Effect of Frying with Edible Oil on Antihypertensive Properties of Hatakeshimeji (Lyophyllum decastes Sing.) Mushroom

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The effects of cooking methods on antihypertensive properties and inhibitory action on angiotensin converting enzyme (ACE) of Hatakeshimeji mushroom (Lyophyllum decastes Sing.) were investigated. The molecular mass of proteins in the fruit body of Hatakeshimeji was lowered by deep-frying with cooking oil, as the free amino groups of the hot-water extractable fraction of Hatakeshimeji were increased. However, there was no change in IC₅₀ for ACE activity. The effect on blood pressure for untreated and fried Hatakeshimeji was investigated by oral administration to spontaneously hypertensive rats (SHR). At 6 hours after administration, systolic blood pressure (SBP) of the fried Hatakeshimeji group was significantly lower than that before administration. At 5 hours after administration, ACE activity in the lungs of the fried Hatakeshimeji group was decreased as was the level of free amino groups of hot-water extractable fraction of Hatakeshimeji. These results show that the antihypertensive effect of Hatakeshimeji peptide was not lost by deep-frying.

Keywords: Hakakeshimeji, ACE, SBP, Lyophyllum decastes, deep-frying

Introduction

Hatakeshimeji mushroom (Lyophyllum decastes Sing.), which have a delicious taste, forms a family of Honshimeji (Lyophyllum shimeji Hongo) and belongs to a highly related genus of Lyophyllum (Kanno and Nishii, 2000).

Several beneficial physiological properties of Hatakeshimeji for human health have been reported: antitumor activity (Ukawa et al., 2001a), blood cholesterol-lowering ability (Ukawa et al., 2001b), antihypertensive effect (Kokean et al., 2002), anti-diabetes mellitus (Miura et al., 2002), and hypolipidemic properties (Ukawa et al., 2002). We showed that hot water-extracts of Hatakeshimeji inhibited angiotensin converting enzyme (ACE) activity and that increase of systolic blood pressure (SBP) was inhibited by feeding dry powdered hot-water extracts of Hatakeshimeji fruit body to spontaneously hypertensive rats (SHR) (Kokean et al., 2002). However, most previous reports describe physiological effects due to the mushroom itself, but not the influences of cooking and processing. Suitable cooking method, without causing the destruction of both physiological effects and the taste, is important.

Methods of cooking Hatakeshimeji include boiling, deep-frying, stir-frying, and grilling. The texture of Hatakeshimeji is changed by stir-frying and grilling. The antihypertensive constituent of Hatakeshimeji is dissolved in water during boiling and thus decreased. The high temperature of oil (around 180°C) allows rapid heat transfer and very short cooking time (only a few minutes). The temperature inside the mushroom does not usually exceed 100°C (Fillion and Henry, 1998).

In the present study, the effects of deep-frying on antihypertensive properties of Hatakeshimeji were investigated.

Materials and Methods

Hatakeshimeji fruit body Hatakeshimeji (Lyophyllum decastes Sing.) were cultivated in medium containing bark compost, rice bran, and brewer's grain at Mie Prefectural Science and Technology Promotion Center (Mie, Japan).

Preparation of the Hatakeshimeji powder Untreated Hatakeshimeji powder was prepared by pulverizing freeze-dried Hatakeshimeji fruit body with a mixer (Miller IFM-170G, Iwatani, Nagoya). Fried Hatakeshimeji powder was prepared by heating the fruit body in an oil bath at 180°C for 0.5, 1, and 2 minutes. The powder was defatted with n-hexane. After removing the hexane, the powder was dried and analyzed.

Preparation of Hatakeshimeji hot-water extract (Ohtsuru et al., 2000; Tsuda et al., 2000) Each 10 g aliquot of Hatakeshimeji powder was suspended in 300 ml of boiling water and kept in a boiling water bath for 10 min. After cooling, they were centrifuged at 18,000 xg, 4°C for 40 min.
The supernatant was added to acetone at 75% of final concentration. The insoluble matter was removed by centrifugation. The supernatant was dried by an rotary evaporator. The concentrates were suspended in distilled water. The insoluble matter was removed by filtration (PTFE membrane, Millipore, Bedford, MA). Protein concentration of the filtrate was measured by the Lowry method (Lowry et al., 1951).

**Gel filtration**  Dry powder and fried dry powder of Hatakeshimeji were suspended in distilled water and fractionated by gel filtration through the column of Superdex Peptide HR10/30 (Amersham Biosciences, Piscataway, NJ). The elution was performed by distilled water at a flow rate of 0.5 ml/min. Each 1-ml fraction was collected in separate test tubes. Protein concentration in the fractions was measured by the Bradford method (Bradford, 1976).

**Determination of amino groups**  Amino groups of the Hatakeshimeji proteins were determined by the method of Fields (1971, 1972).

**Experimental animals**  Animal experiments were carried out in accordance with the Mie University animal experiment guideline. Male spontaneously hypertensive rats (SHR/1zm), were purchased at 9 weeks of age from Funahashi Nojo (Funahashi, Japan) and fed with commercial powder diet (Type SP, Oriental Yeast, Osaka, Japan) for 9 weeks. All rats were housed in a room under controlled lighting (lights on from 8:00 to 20:00) at 22°C with free access to food and water.

**Measurement of blood pressure**  SBP and heart rate were measured by the tail-cuff method with a electrophysiyogonometer (MK-1030, Muromachi Kikai, Tokyo).

**Intragastric administration of Hatakeshimeji hot-water extract to SHR**  Hot-water extract (500 mg protein/kg body weight in 2 ml) was administrated to SHRs of 18 weeks of age by stomach tube. Physiological saline was used as a control. The measurement of SBP was carried out just before and at 1, 5, 3, 4.5 and 6 hours after administration.

**Preparation of lung enzyme extract**  SHRs at 18 weeks old were divided into 3 groups of 6 rats each (control group, untreated Hatakeshimeji group, and fried Hatakeshimeji group). Five hours after intragastric administration, the animals were killed by blood collection from the abdominal aorta under ether anesthesia and their lungs excised. Enzyme extracts were prepared from lung tissue by the method of Masuda et al. (1996) with some modification. Protein concentration of enzyme extracts was measured by the Bradford method using bovine serum albumin as a standard.

**Enzyme assay**  ACE activity was determined according to the method of Cushmann and Cheung (1971) with some modification. Enzyme extracts from lung and serum of SHR were diluted with 0.1 M borate buffer (pH 8.3) containing 0.3 M NaCl. One unit (U) of enzyme activity was defined as the amount of enzyme which released 1 mmol of hippuric acid per min (Tokunaga, 2004).

**Statistical analysis**  The results were expressed as the mean ± SEM. All statistical analyses were evaluated using one way ANOVA followed by Tukey’s multiple comparison (Zar, 1999) and were performed using EXCEL tokei version 5.0 software (Esumi, Tokyo). Differences were considered significant when P values were less than 0.05.

### Results

**Effect of ACE activity inhibition on fried Hatakeshimeji** Effects of deep-frying of Hatakeshimeji in the edible oil on the inhibitory activity for ACE are shown in Table 1. Significant differences were not shown in the values of IC50, and amino groups in Hatakeshimeji protein increased significantly by frying. The effects of frying for molecular weight of the Hatakeshimeji protein and ACE activity with Hatakeshimeji on gel filtration are shown in Fig. 1. Two main peaks, F1 and F2, were separated. Molecular weights of these peaks were less than 1 kDa by comparison with the molecular weight marker. F1 peak overlapped with the peak for the inhibition of ACE activity. Absorbance of F1 became about 80% by deep-frying, however the effect of inhibition of ACE activity was nearly unchanged. The absorbance of F2 was decreased to about half by frying.

**Single oral administration of Hatakeshimeji hot-water extract to SHR**  Blood pressure after the administration of the untreated and fried Hatakeshimeji hot-water extract is shown in Fig. 2. The SBP gradually decreased after administration of untreated Hatakeshimeji hot-water extract, and the lowest value was observed after 3 hours.

### Table 1. Effect of frying on ACE activity of Hatakeshimeji.

<table>
<thead>
<tr>
<th>Time of treatment (min)</th>
<th>dry weight* (g)</th>
<th>-NH2/mg protein (x10^{18})</th>
<th>IC_{50} (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>untreated</td>
<td>9.3 ± 0.15</td>
<td>2.8 ± 0.06^a</td>
<td>1.7 ± 0.21</td>
</tr>
<tr>
<td>0.5</td>
<td>8.9 ± 0.12</td>
<td>7.7 ± 0.05^b</td>
<td>1.3 ± 0.12</td>
</tr>
<tr>
<td>1</td>
<td>9.1 ± 0.25</td>
<td>6.6 ± 0.21^b</td>
<td>1.0 ± 0.08</td>
</tr>
<tr>
<td>2</td>
<td>8.5 ± 0.10</td>
<td>6.7 ± 0.19^c</td>
<td>1.5 ± 0.03</td>
</tr>
</tbody>
</table>

ACE assay was performed in duplicate. Values are expressed as the mean±SEM (n=6). Significant differences among the untreated, 0.5, 1, and 2 min groups were analyzed using one-way ANOVA followed by Tukey’s multiple comparison. Values with different letters are significantly different at p<0.05.

*: Weights of 100 g of raw Hatakeshimeji fruit body after deep-frying for each length of time, freeze-drying and defatting.
Then, the significantly lower SBP was maintained for 6 hours after administration. For administration of fried Hatakeshimeji hot-water extract, SBP continued to be lower for 6 hours after administration. The antihypertensive effect of fried Hatakeshimeji was shown as well as that of untreated Hatakeshimeji.

**Discussion**

The ACE inhibitory substances of Hatakeshimeji have been identified as being a protein or peptide (Ukawa et al., 2001a). Moreires-Valera et al., (1984) described that the protein digestibility of foods was not affected by deep-frying (around 180°C) without any additional ingredients. However Bender and Husaini (1976) found that the nutritive values of proteins only decreased when reducing substances (glucose and wheat flour) were added to the meat. Soluble protein in Hatakeshimeji was decreased by deep-frying. Hatakeshimeji protein might be partially decomposed by deep-frying, as amino groups in Hatakeshimeji hot water-extract were increased compared to deep-frying. Hatakeshimeji contains large amounts of carbohydrates. These carbohydrates might affect the decrease of protein by frying.

The results on the degradation of Hatakeshimeji protein were consistent with those on gel filtration. It seems that peptides of Hatakeshimeji with ACE activity inhibition are difficult to degrade by deep-frying. Previous studies on frying for protein (Ammu et al., 2000; Fillion and Henry, 1998) were on the effects of changes in nutritional value. There are few reports on the changes
of protein content and physiological effects of food constituents by deep-frying. It is interesting that the inhibition of ACE activity of the peptide in Hatakeshimeji is not affected by deep-frying.

In the organs of mammals, ACE is abundantly incorporated in pulmonary vascular endothelial cells (Nakamura et al., 1996). It is known that ACE activity in the lung is particularly higher. Accordingly, in this study, effects on ACE activity in the lung were investigated. Both of SBP values for SHR and ACE activity in the lung were decreased by the administration of fried and untreated Hatakeshimeji hot-water extracts. After administration of Hatakeshimeji hot-water extracts to SHR, ACE activity for the lung of the fried Hatakeshimeji group as well as that of the untreated Hatakeshimeji group was decreased, and IC\textsubscript{50} of ACE did not change by deep-frying in vitro. Based on these results, generation of peptides from protein by deep-frying might affect ACE activity in the lung of SHR. Thus, the advantage of deep-frying was demonstrated in the results of this study.

If the prevention of life style diseases without depending upon the medicine were possible and it were possible to eat diets containing food ingredients with physiological effects, such foods would increase the pleasure of eating. These findings lead to the conclusion that deep-frying did not adversely affect the taste, the texture and the antihypertensive property of Hatakeshimeji.

References


