correlation between the polyphenol content and the antioxidant activity of the mushroom samples. Thus, other ingredients in mushrooms may be related to antioxidant activity.

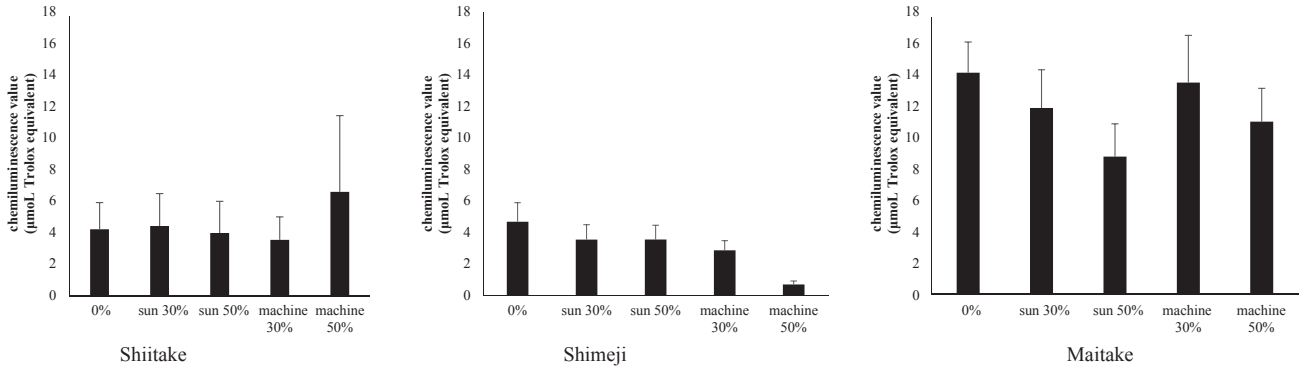

Fig. 3 Peroxyl radical scavenging activity of boiled soup prepared from each mushroom samples during the different drying processes
Each value is mean ± SD (n = 3)

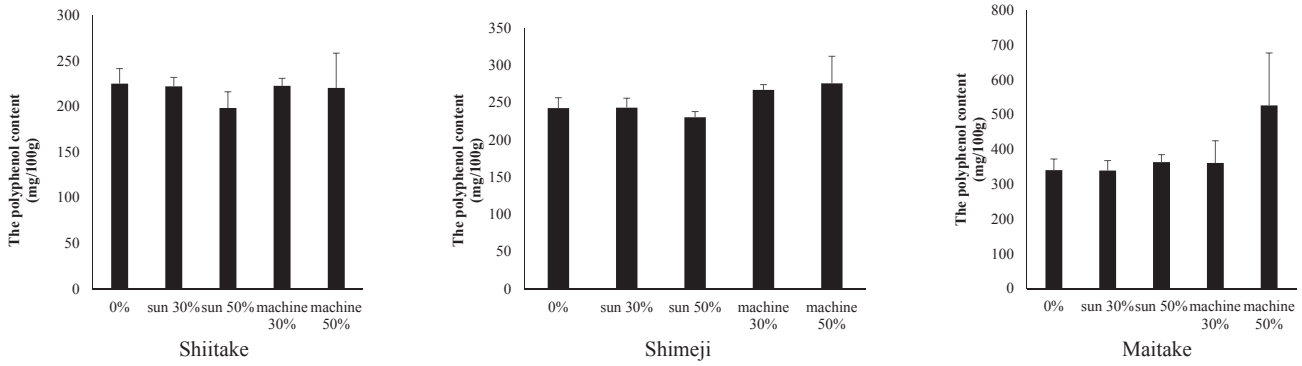

Fig. 4 Polyphenol amount in the boiled soup prepared from of each mushroom sample during different drying processes
Each value is mean ± SD (n = 3)

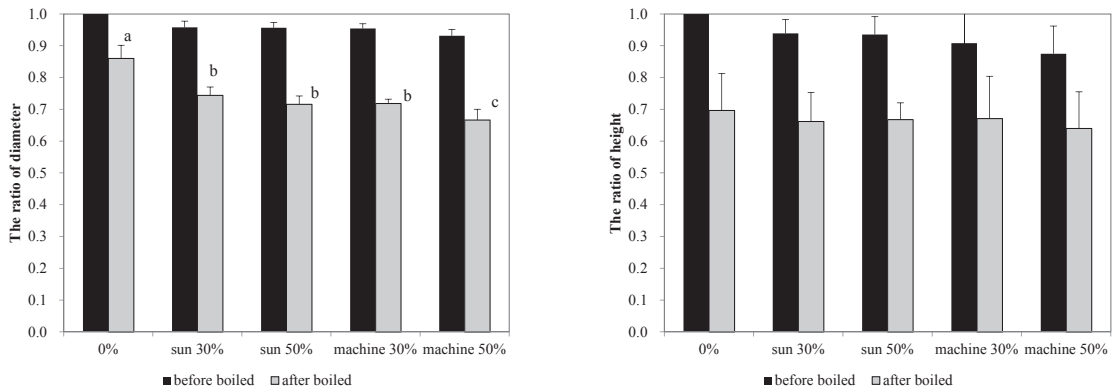

Fig. 5 Comparing the apparent ratio of the diameter and the height before and after boiling treatments of shiitake samples subjected to different drying processes
This figure represents the ratio of the diameter and the height of each sample with that of the non-dry sample.
Different letters on the bar indicate significant differences ($p < 0.05$).
Each value is mean ± SD (n = 12)

Measurement of the changes in shape

We examined why the extraction of water-soluble materials from the mushrooms was decreased. We found that the size of the boiled mushroom changed after partial drying by more than that at the time of sample preparation. Such changes in diameter and height were measured before drying, before boiling, and after boiling for the Shiitake sample (Figure 5). A standard sample and a half-dried sample showed greater changes in diameter than in height. For a half-dried sample, the diameter was generally decreased after boiling. Thus, the components were eluted from the mushroom, causing the size to decrease[12].

Conclusion

The antioxidant activity was lower in the boiled half-dried mushroom than in the standard sample. There was no correlation between polyphenol content and antioxidant activity in the mushroom samples. Non-polyphenol ingredients may be involved in the antioxidant activity of the mushroom.

In this study, the antioxidant activity of the boiled soup of the half-dry Maitake sample was low, suggesting that the elution of the antioxidant component of the boiled soup was suppressed. A previous study indicated that mushrooms with high palatability and functionality are desired by consumers[13]. Thus, it can be proposed that half-drying of mushroom enhances its health functionality, and can be easily prepared in homes. Further studies are required to determine the changes in the antioxidant activity of mushrooms caused by the half-drying process.

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