RETORT PROCESSING MAY INDUCE PROTEIN DEGRADATION AND IMPROVE FOOD FUNCTIONALITIES OF MEAT PRODUCTS

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***Abstract*: Food functionality of a retort-processed model pork sausage was evaluated by analyzing its protein and peptide distribution, inhibitory activity against angiotensin I-converting enzyme (ACE), and growth-promoting activity in an osteoblast-like cell culture. The sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) pattern of whole homogenate from the retorted sausage showed that the band corresponding to the myosin heavy chain disappeared and the intensities of the actin and α-actinin bands decreased after retort processing compared with those obtained using the homogenate of the sausage heated at 75°C. SDS-PAGE analysis also showed that the band intensity of low-molecular-weight (<10 kDa) peptides obtained from the retorted sausage was greater than that of the low-molecular-weight peptides obtained from the sausage heated at 75°C. Furthermore, free amino acid and peptide contents significantly increased during retort processing compared to heating at 75°C. Therefore, we suggest that retort processing may lead to degradation of myofibrillar proteins as a result of exposure to extremely high temperatures. The inhibitory effect of the soluble protein fractions obtained from the model pork sausage on ACE activity was measured using an *in vitro* assay system. The retorted sausage was found to have a higher ACE inhibition activity than that heated at 75°C. Furthermore, alkaline phosphatase and collagen synthesis activities of osteoblast-like cell cultures were significantly higher in the retorted sausage than those in the sausage heated at 75°C. These results suggest that retort processing may contribute to an improvement in functionalities of meat products.**

Index Terms—meat product, muscle protein degradation, physiological functions of meat, retort processing

I. INTRODUCTION

Types of food that people desire has diversified in late recent years, and as a result the consumer demand for retort-processed foods, which are safe and convenient (Ramesh, 1995), has increased. Although the sterilization efficiency of retort processing is high, wet heating over 100°C causes deterioration and loss of food ingredients, and consequently leads to decrease in palatability. The optimal temperature for heat-induced gel formation of myofibrillar proteins, which serve as important determining factors for water-holding and binding capacities in a meat product, is around 70°C (Asghar, Samejima and Yasui, 1985). Therefore, high-temperature treatments such as retort sterilization are believed to impose severe conditions during meat processing. In recent years, we have performed various quality evaluations of processing methods, such as fermentation using lactic acid bacteria or retort sterilization with the aim of developing meat products with a greater added value. The microbiological safety, color, and physical properties of meat have been markedly improved due to lactic acid fermentation, and data have been obtained demonstrating improvements in functionality such as generation of peptides with inhibitory activity against angiotensin I-converting enzyme (ACE), which may counteract elevation of blood pressure, and anti-oxidant properties and so on (Haga, Kato and Kotsuka, 1994; Teramoto and Haga, 2001; Hayashi, Kato and Haga, 2008). On the other hand, retort heating leads to reduction in physical properties such as water-holding capacity and the breaking strength of the myofibrillar protein gel due to high temperatures used in the process; protein degradation may occur simultaneously (Fujita, Hayashi and Haga, 2006; Hayashi, Kato, Ogawa, Uno and Haga, 2005). Research on bioactive peptides originating from food proteins has been recently reported (Chen, Peng, Jiao, Wong, Yang and Huang, 2009; Arihara, 2006; Okamoto, Hanagata, Matsumoto, Kawamura and Koizumi, 1995). Therefore, it is reasonable to expect that functional low-molecular-weight peptides can be formed as a result of protein decomposition that occurs during retort heating. Although some reports have indicated the occurrence of syneresis and a flavor change in retort-heated meat (Einerson and Reineccius, 1977; Funami, Yada and Nakao, 1999), no reports have focused on protein decomposition and functionality of retort-processed meat.

In the present study, we evaluated food functionality of a retort-processed model pork sausage by analyzing its protein and peptide distributions, inhibitory activity against ACE, and growth-promoting activity in osteoblast-like cell cultures as the index of physiological activity.

II. MATERIALS AND METHODS

A. Sample preparation

Fresh commercial pork (ham) was purchased from a local wholesaler. Visible fat and connective tissue were

carefully removed, and the pork was cut into small cubes (approximately 3 cm in diameter). The meat was cured using the following agents: 2.0% sodium chloride, 0.02% sodium nitrite, 0.05% potassium nitrate, 0.3% sodium tripolyphosphate, 0.05% sodium ascorbate, and 2.0% glucose. Cured meat with 5% crushed ice was immediately cut for 60 s using a silent cutter and stored at 5°C for 3 days. The cured meat emulsion (100 ± 5 g for the model sausage) was stuffed into a polyvinylidene chloride casing (40 mm in diameter). Then, half of the resulting raw sausage was subjected to retorting at 120°C for 15 min, and the remaining half was cooked at 75°C for 30 min after reaching an internal temperature of 63°C. After cooking, the retorted and heated sausages were cooled under ice water for 30 min.

Both heated and retorted samples were defatted with diethyl ether, homogenized in a 6-fold volume of distilled water, and centrifuged (35,000 ×*g*, 15 min, 4°C). The resulting supernatants were used as water-soluble protein fractions. The protein concentration of each fraction was determined by the biuret method (at 550 nm).

B. Free amino acid and peptide contents

A fraction soluble in 2% trichloroacetic acid (TCA) was prepared by adding an equal volume of 4% TCA to the water-soluble protein fraction. The mixtures were incubated at 37°C for 30 min and filtered through a 5C filter paper. The resulting filtrate was analyzed for its amino acid and peptide contents. Free amino acid and peptide contents were determined by the ninhydrin method and that described by Mikami, Yamada, Wakahar, and Miura (1991), respectively.

C. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

The protein distribution of whole sausage homogenates was analyzed by SDS-PAGE on a gradient slab gel (7.5–20%) according to the method described by Laemmli (1970). The distribution of low-molecular-weight proteins was analyzed by tricine-SDS-PAGE (Schägger and von Jagow, 1987). The gels were stained with Coomassie Brilliant Blue R-250.

D. ACE inhibitory activity

Water-soluble protein fractions obtained from retorted and control (heated at 75°C) sausages were dialyzed in distilled water using a dialysis membrane (Spectra/Por, M.W. cutoff 500) and then lyophilized. The lyophilizates were dissolved in distilled water, filtered through a syringe-driven filter (0.22 μm), and used as samples for the ACE inhibitory activity assay. ACE inhbitory activity was measured according to the method described by Cushman and Cheung (1971). In this method, ACE inhibitory activity is quantified by the reaction of hippuryl-histidyl-leucine with ACE to form hippuric acid, in the presence or absence of the sample by measuring the absorbance at 228 nm. The decrease in absorbance is proportional to the inhibition of ACE caused by substances having inhibitory activity in the sample. The peptide concentration (mg/ml) required to inhibit 50% of ACE activity was defined as the IC_{50} value. The IC_{50} value was defined as 1 unit, and the number of units contained in 1 g of sausage was calculated as the total activity (units/g meat) (Ibe, Yoshida and Kumada, 2006).

E. Osteoblast cell growth-promoting activity

MC3T3-E1 cells were obtained from the RIKEN Cell Bank (RCB1126). MC3T3-E1 cells were cultured at 37°C in a 5% CO_2 atmosphere in α-modified minimal essential medium (α-MEM). Unless specified otherwise, the medium contained 10% fetal bovine serum (FBS), 100 U/ml penicillin, and 100 μg/ml streptomycin. Cell proliferation was evaluated by the WST-8 assay using a Cell Counting Kit-8 (Dojindo). The concentration of added water-soluble protein sample in the culture medium to be tested ranged from 0.004 to 0.5 mg/ml as final concentration.

Alkaline phosphatase (ALP) activity and collagen content were determined according to the method described by Choi (2005) with slight modifications. In short, after the cells were cultured at a density of 2.5×10^5 cells/ml in a culture dish for 3 days, the medium was replaced with FBS-free α-MEM, and the cells were cultured in this medium in the presence or absence of water-soluble protein fractions (0.5 mg/ml) for 3 days.

ALP activity: After incubation, the medium was removed and the cell monolayer was gently washed 3 times with PBS. The cells were recovered and lysed by sonication in a lysis solution (0.25 M sucrose), and the cell lysates were centrifuged at 2000 ×*g* for 3 min. The resulting supernatants were used for measurement of ALP activity and protein concentration. ALP activity and protein concentration were measured by an ALP Assay Kit (Wako) and a BCA Protein Assay Kit (Pierce), respectively. ALP activity was calculated according to manufacturer's protocol and expressed as nmol of p-nitrophenyl phosphate per min per μg cellular protein.

Collagen content: After 3 days of incubation in the presence or absence of the water-soluble protein fractions, the collagen content was measured using the Sircol Collagen Assay Kit (Biocolor Ltd.). This assay uses a quantitative dye-binding method designed for analysis of collagens extracted from mammalian cell cultures. The dye reagent specifically binds to the [Gly-X-Y]n helical structure of mammalian collagens. The collagen content was calculated according to manufacturer's protocol and data were expressed as mg of collagen per ml of culture supernatant.

III. RESULTS AND DISCUSSION

Changes in muscle protein distribution after retort heating were investigated by SDS-PAGE (Fig. 1). The SDS-PAGE pattern of whole homogenates obtained from the retorted sausage showed that the band corresponding to the myosin heavy chain disappeared and the intensities of the actin and α-actinin bands decreased after retort processing

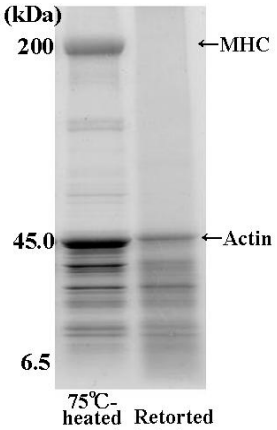

Fig. 1. SDS-PAGE pattern of whole homogenates prepared from 75℃-heated and retorted model pork sausage.
MHC; myosin heavy chain

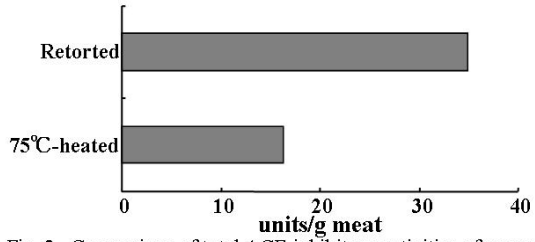

Fig. 2. Comparison of total ACE inhibitory activities of sausage heated at 75℃ and retorted sausage.
One unit of ACE inhibitory activity is defined as the amount of sample needed for 50% inhibition of ACE.

compared with those obtained using the homogenate of the sausage heated at 75°C. On the other hand, low-molecular-weight bands (<10 kDa) obtained from the retorted sausage homogenate were stained imprecisely but strongly than those obtained from the control sausage homogenate. Furthermore, the distribution of low-molecular-weight peptides was analyzed by tricine-SDS-PAGE. The results showed that the band intensity of low-molecular-weight peptides (<6 kDa) obtained from the retorted sausage was greater than that of those obtained from the sausage heated at 75°C (data not shown). The free amino acid and peptide contents in the water-soluble protein fractions of the retorted sausage were significantly higher than those of the control sausage ($p < 0.01$); the difference was particularly large for the peptide content (data not shown). Tajima, Ito, Arakawa, and Parrish Jr. (2001) reported that myosin was degraded into fragments when bovine meat was simmered at a sub-boiling temperature (95°C). The data from the present study suggest that retort processing leads to degradation of myofibrillar proteins as a result of exposure to extremely high temperatures.

Many biologically active peptides have been recently discovered from various food proteins including those of animal muscle origin (Chen et al., 2009; Arihara, 2006). Therefore, we examined whether retort processing alters the biological activity of meat in addition to protein degradation. The inhibitory effect on ACE activity in soluble protein fractions obtained from the model sausage was measured by an *in vitro* assay. ACE inhibitory activity increased when the water-soluble protein fractions from both types of sausages were added in a concentration-dependent manner, and the IC_{50} values of the retorted sausage were lower than those of the sausage heated at 75°C (data not shown). The retorted sausage showed higher ACE inhibitory activity than the control sausage (Fig. 2). Therefore, we conclude that more peptides exhibiting ACE inhibitory activity may be formed in pork sausages when subjected to retort heating.

Next, the effect of water-soluble protein fractions on the growth of a culture of osteoblast-like cells (MC3T3-E1) was examined. The proliferation of these cells was slightly suppressed when the fraction obtained from the retorted sausage (0.5 mg/ml protein) was added compared with when the fraction obtained from the sausage heated at 75°C (0.5 mg/ml protein) was added (data not shown). The effects of retorted sausage (0.5 mg/ml protein) on ALP activity and collagen synthesis in MC3T3-E1 cells are shown in Fig. 3-A and -B, respectively. ALP activity and collagen synthesis are both widely recognized as biochemical markers of osteoblastic activity. Cells cultured in the presence of the water-soluble protein fraction from the retorted sausage (0.5 mg/ml protein) exhibited significantly higher ALP activity than the control cells cultured with the vehicle control (PBS). This activity was comparable to that of β-cryptoxanthin (10^{-7} M) reported previously (Uchiyama and Yamaguchi, 2005). However, water-soluble protein fraction from the sausage heated at 75°C had no effect on ALP activity. The collagen content was significantly increased by stimulation with water-soluble protein fractions from both types of model sausages; the value for the retorted sausage was significantly higher than that for the sausage heated at 75°C. Thus, the effect of pork sausage on osteoblast differentiation was significantly promoted by retort processing. These results indicate that retort processing may contribute to an improvement in functionalities of meat products rather than compromising their functionalities.

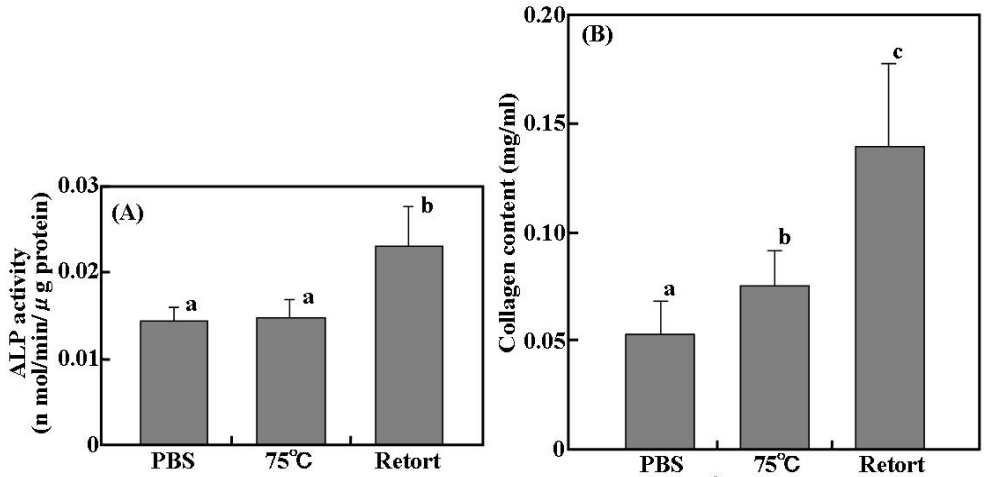

Fig. 3. Effect of water-soluble protein fractions from sausage heated at 75℃ and retorted sausage on ALP activity (A) and collagen synthesis (B) in MC3T3-E1 cells.
PBS was used as a vehicle control.
Values with different superscript letters are significantly different ($p < 0.05$).

IV. CONCLUSION

The results of the present study indicate that retort-processed pork sausages may contain more functional peptides with osteoblastic and anti-hypertensive activities compared to cooked sausages. Therefore, retort processing may be a useful heat treatment not only for the preservation of food safety but also for improvement of food functionality in meat products.

ACKNOWLEDGMENTS

This study was supported in part by a research grant for Meat and Meat Products (The Ito Foundation) and by a grant of the Strategic Research Foundation Grant-aided Project for Private Universities from Ministry of Education, Culture, Sport, Science, and Technology, Japan (S0801056).

REFERENCES

Asghar, A., Samejima, K. and Yasui, T. (1985) Functionality of muscle proteins in gelation mechanisms of structured meat products. *CRC Critical Reviews in Food Science and Nutrition*, 22, 27-106.

Arihara, K. (2006) Strategies for designing novel functional meat products. *Meat Science*, 74, 219-229.

Chen, Z.-Y., Peng, C., Jiao, R., Wong, Y. M., Yang, N. and Huang, Y. (2009) Anti-hypertensive nutraceuticals and functional foods. *Journal of Agricultural and Food Chemistry*, 57 (11), 4485-4499.

Choi, E.-M. (2005) The licorice root derived isoflavan glabridin increases the function of osteoblastic MC3T3-E1 cells. *Biochemical Pharmacology*, 70, 363-368.

Cushman, D.W. and Cheung, H.S. (1971) Spectrophotometric assay and properties of the angiotensin-converting enzyme of rabbit lung. *Biochemical Pharmacology*, 20, 1637-1648.

Einerson, M.A. and Reineccius, G.A. (1977) Inhibition of warmed-over flavor in retorted turkey by antioxidants formed during processing. *Journal of Food Processing and Preservation*, 1 (4), 279-291.

Fujita, T., Hayashi, T. and Haga, S. (2006) Research on changes of physical properties of the actomyosin gel by retorting. *Nippon Shokuhin Kagaku Kogaku Kaishi*, 53(8), 423-429 (in Japanese).

Funami, T., Yada, H. and Nakao, Y. (1999) Effect of curdlan on rerort stability of a pork meat gel system. *Food Science and Technology Research*, 5 (1), 24-29.

Haga, S., Kato, T. and Kotsuka, K. (1994) Effects of curing and fermenting time on the quality of hams fermented with psychrotrophic lactic acid bacteria. *Nippon Shokuhin Kogyo Gakkaishi*, 41(11), 797-802 (in Japanese).

Hayashi, T., Kato, K. and Haga, S. (2008) Angiotensin I-converting enzyme inhibitory peptide derived from porcine skeletal muscle myosin fermented by *Lactococcus lactis* subsp. *lactis* IFO-12007. *Journal of Research Institute of Meijo University*, 7, 71-80.

Hayashi, T., Kato, K., Ogawa, Y., Uno, K. and Haga, S. (2005) Sutudies on evaluation of food functionality of retort processed meat product. "Final Reports for Research Grants for Meat and Meat Products (The Ito Foundation)", 23, 233-237 (in Japanese).

Ibe, S., Yoshida, K. and Kumada, K. (2006) Angiotensin I-converting enzyme inhibitory activity of natto, a traditional Japanese fermented food. *Nippon Shokuhin Kagaku Kogaku Kaishi*, 53 (3), 189-192 (in Japanese).

Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, 227, 680-685.

Mikami, M., Yamada, Y., Wakahara, Y. and Miura, H. (1991) Effects of electrical stimulation on the sarcoplasmic proteins, peptide and amino acid contents of beef. *Animal Science Technology* (Japan), 62 (6), 519-528.

Okamoto, A., Hanagata, H., Matsumoto, E., Kawamura, Y. and Koizumi, Y. (1995) Angiotensin I converting enzyme inhibitory activities of various fermented foods. *Bioscience*, *Biotechnology*, *and Biochemistry*, 59 (6), 1147-1149.

Ramesh, M.N. (1995) Optimum sterilization of foods by thermal processing - a review. *Food Science and Technology Today*, 9 (4), 217-227.

Schägger, H. and von Jagow, G. (1987) Tricine-sodium dodecyl sulfate-polyacrylamide gel electrophoresis for the separation of proteins in the range from 1 to 100 kDa. *Analytical Biochemistry*, 166, 368-379.

Tajima, M., Ito, T., Arakawa, N. and Parrish Jr., F.C. (2001) Heat-induced changes of myosin and sarcoplasmic proteins in beef during simmering. *Journal of Food Science*, 66 (2), 233-237.

Teramoto, K. and Haga, S. (2001) Effect of lactic acid fermentation on formation of myoglobin derivatives. *Scientific Reports of the Faculty of Agriculture*, *Meijo University*, 37, 129-134.

Uchiyama, S. and Yamaguchi, M. (2005) β-Cryptoxanthin stimulates cell differentiation and mineralization in osteoblastic MC3T3-E1 cells. *Journal of Cellular Biochemistry*, 95 (6), 1224-1234.