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THE IMPROVEMENT OF GEL STRENGTH OF SAUSAGES PREPARED FROM THREE TYPES OF MEAT WITH MICROBIAL TRANSGLUTAMINASE

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Abstract

An attempt to improve the texture of chicken, beef, and pork sausages by using the microbial transglutaminase (MTG) has been investigated. In regards to the proteins, actin and myosin are the uppermost capable myofibril proteins for meat gelation, and pursuant to the affinity of MTG to these proteins; that promotes the improvement of the texture of the meat. Sausages were treated for breaking strength at two different temperatures, 40°C and 80°C**,** for 30 min. From the data we saw that the breaking score increased by adding MTG. That data gave the concept that the type of meat that reacted best was beef. The sausages were incubated at 40°C, so that shows the content of ε-(γ-glutamyl) lysine content (G-L) of chicken, beef, and pork after adding MTG has been significantly increased and the values were mostly the same in pork as well as beef. On the other hands, the G-L content of chicken was lower than beef and pork. To shore up the tasks of MTG, the characteristics of protein solubility of chicken, beef, and pork were investigated using Guba-Straub ATP solution. The extractability of myosin heavy chain (MHC) was significantly decreased in beef and pork, that leads to the high interaction of MHC with MTG; there was a good reaction binding ability between MTG and myofibrillar proteins, especially, MHC. Lastly, Cross-linking of proteins by using MTG may be useful in texturization in beef and pork better than chicken.

Background: Eating healthy food is an important initial process to the maintenance of life itself. Recently, many institutions have been involved in achieving a lot of new food with high quality and nutritional value to satisfy the appetite of people; especially in the 21st century, the sense of people has arisen. The factories producing foods came to rely somewhat on the MTG productions, for good reason-the cost to produce MTG itself is not expensive compared to the original TG, and it has proven its competence as a food texture additive [1)].

Objectives:

In our quest for completeness we have attempted to provide information on sausages of chicken, beef, and pork. The aim of our research was focused on investigating the differences and abilities of sausage texture improvement by using the MTG. This study

has involved determining G-L content and evaluating the reactability of MHC as well as the extractability of the myofibillar proteins.

Methods and materials:

Meat and sausage preparations: Chicken, beef, and pork meat was purchased from local markets in Japan; the meat, before using it, was kept in the refrigerator after slaughtering. The skeletal muscle blocks were minced in a meat grinder; also the fat was degreased and the temperature was maintained at 4°C. The sausages were prepared by adding distilled water, 1M imidazole-HCl (pH6.0), NaCl, and sodium pyrophosphate, and then MTG [2]. The MTG in meat was 0.005%. All the additives and the minced meat were homogenized and the paste was put in a cloth rain rest (funnel-shaped bag) and ended up in a clear plastic casing. The paste was stuffed into casing until the sausage was a length of 12-14 cm, and the ends of the casing were fastened well with string. The sausage was divided into two groups. Each group contained -MTG and +MTG. The first group was incubated at 40°C for 30 min; and the second group was incubated at 80°C for 30 min.

Protein extraction: Since we were expecting that the muscle proteins became a G-L complex by MTG reaction; that gives us a concept to extract the proteins by low ionic strength solution [A-solution: 50mM Imidazole-HCl (pH6.0) 2mM EDTA], as well as high ionic strength solution [Guba-Straub-ATP solution: 0.09M KH_2PO_4, 0.06M K_2HPO_4, 0.3M KCl, 1mM ATP]. The purpose of adding ATP was to increase the MHC extractability, in general cases ATP in the postmortem state reduces. It might be that ATP broke the bonds between MHC and actin, especially the tight bond. Consequently, 28 ml of both solutions were respectively added to 2g of the sausage which had been divided into two groups as mentioned previously. Thereafter, the melange was and then centrifuged at 15,000rpm for 20min. Eventually the supernatant was taken out and filtrated with a filter paper; thus the final solution was used as an extracted protein solution. The protein concentration was determined by the biuret method [3].

Analysis of SDS-polyacrylamide gel electrophoresis (SDS-PAGE): SDS-PAGE was carried out on gradient slab gel (7.5-17.5% acrylamide) with 2-mercaptoetanol at 20 mA/gel employing the discontinuous buffer system of Laemmli [4].

Assay of ε-(γ-glutamyl) lysine content: Determination of G-L content proteolytic digestion was carried out by the method of Kumazawa et al. [5]. Briefly, 100g of sausage was homogenized and then kept in a freeze-dryer for drying. That was followed by dissolving it in a buffer (0.1M borate pH8.0). Pronase (0.2 unite/mg proteins) was mixed with the melange that was incubated for 24 hr. In this step the leucine aminopeptidase (0.4 units) and prolidase (0.45 units) were added after inactivating the pronase by boiling it for 10 min. After incubation for 24 hr, leucine aminopeptidase (0.4 units) was added with further incubation for 24 hr. The mixture was finalized by adding carboxypeptidase's A (0.4 units), and then the mixture was incubated another 24 hr. All this was performed at 37°C. Before lyophilized and dried digests were filled up to a volume of 7.5ml with distilled water, digested samples were heated at 100°C for 10 min. A part of the samples were subjected to reverse-phrase HPLC, sent after collecting the fractions which contained G-L. Then the G-L fraction was evaporated to enhance the concentration. Afterward, we mixed in *o*-phthalaldehyde (OPA); lastly, the rest of the samples were subjected to reverse-phrase HPLC**.**

Results and Discussions

The chemical and physical properties of muscle tissue and the interpenetrated connective tissue are of uppermost importance in the usefulness of meat as a food. The following discussion is intended only to point out the differences of improvement in sausage and how some factors can facilitate the methods to improve meat textures. Changes in breaking strength and deformation of meat gels (Fig.1) shows the breaking strength of sausages were treated at two different temperatures, 40°C and 80°C for 30 min; the breaking score increased by adding MTG as well as increasing the temperature. The sausage was subjected to the puncture test, which was measured by a knife of creep meter (Rheoner II, Yamaden Co. Ltd., Tokyo) at room temperature. The values presented in Fig.1 give the concept that the type of meat that reacted best was beef, and the deformation increased for beef from 39% in the case of -MTG to 51% in the case of +MTG. For chicken it was slightly decreased in the case of -MTG by 34% (data not shown). So from the categorized data we illustrated that the temperature was affected significantly on the breaking strength score, even though pork reacted formidably at 40°C; but we must consider the sausages that were treated at 80°C. A particularly interesting approach was proposed by the cooking temperature to prepare well-cooked food which is around 80°C. That was the reason for concern about this temperature itself. Therefore, this leads to the fact that the best meat type that can be improved as far as texture is concerned, is beef.

When we compared the extractability of the proteins in two different solutions, A-solution and GS-ATP solution, a difference was realized, and the changes of the extractions were significantly increased between the solutions. Values which are provided in Fig. 2 clarify that the protein concentration of the GS-ATP solution was higher than the protein concentration of A-solution. It was clear that chicken in the A-solution was less than beef and pork, but in the GS-ATP case was higher than the others. That might lead to considerations of the mechanisms and the high affinity reaction between MTG and MHC of beef and pork. So probably ATP played an important rule in the extractability, which might release some proteins and make some cleft in all the structures.

SDS-PAGE analysis of sausage's proteins extracted in A-solution and GS-ATP solution and their reaction with the MTG was shown in Fig. 3.The analysis of extracted protein solutions illustrated the density of MHC; the band reduced significantly, which clearly showed what the differences were between truancy and presence of MTG itself. The pattern shows the values which were obtained from sausage treated at 40°C for 30 min. This suggested that the binding ability of myofibrillar proteins with MTG might be formed and fabricated predominantly by MHC. It is known that MHC is the most important capable protein for meat gelation, and even myosin constitutes approximately 50-55% percent of the myofibrillar protein, and is characterized by a high proportion of basic and acidic amino acid, making it a highly charged molecule (Fig. 3). As shown in the lanes of chicken the MTG did not react well with MHC. At the same time the reaction in beef and pork significantly increased by adding MTG and that is seen clearly from the disappearance of the label in the MHC. It must however be recognized that there are other influential proteins; similarly they can react as the influencing factors of the cross liking battlefield such as actin.

G-L content (Fig. 4) contributed a great value of intelligibility of the reactions in the presence of MTG itself. This leads us to realize how different the reaction was between these types of meat sausages with the MTG. The sausages were incubated at 40°C, so the content of G-L of sausages after adding MTG was significantly increased and the values were mostly the same in pork as well as beef. The glutamine residues are the acyl donor and the MTG catalyzed an acyl transfer reaction in the carboxyamide group of proteins, and the difference refers to the glutamine residues reacting at different rates depending on their location in the protein and obviously resultants from the shape of the muscle.

Conclusion

A few concepts will be discussed. To sum up all the values which were provided in our data. All the figures gave very clear differences between the three types of meat, although the meat proteins are same of the compositions. For instance, MTG was a good additive for beef and pork to improve their texture as well as gelation, and that was different to chicken. Cross-linking of proteins by using MTG may be useful in texturization and in modification of solubility and emulsifying properties in beef and pork better than chicken. Finally, we recommend improving upon our methods in experiments to get more clarification in the future.

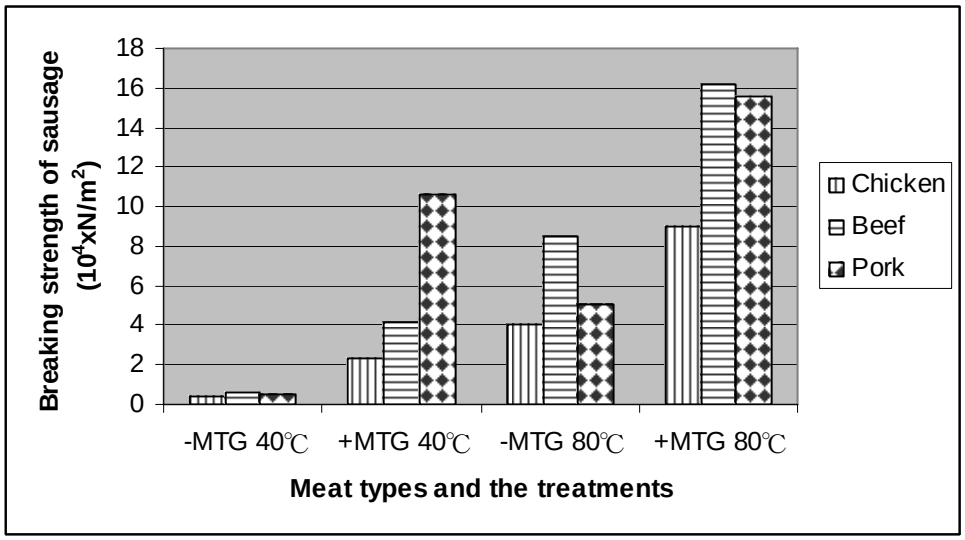

Fig. 1 Changes in breaking strength and deformation of chicken, beef, and pork sausage as the functions of MTG and temperature. The sausages were set at 40°C and 80°C for 30 min.

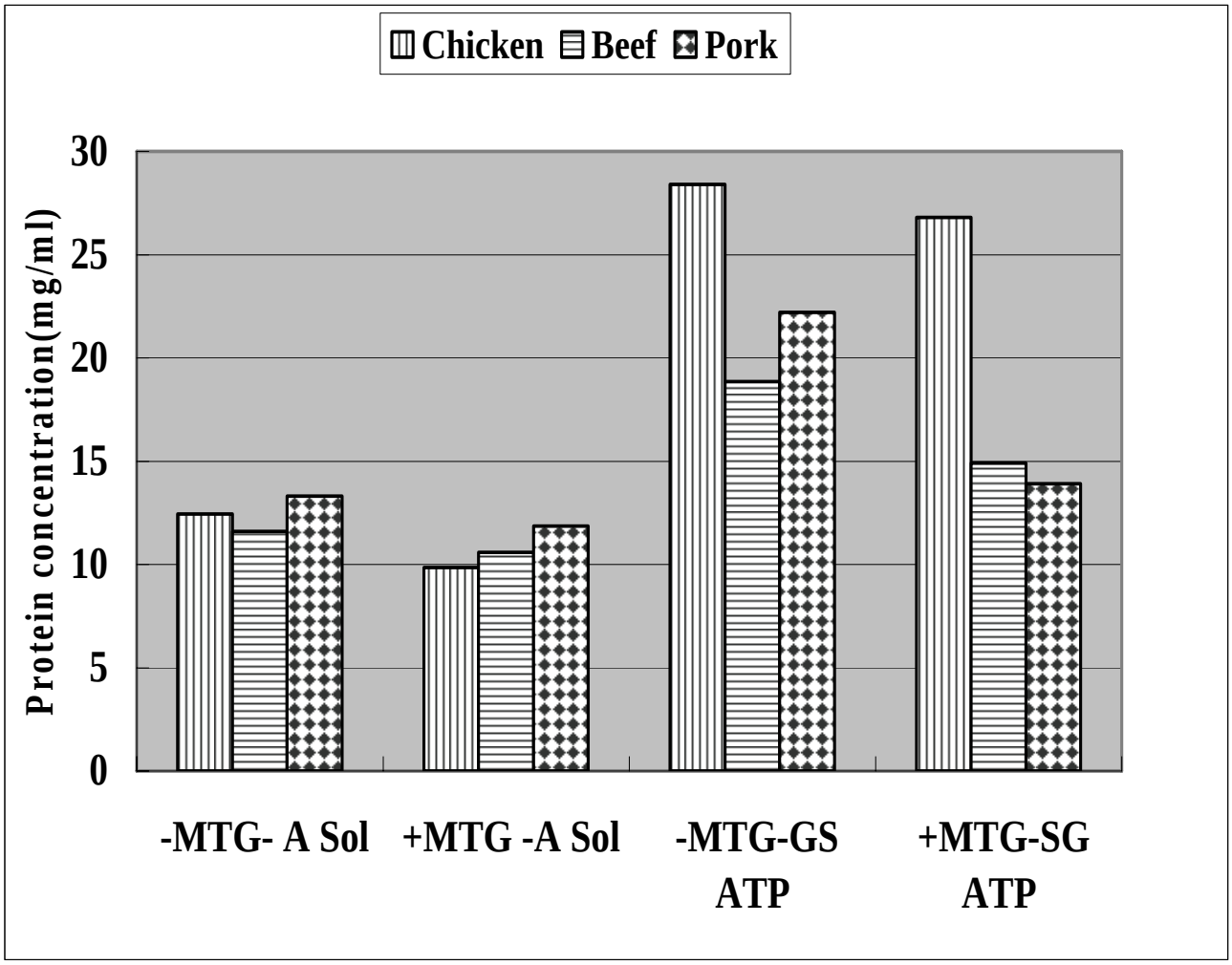

Fig. 2 Extracted protein concentration of chicken, beef, and pork in A-solution (50mM Imidazole-HCl (1M pH6.0), 2mM EDTA and GS-ATP solution (0.09M KH_2PO_4, 0.06M K_2HPO_4, 0.3M KCL, 1mM ATP). The sausages were set at 40°C for 30 min.

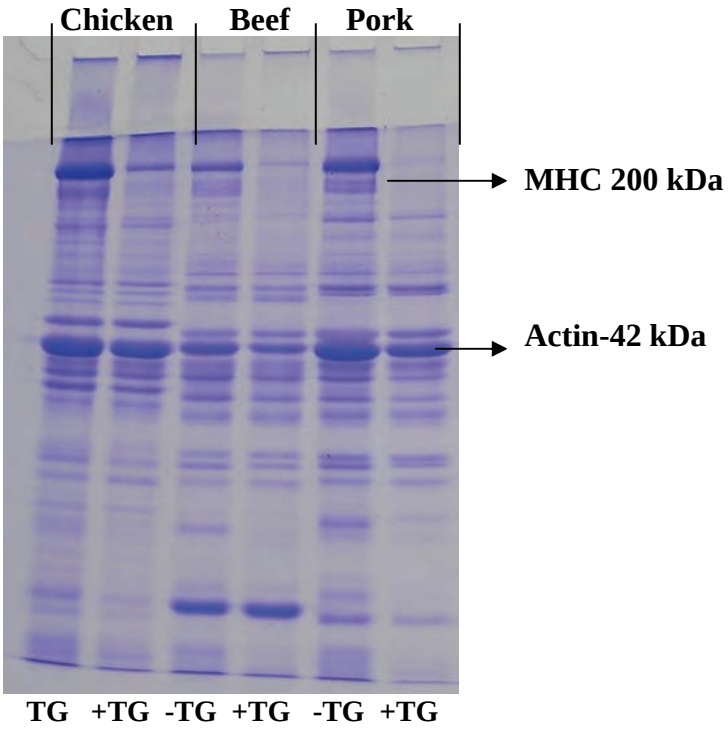

Fig. 3 Changes in SDS-page pattern of chicken, beef, and pork sausage proteins. Illustratation of the influence of MTG on MHC of the proteins which were extracted from sausage samples which were incubated at 40°C for 30 min, and dissolved in GS-ATP solution.

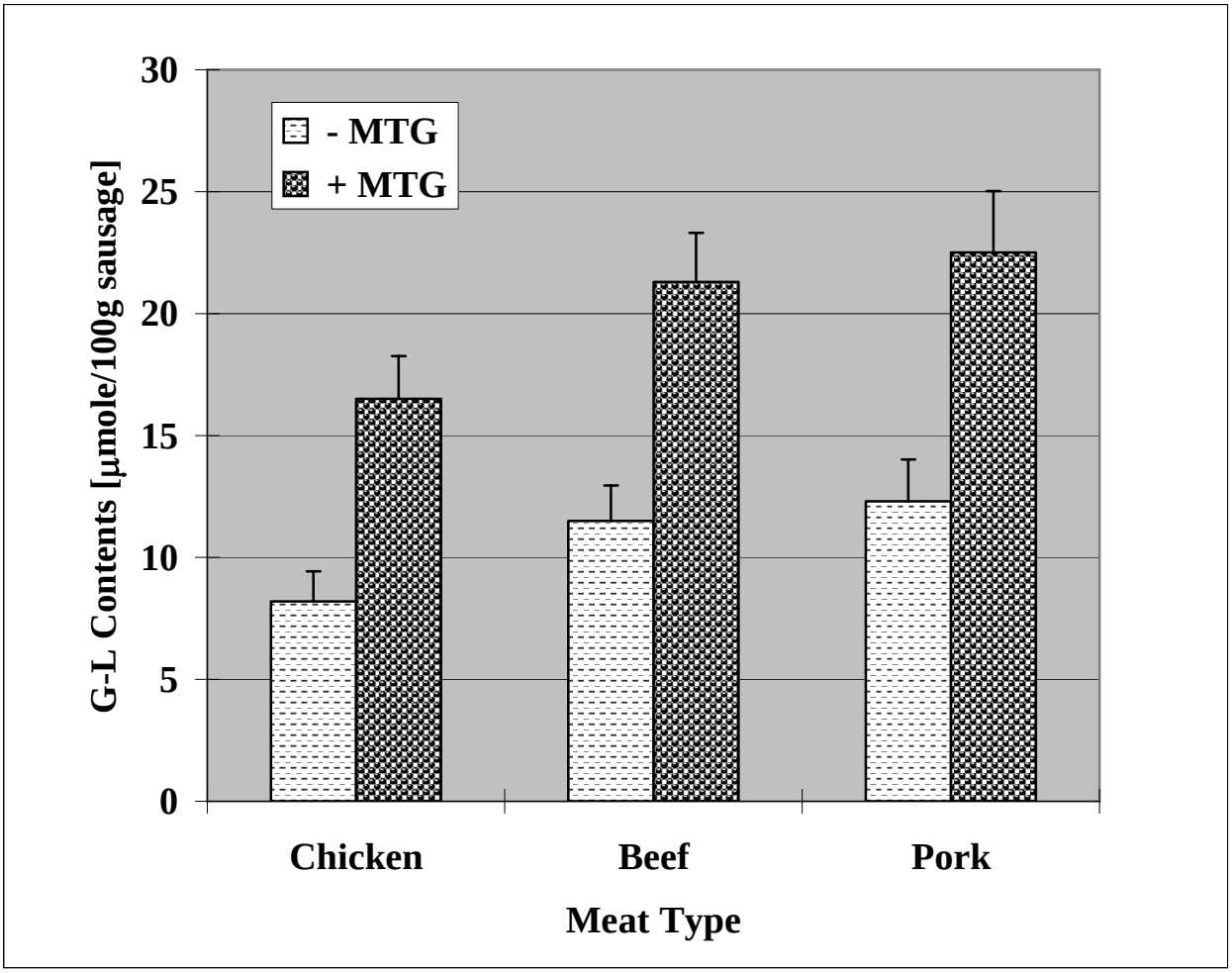

Fig. 4 Changes in ε-(γ-glutamyl) lysine content (µmole/100gm of sample). The sausage samples were treated at 40°C for 30 min.

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