PHYSICOCHEMICAL CHANGES IN MUSCLE PROTEINS OF CURED BEEF PRODUCTS MADE FROM *M. CUTANEOUS-OMO BRACHIALIS* MUSCLE: PART II

A. Ahhmed[1,2,3], G. Kaneko[1], H. Ushio[1], S. Kawahara[4], M. Muguruma[4], R. Sakata[2] and S. Watabe[1]

[1]Department of Aquatic Bioscience, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo 113-8657, Japan; [2]Department of Animal Science and Biotechnology, School of Veterinary Medicine, Azabu University, Sagamihara 252-5201, Japan; [3]Food Technology Department, Gheran Higher Centre for Agricultural Technologies, Tripoli P.O. Box 151, Libya ; [4]Department of and Biochemistry and Applied Biosciences, Interdisciplinary Graduate School of Agriculture and Engineering, University of Miyazaki, Miyazaki 889-2192, Japan

Abstract - We examined changes in the physicochemical parameters of muscle proteins as a result of beef processing to produce Pastırma by traditional method. Pastirma is a traditional Turkish dry-cured beef product. Protein extractability and concentrations were significantly increased in processed muscles, as a result of curing and drying. The surface hydrphobicity of processed samples were higher than those of the control samples at all Gu-HCl concentrations. The hydrophobicity also increased as new compounds were created during the Pastirma-making process. The metmyoglobin content was greatly increased in Pastirma samples compared to the unprocessed samples. The results of this study demonstrated that meat processing promoted the enzymatic digestion of some proteins, and the differences in composition between the control and Pastirma samples were thus likely to be attributable to protein degradation.

Key Words - Pastirma, Turkish meat product, Protein degradation

I. INTRODUCTION

Pastirma is a popular dry-cured beef product made from whole muscle (Gok, *et al*.,)[1]. It is prepared by the preservation of beef muscle by salting and drying, with the most famous product coming from the province of Kayseri in central Turkey. The different large muscles are considered to produce about 20-25 different types of Pastirma, depending on the cut. The beef cuts then undergo a series of processes and treatments lasting about a month. The first stage of Pastırma processing is dry curing, during which the meat strips are rubbed and covered with a curing mixture (salt). The salting and drying procedure affects the structure of the proteins and the enzyme mechanisms, potentially increasing the nutritional and organoleptic values of Pastirma. The muscles undergo physicochemical changes during the course of the traditional dry-curing process. These changes occur especially during the ripening period, contributing to the improvement of some organoleptic properties. However, little information is available regarding the chemical changes in proteins during the traditional Pastırma-making process, including its effects on protein degradation and the generation of new bioactive peptides. Changes in myoglobin formation in relation to the metmyoglobin genation process need to be verified. This study therefore examined the physicochemical properties of Pastirma made from the *M. cutaneous-omo brachialis* (COM) muscle of cattle, in order to identify the potential degradation of proteins as a result of the Pastirma-making process.

II. MATERIALS AND METHODS

Meat cuts and Pastırma-manufacturing process: The Pastırma was produced in a factory in Kayseri city using the traditional process. Shoulder rose muscles were sourced from male cows at 30 months old. Fresh and processed meat samples were sourced from the same animals, and the fresh samples (control samples) were kept at -30°C until the experiment. Meat cuts were processed by removing most of the subcutaneous fat layers then the muscles placed on a curing mixture (salt 1,000g + 15g nitrate/kg

of meat) at room temperature. Muscles were salted on one side, stacked, left for about 24 h, then salted on the other side, stacked and left for a further 24 h. After salting, the samples were washed thoroughly using fresh water and the cuts were then placed in a plastic net and dried in the open air for a period of 6-12 days. Cuts were further processed by hanging to dry in the shade for 4-6 days at 15-20°C. The cured and dried cuts were put in a bowl of seasoning mixture containing 12% milled fenugreek seeds, 20% crushed garlic, 13% red pepper and 55% H_2O, and left to cure for 12-24 h in hot weather, and an extra 1–2 days in cooler weather.

Protein extraction: Proteins were extracted from the fresh and pastirma samples by separately adding 28ml of three different solutions (distilled water: H_2O P-ex), (WSP) and (GS-ATP) to 2g of meat cuts. The protein concentration of the extracted solution was determined using the biuret method.

pH values: The pH hydrogen ion concentration was measured in aqueous solutions of extracted proteins and meat cuts.

Fluorescence intensity: Surface hydrophobicity of the proteins extracted from the fresh and Pastirma samples was determined by ANS method (Ahhmed, *et al*.,) [2].

SDS–PAGE: The proteins were separated according to their size after extracting them in different ionic-strength solutions [2]. It was carried out on two different gradient slab gels {7.5% and 7.5–17.5% acrylamide} with 2-mercaptoethanol at 20 mA/gel.

Metmyoglobin %: Metmyoglobin concentration in sample was evaluated using a modification of procedures by Krzywicki [3].

Histological studies: Histological imaging was carried out essentially as described earlier [2]. Statistical analyses were conducted by SAS software using Duncan multiple comparison method.

III. RESULTS AND DISCUSSION

Protein extractability and concentration: Samples from processed muscles showed significantly increased concentrations of extracted proteins, especially of WSP, as a result of the salting process (data not shown). The salting and curing process was likely to have an important effect on the extractability of muscle proteins such as MHC and WSPs, possibly as a result of releasing some proteins from each other and cleaving the structures between certain proteins. Proteins extracted in GS-ATP solution increased as a result of the degradation of large proteins, and ATP was likely to play a crucial role by cleaving the strong bonds between myosin and actin. Surprisingly, more proteins were extracted from Pastirma in H_2O compared to fresh cuts, probably because of certain enzymes activity in the course of processing. In general, the Pastirma-making process increased the amounts of proteins extracted by all three solutions, implying that the salt-curing process had a beneficial effect.

Measurements of pH: In general, the Pastirma-making process had no negative effect on the pH values, with only a slight increase among all samples compared to fresh samples. The increase might have been associated with the degradation of some proteins and enzymes (table 1). The pH values of raw meat and Pastirma, measured by inserting a pH meter probe directly into the meat cuts. Surprisingly, the measurements were similar, indicating that the acidity of the Pastirma was the same as that of the original fresh-cut meat (5.48 and 5.7, respectively).

Table 1. pH values of protein extractions in fresh meat cuts and Pastirma made from COM muscle.

Muscles	Sample type					
	GS-ATP		WSP		H2O P-ex	
	mean	sdv	mean	sdv	mean	sdv
Fresh	6.27	0.04	5.65	0.07	5.78	0.01
Pastirma	6.40	0.04	5.78	0.03	5.88	0.01

Fluorescence intensity: Fluorescence intensity measurements indicated that SH and the proportion of hydrophobic amino acids relative to all amino acids in the proteins extracted from fresh muscle increased with increasing guanidine hydrochloride (Gu-HCl) concentration (Fig. 1). In general, the SH of the control-sample H_2O P-ex (protein extracted in water) was higher than those of proteins extracted in GS-ATP and WSP. In relation to the changes of physicochemical properties, we suggest the

increase in disassociated proteins was associated with an increase in protein hydrophobicity.
The fluorescence intensities of the processed samples were higher than those of the control samples at all Gu-HCl concentrations (Fig. 2).

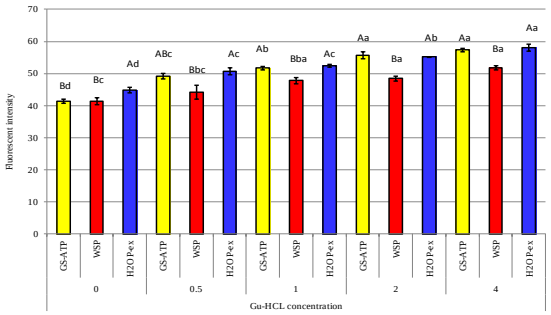

Figure 1. Effect of Gu-HCl on the fluorescence intensity of native proteins (GS-ATP extracts) extracted from fresh COM muscle of beef. Different capital and small letters in each concentration level and buffer type, respectively show the statistical differences at p< 0.01.

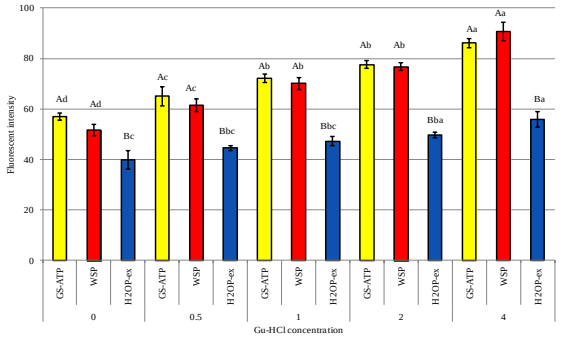

Figure 2. Effect of Gu-HCl on the fluorescence intensity of native and degraded proteins (GS-ATP extracts) extracted from Pastirma cut made from COM muscle. Different capital and small letters in each concentration level and buffer type, respectively show the statistical differences at p< 0.01.

An increase in the SH of the treated samples indicates an increase in the polarity of the amino acids. The creation of new molecules by the traditional Pastirma making process is associated with an increase in SH and changes in the physical properties. This suggests that changes in the protein composition in the meat cuts resulted in increased protein content and alterations in the surface hydrophobicity.
Metmyoglobin: The results of the current study indicated that the metmyoglobin percentage in Pastirma samples was increased by as much as 51% compared to control, fresh samples (Fig. 3). The traditional Pastirma-making process lasts about a month, which is long enough to allow most of the myoglobin to be converted to oxymyoglobin and then to metmyoglobin. However, changes in myoglobin in meat cuts are one of the fastest chemical reactions occurring during food processing. The mechanism is complex, as myoglobin is converted to oxymyoglobin, which is subsequently converted to metmyoglobin within a few hours.

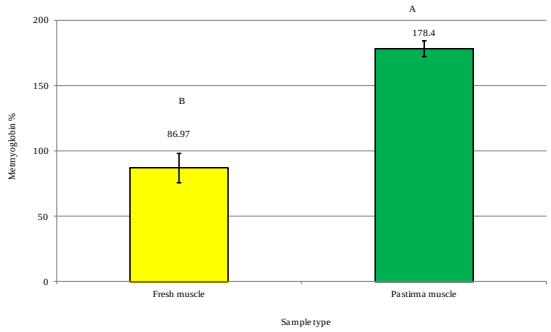

Figure 3. Metmyoglobine percentage in fresh meat and Pastirma products, both samples originated from *M. cutaneous-omo brachialis* muscle of beef. Different capital letters show the statistical differences at p< 0.01.

Electrophoresis: In the GS-ATP extract of fresh meat cuts, the myosin heavy chain (MHC) and β-galactosidase bands were clear (Fig. 4-A). There are similarly to be several bands of different molecular weights. However, the MHC and β-galactosidase bands were absent in pastirma samples, indicating degradation of the muscle proteins during the Pastirma-making process. Other bands representing phosphorylase-B (97kDa) bovine albumin (66kDa), and carbonic anhydrase (29kDa), were present in the GS-ATP, WSP, and distilled-water extracts of fresh meat, but absent from the Pastirma samples, probably as a result of degradation into smaller bioactive peptides and possibly functional nutreaceuticals (black dotty box) (Fig. 4-B). In general, the SDS-PAGE pattern indicated that most muscle proteins were metabolized to new, smaller molecules such as amino acids and peptides. Furthermore, separation of the same samples on different gradients (7.5–17.5%) showed further

degradation of low-molecular-weight proteins (Fig. 5-A and 5-B).

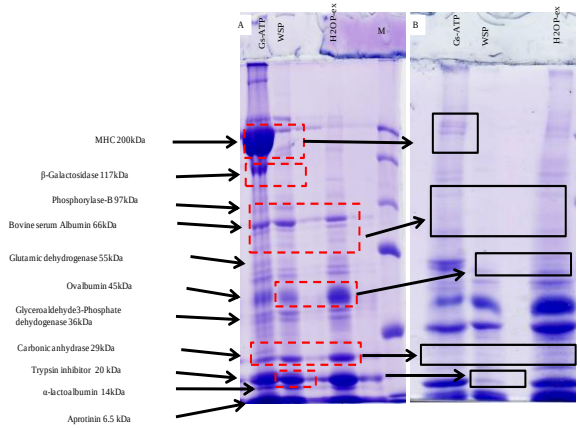

Figure 4. SDS–PAGE pattern shows muscle proteins bands of samples extracted in GS-ATP solution. (A: fresh; B: Pastirma. Gradient slab gels (7.5-17.5%).

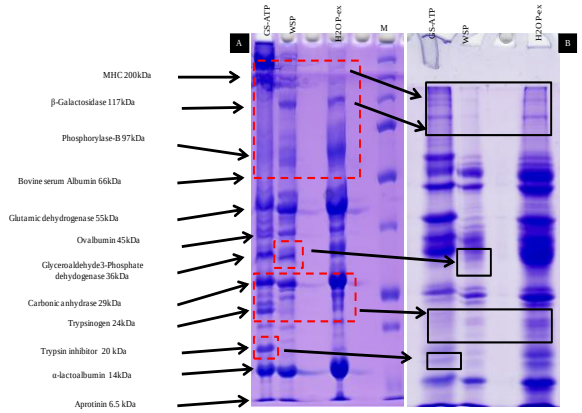

Figure 5. SDS–PAGE pattern shows muscle proteins bands of samples extracted in GS-ATP solution. (A: fresh; B: Pastirma. Gradient slab gels (7.5-17.5%).

Some major small proteins have been changed: the WSP 36kDa glyceroaldehyde3-Phosphate dehydogenase band disappeared in the Pastirma samples, as did carbonic anhydrase (29kDa), trypsinogen (24kDa), and trypsin inhibitor (20kDa) in the three extracts, all of which were apparently degraded into smaller peptides. These results thus demonstrate that certain proteins were degraded by enzymes activated during or after the processing (black dotty boxes) (Fig.5-B).

Histological: The histological analyses indicate that structures of the muscle protein in the Pastirma samples were slightly changed; the intracellular and tubular gaps were smaller than in fresh muscles. Figure 6-B shows that the muscle protein filaments were attracted to each other and were closer than in fresh samples. The filaments and myofibrillar proteins are magnetized due to the hydration process. However, the Pastirma-making process had no negative impact on the SEM structure.

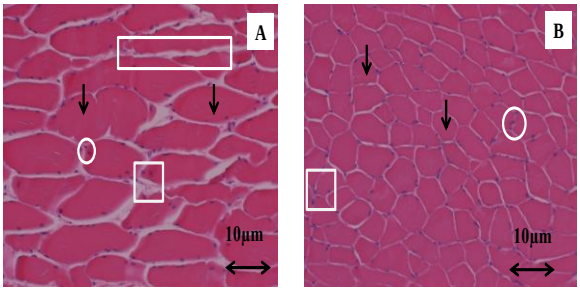

Figure 6. Histological analyses images show the structure of *M. cutaneous-omo brachialis* muscle. A: fresh and B; Pastirma. Box: arteries; circles: nucleus; arrows: fibers or filaments; rectangle: endomysin.

IV. CONCLUSION

The traditional Pastirma-making process facilitates the degradation of some muscle proteins into smaller compounds, such as peptides. These results suggest that pastirma may contain newly-generated peptides that can serve as nutraceuticals suitable for reducing some life style-related diseases. Also the salting and curing process had no negative impact on the texture and firmness of the cured-meat product made from *M. cutaneous-omo brachialis* muscle of cattle.

REFERENCES

1. Gok, V., Obuz E., & Akkaya, L. (2008). Effects of packaging method and storage time on the chemical, microbiological, and sensory properties of Turkish pastirma - A dry cured beef product. Meat Science 80: 335-344.
2. Ahhmed, M., A., Nasu, T., Mguruma, M. (2009). Impact of transglutaminase on the textural, physicochemical, and structural properties of chicken skeletal, smooth, and cardiac muscles. Meat Science 83: 759-767.
3. Krzywicki, K. (1979). Assessment of relative content of myoglobin, oxymyoglobin and metmyoglobin at the surface of beef. Meat Science 3:1-10.