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MEAT TENDERIZATION BY PROTEOLYTIC ENZYMES AFTER OSMOTIC DEHYDRATION

Gerelt*, Yoshihide Ikeuchi and Atsushi Suzuki

* Master's Program in Functional Biology, Graduate School of Science and Technology, and Department of Applied Biological Chemistry, Faculty of Agriculture, Niigata University, 2-8050 Ikarashi, Niigata 950-2181, Japan

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Background and objectives:

The treatment of proteolytic enzyme is one of the popular method for meat tenderization. In this case, it is very important how to penetrate the enzymes into meat. There are three methods to penetrate the proteolytic enzymes into meat cuts postmortem, such as dipping in a solution containing proteolytic enzymes, pumping enzyme solution into major blood vessel of meat cut and rehydration of the freeze-dried meat in a solution containing a proteolytic enzyme. Except the rehydration of freeze-dried meat, first two methods are somewhat unsatisfactory, since they over-tenderized the surface, producing a mushy texture, and, since they were unable to penetrate within meat, left the interior unaffected (Lawrie,1998). The rehydration of freeze-dried meat showed a much better distribution of the enzymes than did dipping or perfusion, but it was not still ideal. Instead of introducing the enzymes into meat post-mortem, pre-slaughter injection of the enzymes into live animal was developed and was proved to be most effective method of introducing the enzymes into meat. Since the appearance of the patent by Beuk *et al.*(1959), many papers describing this method have been published. In this case, it is an important to select an enzyme suitable for live animal. If unsuitable enzymes were injected, it may cause a shock to the animal, resulting in the production of low grade of meat. This method is not acceptable with ease, because handling live animal requires a skillful operator. Osmotic dehydration with a contact dehydration sheet (Numamoto & Kasai, 1983) is simple method of dehydration of meat. None of special equipment and skillful operator are required.

This paper describes meat tenderization by dipping meat cut in a solution containing proteolytic enzyme after the osmotic dehydration.

Materials and methods:

Lean meat from culled cow was excised from the shoulder part of a beef carcass 2 days after slaughter and stored at -25℃. As required, it was tempered overnight in a cold room (3~4℃) and cut into small pieces (30×30×20 mm).

Osmotic dehydration

Pichit, a contact-dehydrating sheet consists of a high osmotic pressure substance (e.g. thick malt syrup, glucose, sorbitol, etc.), a polymeric water absorbent (e.g. acrylic acid salt, acrylic acid esters, polyvinyl pyridine, etc.) and a hydrophilic alcohol (e.g. propylene glycol, glycerol, etc.) which are integrally covered with a semipermiable membrane allowing selective permeation of water. Each piece of meat covered with a cellophane sheet was placed between contact-dehydrating sheets and stored for 18 hr in a cold room. After the dehydration, each sample was dipped in a two volume (w/w) of solution containing proteolytic enzymes, such as papain and proteinases from *Aspergillus oryzae* and *Aspergillus sojae*, which are traditionally used for soy sauce production for 3 hr in a cold room. Taking out from the solution, each sample was stored for 24, 48 and 168 hr in a cold room. The concentration of enzyme solution is 0.1% for papain and 1% for proteinases from *Aspergillus*.

Texture measurement

Hardness of meat was measured by Rheometer with a conical plunger. Five places of each sample were measured.

Fragmentation

Myofibrils were made from each muscle by the method of Busch *et al.* (1972). After adjusting the protein concentration to 0.5 mg/ml of 100 mM KCl, turbidity at 540 nm of the solution was measured as fragmentation index (Moller *et al.*, 1973).

Scanning electronmicroscope(SEM) studies on intaramuscular connective tissue

Specimens for SEM of intramuscular connective tissue were prepared by the cell-maceration method of Ohtani *et al.* (1988) and examined using a SEM ABT-55 (Akashi Beam Technology Co., Tokyo) with an accelerating voltage of 10 kV.

Results and discussion:

Dehydration and absorption of enzyme solution

The absorption ratio of enzyme solution [absorption of enzyme solution(ml) / dehydration of water (ml) X 100] was about 80 % of the water dehydrated. The penetration efficiency of enzyme solution after the contact-osmotic dehydration of meat seems to be sufficient.

Texture measurement

The changes in the relative hardness of enzyme-treated meat as expressed as a percentage of that of the control (untreated meat) stored for 24 hr is shown in Fig. 1. The decrease in the relative hardness was observed during storage irrespective of enzyme treatment or not, but it happened more rapidly and largely in the enzyme-treated meat. Among the enzymes tested in this experiment. the meat tenderizing activity of papain was the best and the difference in the activity was not observed between the proteinases from *A.oryzae* and *A.sojae*.

Fragmentation of myofibrils

The changes in the relative fragmentation of myofibrils prepared from enzyme-treated meat as expressed as a percentage of that of the control (untreated meat) immediately after thawing are shown in Fig. 2. As compared with the myofibrils from the control, the rapid increases of the fragmentation of myofibrils from the enzyme-treated meats were observed at first 24 hr storage. The relative fragmentation ratio of the myofibrils treated with the protease from *A.sojae*, *A.oryzae* and papain reached about 260 %, 200 % and 190 %, respectively. After that the gradual increases of the fragmentation were observed in the myofibrils treated with papin and proteinase from *A.oryzae* up to 48 hr storage. From 48 hr to 168 hr storage, a slight increase and decrease of the fragmentation were observed in the myofibrils treated with papain and *Aspergillus* proteinases, respectively. In the control, gradual increase of the fragmentation was observed throughout the storage, but the ratio of the fragmentation was lower than that of the enzyme- treated myofibrils at any stage of the storage. Especially, at first 48 hr storage, the fragmentation ratio of the control was about half of that of the enzyme-treated myofibrils. The acceleration of fragmentation of myofibrils from the meats treated with proteolytic enzymes is one of the reasons of the meat tenderization as obtained in the texture measurement.

Scanning electronmicrographs of intramuscular connective tissue

SEM of the intramuscular connective tissue in the meats treated with proteolytic enzymes are shown in Fig. 3. The remarkable deformation and disruption of honeycomb like structure of endomysium were observed at 24 hrs of storage as compared with that of the untreated meat. The disruption of intramuscular connective tissue is another reason of meat tenderization.

Conclusion :

From the results, it was proved that the proteolytic enzyme treatment after the osmotic-contact dehydration was useful to tenderize tough meat. These results are in good agreement with that of sensory evaluations (data not shown).

Pertinent literature:

Beuk, J.F., Savich, A.L., Goeser, P.A. & Horgan, J.M., US Patent 2903362 (1959).

Busch, W.A., Stromer, M.H., Goll, D.E. & Suzuki, A., *J.Cell Biol.*, **52**, 367 (1972).

Lawrie, R.A. (1998), In " Lawrie's Meat Science", 6th Edition, pp.244, Woodhead Publishing Ltd., UK.

Moller, A.J., Vestergaard, T. & Wismer-Pedersen, J., *J.Food Sci.*, **38**, 824 (1973).

Numamoto, Y. & Kasai, J., US Patent 4383376 (1983).

Ohtani, O., Ushiki, T., Taguti, T. & Kikuta, A., *Arch.Histol.Cytol.*, **51**, 249 (1988).

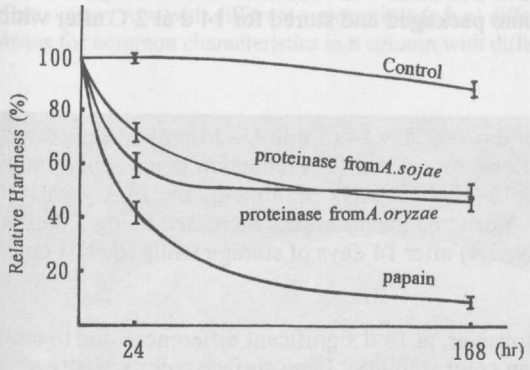


Fig.1 Changes in Hardness of Enzyme-Treated Meat

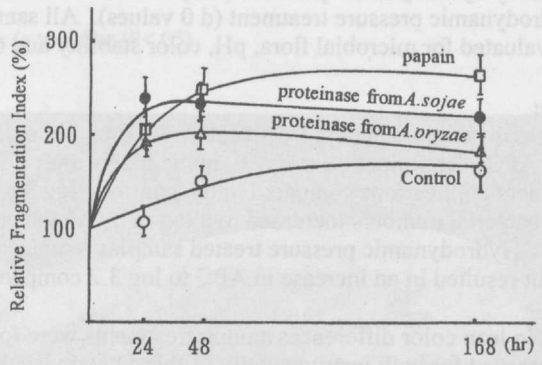
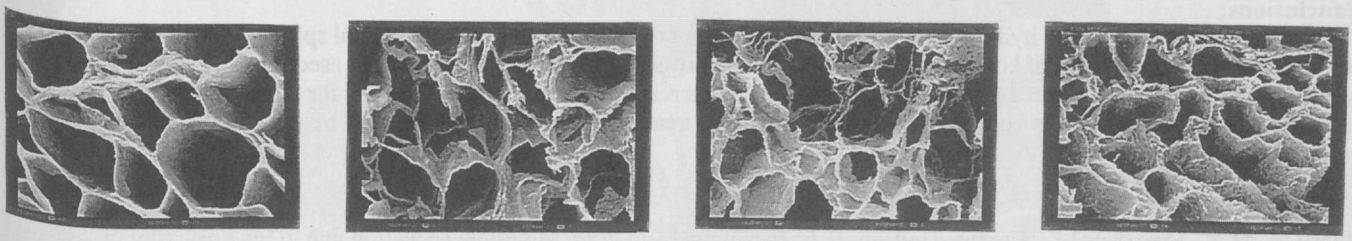


Fig.2. Changes in Fragmentation of Myofibrils from Enzyme-Treated Meat



Control papain proteinase from *A.sojae* proteinase from *A.oryzae*

Fig.3 Intramuscular Connective Tissue of Enzyme-Treated Meat stored fo 24 hr