

Tenderizing of Meat by Using *Maitake* (*Grifola frondosa*) Extract with Low Temperature Steam Cooking

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Cooking of various food materials at lower but acceptable temperatures produces not only tender and tasty food but also nutritionally valuable food relative to food cooked by ordinal methods, i. e. boiling and baking.

Maitake (*Grifola frondosa*) extract contains proteases which have a high heat resistance as measured by casein hydrolysis assay. The optimal activity was observed at a temperature range between 50–70°C, and 30 to 40 percent of the original activity remained after heating at 70°C for 8 hours. Inside round cuts of beef cooked using low temperature steam cooking with *maitake* extracts released more protein into the soluble fraction than cooking with ginger extracts or by boiling. However, free glutamic acid, which contributes to *umami* taste, increased not only in the solubilized fraction but also inside the meat. Histological observation also presents a degradation of protein in the meat treated with *maitake* extract. Both measurements under the rheometer and sensory evaluation showed a remarkable improvement with respect to hardness and chewiness. All the results that are presented here demonstrate the advantage of combinational cooking at low temperature steam with extract from *maitake* for tenderizing meats. This suggests a superior method of cooking many other food materials to be served on the table.

Key word : low temperature steam cooking, *maitake* (*Grifola frondosa*), protease, tenderizing beef

Introduction

Malnutrition among the elderly due to inadequate protein and energy sources (Protein Energy Malnutrition, PEM) is a current social concern while over-eating, especially of rich foods is an other problem (Sugiyama, 2002 a; Sugiyama *et al.*, 2002 b; Teshima, 2005).

Fifteen to twenty percent of meat contains high quality proteins which consist of many amino acids, including those essential to humans as well as providing on energy source. However, meat is a harder food material relative to fish because of it's richness in stroma protein. Beef is especially hard relative to chicken and pork due to a complex of rich collagen and muscle fibers. Meat becomes harder because of denaturation, shrinkage and a loss of lipid and water through the further heating of muscle fibers. On the other hand, it has been shown that a tenderer meat can be prepare by heating at a lower temperature for a longer period (Bramblett *et al.*, 1959; Martens *et al.*, 1982; Nishimura *et al.*, 2004; Sainen *et al.*, 2003). We have also reported that silverside beef, which is not eatable due to it's hardness, can be served as an acceptably tender meat by cooking it with lower temperature steam for a longer period (Ito *et al.*, 2003; Yamaza-

ki *et al.*, 2003; Yamazaki *et al.*, 2006). This tenderizing effect at lower temperature for a long period (low temperature steam cooking) derives from a slow denaturing of protein and the action of an endogenous enzyme. This type of cooking utilizes endogenous enzyme activities at maximum level as well as exogenously added enzyme activities, especially the action of various proteases. This combined protease activity produces tender meat dishes, particularly good for the elderly. Kiwifruit and papaya are tropical fruits known to contain proteases and have been used for tenderizing meats (Nishiyama, 2001; Sugiyama, 2005; Tsutsumi *et al.*, 1994; Watsuji and Miyamoto; 1985). However, the sweetness of fruit may alter the taste of food. Another source of proteases, which does not change taste are mushrooms which contain various proteases in both their fruiting body and hypha (Mizuno and Kawai, 1992). A change of freshness and protease activity in *bunashimeji* mushroom and oyster mushroom was reported (Nakanishi and Naruse, 1997). And proteases from *maitake* have demonstrated degradation of egg white protein (Kimoto *et al.*, 1994). But rarely found is research on the role of mushroom in tenderizing meat. We have developed a combined cooking method of low temperature steam cooking with *maitake* protease, together with the basic analysis of such proteases.

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Materials and methods

1. Materials

An inside-round cut of Australian beef, used 3 weeks after slaughter, was sliced into 1 cm thick round pieces transversally against the muscle fiber and frozen. Then, it was cut to 5×5 cm size in a semi-frozen state in our laboratory and kept in -20°C freezer until use. The meat was transferred to a 4°C environment and kept for 15 hours, followed by one hour at room temperature prior to the experiment. The weight of meat was 27.23 ± 1.91 grams.

Maitake mushroom produced by Yukiguni-Maitake growers, Niigata, and ginger roots produced in Kagoshima prefecture were purchased from a supermarket one day prior to the experiments. The latter was used as a control material. It also contains some proteases (Navennna and Mendiratta, 2001).

2. Preparation of solution to soak the above piece of meat

For the preparation of meat-soaking solution, extracts of *maitake* or ginger root were blended with twice the extracts' weight of distilled water and this was filtered through 3 layers of cheese cloth. The pH of the extracts of *maitake* and ginger were 6.01 ± 0.11 and 6.13 ± 0.12 , respectively and the water used as the control had a pH of 5.60.

3. Heat treatment

Meat soaked with 50 ml of solution was placed in 80×100 mm Nylon-poly bag (Fukusuke Industry Co., Ltd.), shielded under vacuum for 5 minutes using Japan vacuum system, type KN-25. Heating methods were low temperature steam, boiling, and boiling after soaking, for comparison to each other. The temperature of steam cooking was 70°C as used earlier (Yamazaki *et al.*, 2006) and cooking time was 2 hours. Equipment used for low temperature steam cooking was a test type rice cooker (TOSHIBA HA Product. Co., Ltd.) shown in Fig. 1. For boiling, meat was kept in 3 liters of boiling water for 10 minutes. For boiling after soaking, meat was soaked while in a refrigerator, for 110 minutes at 4°C followed by 10 minutes boiling. The total period was 2 hours. At the end of cooking, meat was chilled to room temperature using ice water.

4. Measurement of protease activity

Hydrolysis of casein was employed for determination of protease activity of various mushrooms, ginger, kiwifruit and pineapple (Kobayashi, 2000). Casein was dissolved in 0.1 M phosphate buffer (pH 7.0) at a concentration of



Fig. 1 Low temperature steaming apparatus (TOSHIBA, Test-Type)

1.2% as the substrate solution. 3.0 mls of the substrate was mixed with 0.6 ml of the extract and incubated at 35°C for 30 minutes or for desired period. The reaction was stopped by the addition of 3.0 mls of 10% trichloroacetic acid and stood for 15 minutes at room temperature, followed by filtration. The reduction of absorbance at 280 nm of precipitate fraction dissolved in 3.0 mls of phosphate buffer was measured. An increase of 1.0 at E_{280} for one minute was expressed as one enzyme unit. The heat stability and optimum temperature of enzyme activity were measured at various points between 30°C and 90°C .

5. Determination of protein released from beef

The soluble protein was determined following BCA method using protein assay kit (Bio-rad Co., Ltd.), with BSA (bovine serum albumin) for the standard.

6. Determination of free glutamic acid

Free glutamic acid retained in meat and released from the meat into solution after heating was determined by glutamic acid determination kit (Yamasa Co., Ltd.).

7. Histological observation

A small piece of the meat (raw) was quickly frozen in dry ice-acetone and cross sections were prepared with cliostat with $8\ \mu\text{m}$ thickness. The sections were fixed on slides and were covered with either water, ginger extract or *maitake* extract and kept at room temperature for desired period. The slides were washed with phosphate-buffered saline to stop the reaction followed by hematoxylin-eosin staining. Observations were carried out using an optical microscope.

8. Rupture property

Measurement of rupture property was carried out on the samples cut to 3 cms long and 1 cm wide using Rheoner RE-3305 S (Yamaden Co., Ltd.) with plunger No. 21 knife-type at the speed of 1 mm/sec. The samples

were cut in parallel with the fiber.

9. Sensory evaluation

For sensory evaluation, four samples, 2×2×1 cm in size, prepared by steaming with water, ginger extract or *maitake* extract and boiled with *maitake* extract were tested. Each panelist evaluated them on hardness, ease of biting, ease of swallowing and juiciness according to the ranking test method. The panel consisted of 16 students and professors.

10. Statistical analysis

Each data values is presented as the mean ± standard deviation. The significance tests between samples were performed by a multiple comparison test using Bonferoni's method after one-way analysis of variance (ANOVA). The statistical analysis of sensory evaluation data were carried out using Newell & MacFarlane's test. The Kendall's agreement coefficient was also used to judge the degree of agreement of the panel.

Results and Discussion

1. Protease activity

Fig. 2 shows the casein hydrolysis activities of extracts from mushrooms, ginger, kiwifruit and pineapple at pH 7.0 at 35°C. Oyster mushroom had the highest activity followed by *maitake*, which was equal to pineapple. Ginger extract gave the most scattered value of protease.

Fig. 3 presents the heat stability of oyster mushroom and *maitake* protease activity. Oyster mushroom protease activity was reduced to 20% within 1 hour at 50°C

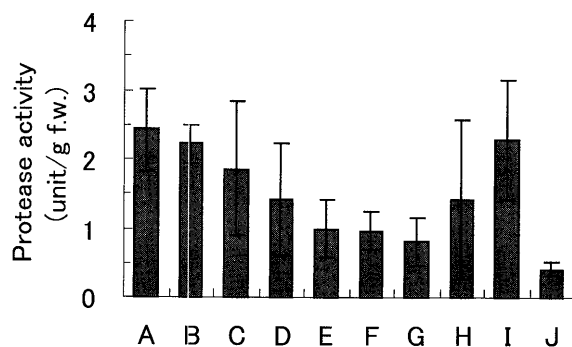


Fig. 2 Protease activity of various mushrooms (n=3-4)

- A: oyster mushroom (*Pleurotus ostreatus*),
- B: *maitake* (*Grifola frondosa*),
- C: *bunashimeji* (*Hypsizygus marmoreus*),
- D: *eringii* (*Pleurotus eryngii*),
- E: winter mushroom (*Flammulina velutipes*),
- F: common mushroom (*Agaricus bisporus*),
- G: *shiitake* (*Leutinus edodes*),
- H: ginger,
- I: pineapple,
- J: kiwifruit

heating, but no activity was found after 1 hour at 60°C and 70°C. In contrast, *maitake* protease activity remained at 50% after 1 hour at 50°C, at 40% at 70°C and 30 to 40 % even after 8 hours. This wide temperature range may suggest the presence of two or more different types of proteases, one with more heat sensitivity and the other with more heat resistance. It should be noted that the highest activity was observed in a wider range from 50 to 70°C (Fig. 4), and this *maitake* extract was usable in cooking of meat because of its high activity and heat sta-

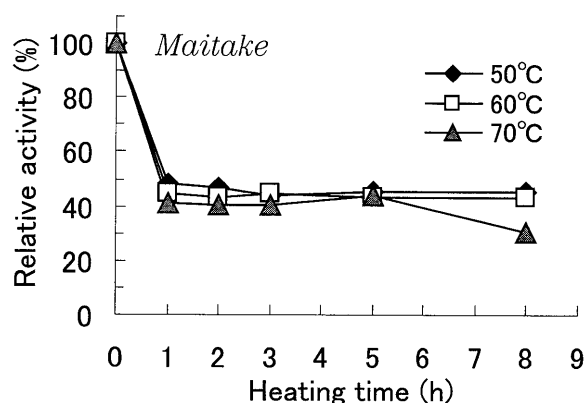
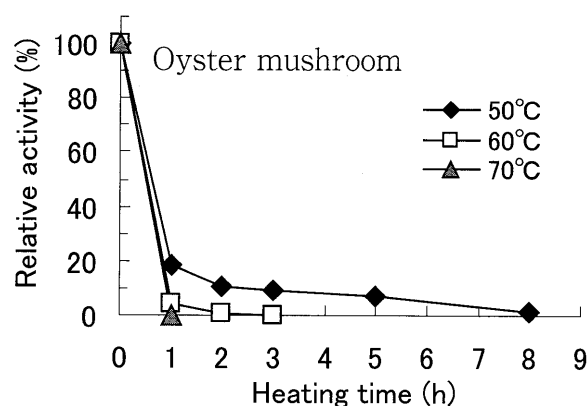


Fig. 3 Stability of *maitake* and oyster mushroom on heating (n=2)

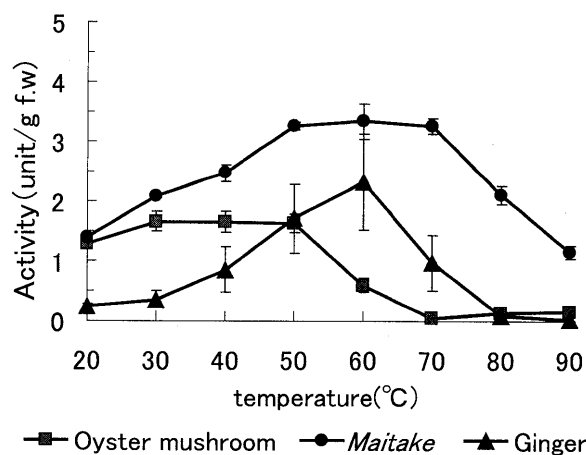


Fig. 4 Optimum temperature curves of protease activity (n=3-4)

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bility.

2. Change in weight of meat and volume of solution after heating

Table 1 shows the weight of the meat after cooking. Comparing the methods of heating, less weight reduction was measured between boiling and steaming, when the meat was soaked in water or with ginger extract. When meat was soaked with *maitake* extract, the reduction was larger in boiling followed by soaking and steaming. Regardless of method, the biggest loss was associated with *maitake* extract. It is clear that reduction of weight of meat occurs with *maitake* extract and the rate is the highest among all treatments.

Table 2 presents the increase of volumes of soaking solution after cooking. An increase of volume was observed with *maitake* extract. The increase was 2.2 to 2.3 times more with *maitake* extract relative to water in boiling and in boiling after soaking, however, 2.7 times more was observed in the case of steam cooking. Further, volume increased more in boiling than with steaming in the case with ginger extract, but in the case of using *maitake* extract, practically no alteration in volume was found between boiling and steam cooking.

It has been considered that the denaturation of protein at lower temperature is slower and the weight loss is

Table 1. Change in weight by heating (%)

Heating method	Soaking solution		
	Water	Ginger	<i>Maitake</i>
Steaming	61.54 ± 3.28	62.93 ± 4.56	58.35 ± 2.50**
Boiling	60.41 ± 2.11	61.13 ± 2.95	59.06 ± 1.64
Boiling after soaking	58.41 ± 1.17	60.20 ± 1.79	56.78 ± 2.42**

Percent of weight remaining after heating versus raw sample (mean ± SD, n = 10–15)

** : Significant difference at $p < 0.01$ between ginger and *maitake* solution.

Table 2. Change in volume of solution after heating (%)

Heating method	Soaking solution		
	Water	Ginger	<i>Maitake</i>
Steaming	8.00 ± 2.65 ^{aA}	13.86 ± 3.54 ^{aB}	21.86 ± 1.93 ^{aC}
Boiling	9.70 ± 1.49 ^{aA}	18.23 ± 2.47 ^{bB}	22.19 ± 2.05 ^{aC}
Boiling after soaking	10.00 ± 1.41 ^{aA}	18.56 ± 2.80 ^{bB}	22.22 ± 1.62 ^{aC}

Ratio of increasing volume in soaking solution after heating versus before heating (mean ± SD, n = 7–12)

a, b : Different superscript in the same column show significant difference ($p < 0.05$)

A, B, C : Different superscript in the same row show significant difference ($p < 0.01$ or 0.05)

smaller. However, in steaming with *maitake* extract, the drip into the soaking solution increased.

3. Amount of proteins and glutamic acids

The amount of protein released after heating was measured (Fig. 5). The values are expressed after the subtraction of the endogenous amounts in ginger or *maitake* extract.

Regardless of method, more protein was eluted with *maitake* extract than with ginger extract or water. In the case of soaking with water, no change of protein content was found, regardless of heating method. But, in soaking with ginger or *maitake* extract, a slight increase was noticed in the case of boiling after soaking compared with the case of direct boiling. In steam cooking, a higher amount of eluted protein from meat was found with *maitake* extract than with boiling.

Amount of free glutamic acid, that is a component of *umami* taste, in the meat and in the soaking solution was measured (Fig. 6 and 7). As one would expect from an increased amount of protein hydrolysis, the amount of free glutamic acid would be larger, at least in elution.

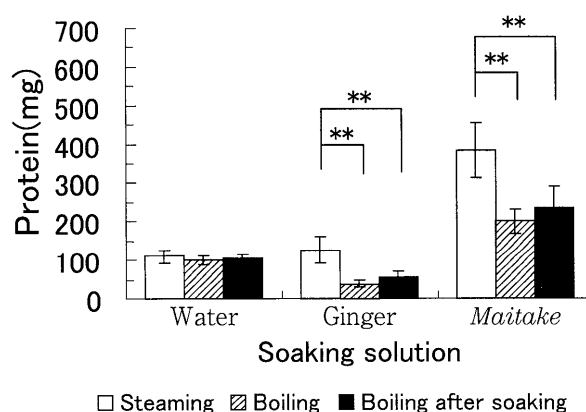


Fig. 5 Amount of protein released from beef during heating

** Significant difference at $p < 0.01$ (n = 6–11)

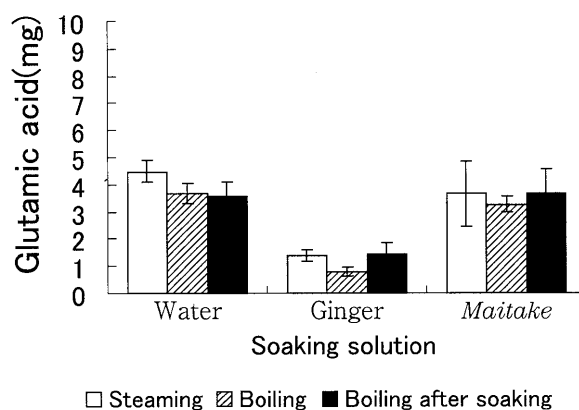


Fig. 6 Amount of free glutamic acid released from beef during heating (n = 3–7)

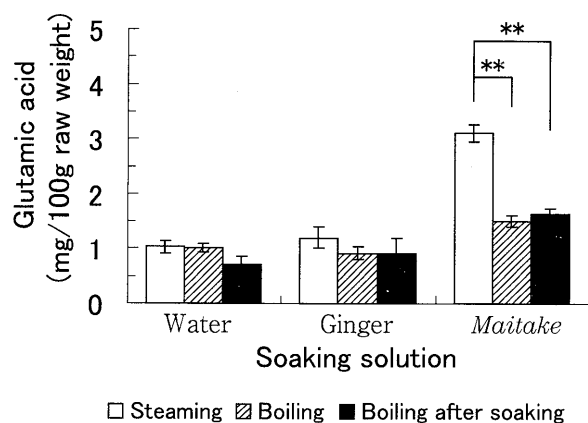


Fig. 7 Amount of free glutamic acid of beef after heating

** Significant difference at $p < 0.01$ ($n = 3-4$)

More free glutamic acid was found in both meat and the soaking solution in steam cooking. A lower amount of free glutamic acid was found in the soaking solution with ginger extract relative to water or *maitake* extract, regardless of heating method (Fig. 6). The amount of free glutamic acid in meat remained the same regardless of method of heating with water or ginger extract (Fig. 7). With the use of *maitake* extract, steaming produced a significant increase of free glutamic acid in meat, in contrast to boiling and boiling after soaking.

Increase of free glutamic acid by steam cooking corresponded with results obtained by using silverside beef as reported earlier (Yamazaki *et al.*, 2006). Involvement of peptidase in production of free amino acids during aging of meat (Okiya, 2000) and retention of amino peptidase M activity in meat after a long period steaming at 70°C (Yamazaki *et al.*, 2005) has been reported. *Maitake* protease contains heat stable amino peptidase that cleaves peptide bonds at the amino end of lysine (Nonaka *et al.*, 1995). Together with these reports, we have speculated a contribution of peptidase on the increase of free glutamic acid in steaming.

4. Histological observation

Fig. 8 presents histological figures of meat sections, non-treated and after 1-2 hours treated with water or ginger or *maitake* extract. Practically no difference was found between the non-treated and treated sections in water or ginger extract. The tissue treated with *maitake* extract showed many holes in the cells' cytoplasm. The size of these holes grew larger after 1 to 2 hours. As the period of treatment with *maitake* extract lengthened a reduction of staining was observed. Such a tendency was also observed in the tissue treated with ginger extract. Staining of collagen showed much less change.

The hardness of meat arises from muscle fiber protein consisting mainly of actomyosin, and connective tissues consisting of collagen. This analysis suggested that tenderization came mostly from a breakdown of muscle fiber protein.

5. Rupture property

Rupture energy is generally smaller in samples cooked with steaming than by boiling (Fig. 9). Only half of energy required in boiling was needed for rupturing meat steamed with *maitake* extract, and it was lower than for steamed meat with ginger extract or water. The rupture energy for meat cooked after soaking in ginger or *maitake* extract was lower than direct boiling. This is conceivably due to the fact that *maitake* enzyme was active even after a long period of heating (Fig. 3 and 4), and because protein released from meat increased, even in case of boiling (Fig. 5). With ginger extract, meat became tenderer in some cases, but not in others. This could be explained by the observation that protease activity of ginger varied widely (Fig. 2).

6. Sensory evaluation

The result of sensory evaluation is shown in Table 3. It is expressed in a total score ranking on hardness, ease of biting, ease of swallowing and juiciness. The steamed meat with ginger or *maitake* extract was significantly tenderer and easier to bite, relative to that with water. The steamed meat with *maitake* extract was tenderer and easier to bite, than the boiled sample, in agreement with data obtained through the rupture property test (Fig. 9). Meat steam-cooked with *maitake* extract was the easiest to swallow among the samples. A difference in score of juiciness was not evident between samples.

From these results, the use of *maitake* extract for tenderizing meat, especially with the application of low temperature steam cooking, has a value in food preparation.

Mushroom is rich in dietary fiber and vitamin D, and is said to have anti-tumor effects, so that the use of *maitake* is increasing in popularity as a healthy food. According to a survey by the Japanese Department of Agriculture and Fishery (2002), 50.6% of people eat mushrooms 2-3 times, a week and 22.4% of people eat it 4-5 times a week. The older people are, the more mushroom is consumed. Another survey by Muramatsu (2004), using students and their families, shows that 70% of people eat mushrooms more than once a week and the preferred ones are *shiitake*, *bunashimeji*, and *maitake* in that order. Of these, *maitake*, which is a special product in the

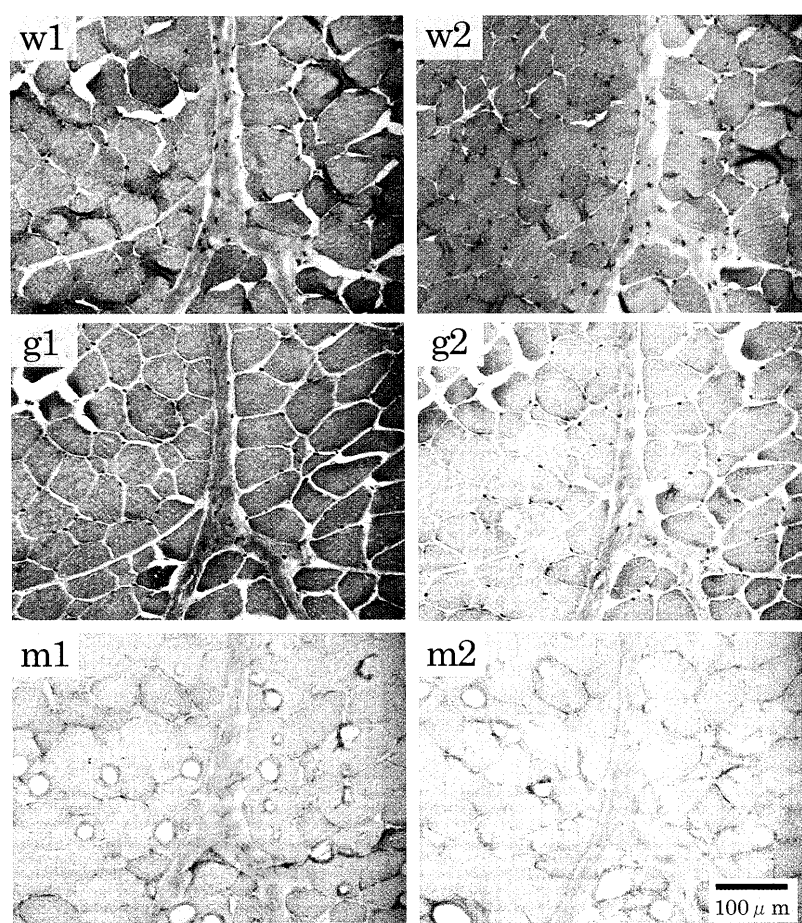
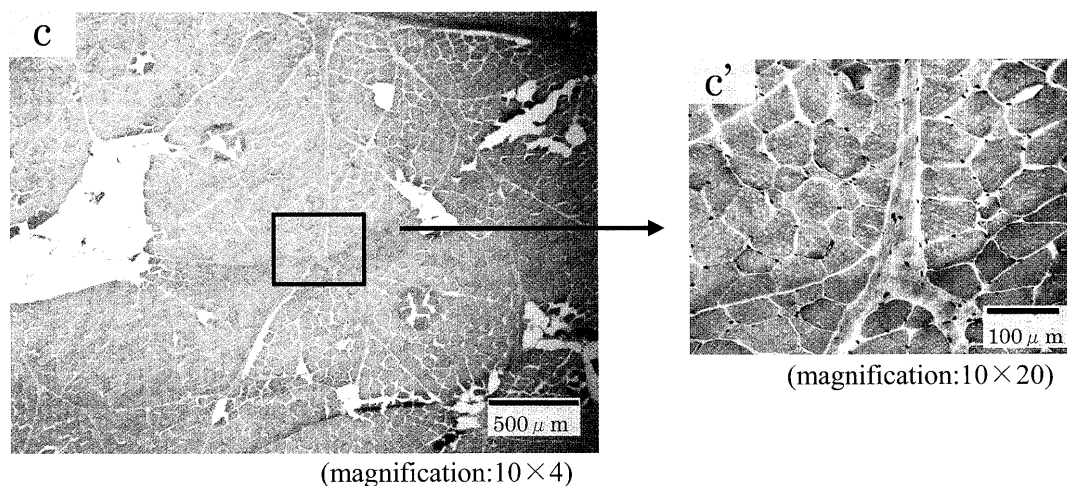


Fig. 8 Cross section of beef after hematoxylin-eosin staining

c, c': non-treatment

w 1: water-treatment 1 h, w 2: water-treatment 2 h,

g 1: ginger-treatment 1 h, g 2: ginger-treatment 2 h,

m 1: *maitake*-treatment 1 h, m 2: *maitake*-treatment 2 h

Niigata area, is preferred in meat cuisine. Use of *maitake* for meat cooking may stimulate the self-sufficiency ratio through the local consumption (*chisan-chisho*, in Japan). There is a report that enzyme treatment of meat produces bitter peptides (Gerelt *et al.*, 2000), but *maitake* has a

special flavor which may eliminate their bitterness. It is known that over-hydrolysis of meat protein by the enzymes may influence the taste and flavor. Thus, careful control of the length of cooking and temperature is indispensable for the cooking of meat, but this can be facilitat-

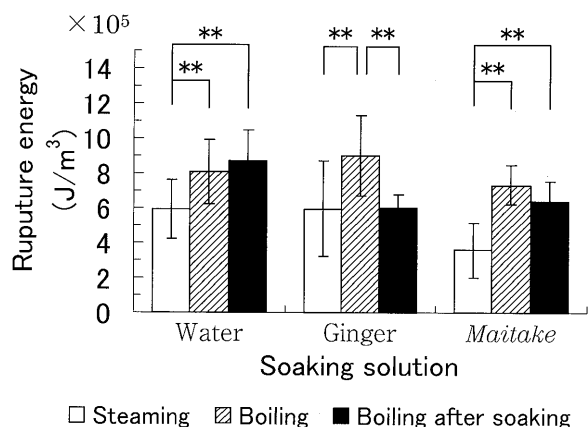


Fig. 9 Rupture property

** Significant difference at $p < 0.01$ ($n = 16-30$)

Table 3. Sensory evaluation

	Steaming			Boiling
	Water	Ginger	Maitake	Maitake
Hardness	21	47 ^{a**}	62 ^{a***b**}	30
Ease of biting	59	33 ^{a**}	20 ^{a***b**}	48
Ease of swallowing	52	38	31 ^{a*}	39
Juiciness	44	40	44	32

Total score of ranking on hardness, ease of biting, ease of swallowing and juiciness ($n = 16$)

a : versus steaming with water, b : versus boiling with maitake

* Significant difference at $p < 0.05$ ** Significant difference at $p < 0.01$

ed by using our low temperature steam cooking.

Successful tenderizing of protein-rich beef, making the meat easy to eat, may relate to an improvement of PEM and a heightening of QOL of the elderly.

Studies along this line have a value to expand the practical use of low temperature steam cooking.

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マイタケ抽出液と低温スチーミング調理併用による食肉軟化について

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和文抄録

マイタケに含まれるプロテアーゼの作用と低温スチーミング調理の併用による牛肉軟化について検討した。

マイタケ抽出液は 50-70℃ で最もカゼイン分解活性が高く、70℃ で 8 h 反応させたあとでも 30-40% の活性が残っており、熱安定性が高かった。マイタケ抽出液とともに牛モモ肉を低温スチーミングすると、茹でた場合や、水またはしょうが抽出液を使った場合に比べて、溶出するタンパク量が多かった。しかし、うま味に関係するグルタミン酸は特に溶出は増えておらず、むしろマイタケとともにスチーミングした肉で有意に増加していた。組織観察の結果、マイタケ抽出液で処理したものはタンパクが分解されている様子が観察された。破断測定および官能評価では、マイタケ抽出液とともにスチーミングしたものは有意に軟らかく、噛み切りやすいという結果となった。

マイタケと低温スチーミング調理を併用すると、効果的に食肉を軟化できることが示唆された。

キーワード：低温スチーミング調理、マイタケ、プロテアーゼ、牛肉軟化

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