INFLUENCES OF THE TRADITIONAL CURED MEAT-MAKING PROCESS ON THE MUSCLE PROTEINS IN *M. LATISSIMUS DORSI* OF BOVINE: PART III

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Abstract – This study investigated the changes in physicochemical properties of cured meat products (Pastirma) made from the *M. latissimus dorsi* (LAT) muscle of cattle. Extractabilities of muscle proteins were significantly increased in processed muscles. The fluorescence intensities of samples derived from processed muscles were higher than those of the samples in fresh muscles at all guanidine hydrochloride concentrations. The hydrophobicity also increased as new compounds were created during the Pastirma-making process. The results of the current study indicated that the metmyoglobin percentage in Pastirma samples was increased by as much as 55% compared to control, fresh samples. In general, the SDS-PAGE pattern indicated that most muscle proteins were metabolized to new, smaller molecules, including peptides. The results of this study showed that Pastirma 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 – Bioactive peptide, Protein hydrophobicity, Rib loin.

I. INTRODUCTION

Pastirma is a traditional Turkish dry-cured beef product. Many travellers, including Turkish and Middle-eastern people, regard Kayserian Pastirma to be the best example of this product. The first step of traditional Pastrima-making process is dry curing, during which the meat strips are rubbed and covered with a curing mixture. The beef cuts undergo a sequence of processes and treatments lasting about 4 weeks. Because of the length of the process traditional dry-curing process, including salt-curing procedure, the muscle structure and proteins undergo many physicochemical changes. Many factors contribute to these changes: the salting, curing and dehydration processes, temperature, spices, time, ripening-chamber type, and oxygen abundance are most important factors affecting the physicochemical properties of the proteins and lipids. Recently, local consumers want information on the functionality and health benefits of Pastirma, including information on its protein content and its beneficial effects on lifestyle-related diseases. 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. We examined changes in the physicochemical parameters of muscle proteins as a result of beef processing to produce Pastırma.

II. MATERIALS AND METHODS

Meat cuts and Pastırma-manufacturing process: The Pastırma was produced in a factory in Kayseri city using the traditional process. Rib loin 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^{o}C until the experiment. Meat cuts were processed by removing most of the visible fat layers and then the muscles placed on a curing mixture (crystallised 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 hanged and dried in the open air

for a period of 6-12 days at 25-30°C. Cuts were further processed by hooking to dry in the shade for 4-6 days at 15-20°C. The cured and dried cuts were put in a bowl of dressing and seasoning mixture containing milled fenugreek seeds, crushed garlic, red pepper and H_2O, and left to cure for 12-24 h in warm weather (25°C), and an extra 24-48 h in cooler weather (10-15°C).

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; water soluble protein: WSP and Guba-straub-ATP: GS-ATP) to 2g of meat cuts. The protein concentration of the extracted solution was determined using the biuret method.

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

Fluorescence intensity: Surface hydrophobicity of the native proteins in fresh muscles and the denaturated proteins after processing was determined by ANS method (Ahhmed, *et al*.,) [1].

SDS–PAGE: The proteins were separated according to their size after extracting them in different ionic-strength solutions [1]. 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 [2]. The filtrate of samples was measured at an absorbance of 525, 572, 700 nm using a spectrophotometer.

Histological studies: Histological imaging was carried out essentially as described earlier [1]. Histological stains and fixative materials were used to enhance the visibility of the microscopic structures in the cells. The samples were cut into thin sections and then stained by hematoxylin and eosin-safran. The stained slices were mounted on a glass microscope slide and photographed. Statistical analyses were conducted by SAS software using Duncan multiple comparison method.

III. RESULTS AND DISCUSSION

Protein extractability and concentration: Samples from cured meat cuts showed markedly increased concentrations of extracted proteins, especially of GS-ATP and H_2O P-ex as a result of the salting process (Fig. 1). In both fresh and processed muscles (Pastirma), the extractability of WSPs was lower than that of proteins extracted in GS-ATP solution H_2O P-ex. Proteins extracted in GS-ATP solution increased as a result of the degradation of large proteins. Surprisingly, more proteins were extracted from Pastirma in distilled water compared to fresh cuts, probably because of the activities of certain enzymes in the course of processing. One possible explanation for the difference in extracted protein concentrations between the two types of muscles is the initiation of a reaction activated by certain proteases, such as aminopeptidase. In general, the Pastirma-making process increased the amounts of proteins extracted by all three solutions, suggesting that this process might lead to the generation of new biologically-active peptides.

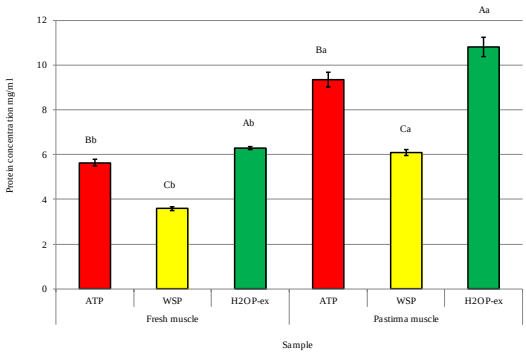

Figure 1. Extractabilities of proteins from Pastirma made from the LAT muscle in GS-ATP, WSP buffer, and distilled water (mg/ml). Different capital and small letters in each muscle and buffer type, respectively show the statistical differences at $p< 0.01$.

pH : The pH values of fresh samples showed common readings of fresh beef cuts, except that those extracted in GS-ATP solution were higher than those extracted in WSP and distilled water (data not shown). In general, the Pastirma-making process had no 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, as indicated by fluorescence intensity values and SDS-PAGE.

The pH values for raw meat and Pastirma, measured by inserting a pH meter probe directly into the meat cuts (Data not shown). Unexpectedly, the measurements were similar, indicating that the acidity of the Pastirma was the same as that of the original fresh-cut meat (5.55 and 5.40, respectively).

Fluorescence intensity: In general, the surface hydrophobicity (SH) of the control-sample proteins extracted in water were higher than those of proteins extracted in GS-ATP and WSP (data not shown). Results suggest that the increase in disassociated proteins was associated with an increase in protein hydrophobicity.

The SH of the processed samples were higher than those of the control samples at all Gu-HCl concentrations (Fig. 2). An increase in the SH of the treated samples indicates an increase in the polarity of the amino acids. The SH of the metabolized proteins during processing was an indicator of the polarity of the muscle proteins, and the results indicate that the hydrophobicity of the Pastirma samples increased as the extracted protein content increased.

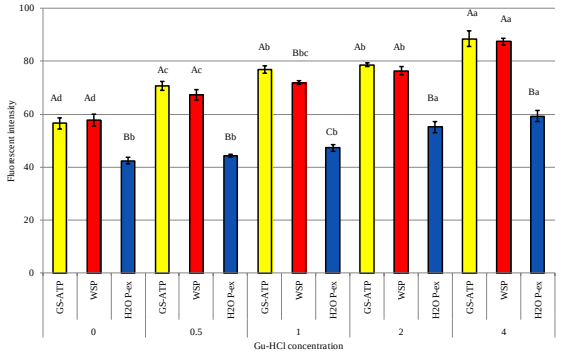

Figure 2. Effect of Gu-HCl on the fluorescence intensity of native and degraded proteins (both were extracted in the GS-ATP) extracted from Pastirma made from LAT muscle. Different capital and small letters in each concentration level and buffer type, respectively show the statistical differences at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

Metmyoglobin: The results of this study indicate the metmyoglobin percentage in Pastirma samples was increased by as much as 55% compared to control, fresh samples (Fig. 3).

The duration of Pastirma-making process was long enough to allow most of the myoglobin to be converted to oxymyoglobin and then to metmyoglobin. However, we suggest that the spice paste to some extent restored oxymyoglobin level.

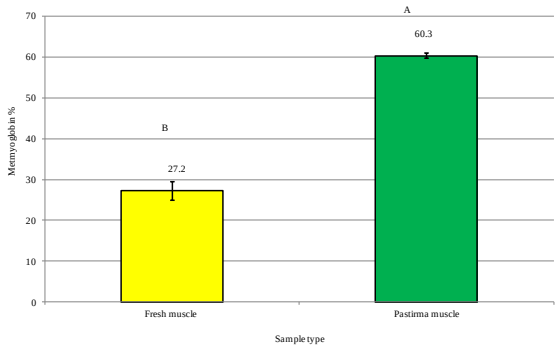

Figure 3. Metmyoglobine percentage in fresh meat cut and Pastirma product, both samples originated from *M. latissimus dorsi* muscle of beef. Different capital letters show the statistical differences at p< 0.01.

Electrophoresis: The extracted proteins (GS-ATP, WSP, and H_2O P-ex) from the fresh and cured meat cuts were separated by SDS-PAGE to detect changes in the native proteins (Fig. 4). In the fresh meat cuts, the myosin heavy chain (MHC) protein band in the GS-ATP sample (Fig. 4-A) was clear, with another band representing a β-galactosidase (β-g). There are likely to be several bands of different molecular weights. In contrast, the MHC and β-g bands were absent in Pastirma samples, suggesting degradation of the muscle proteins during the Pastirma-making process. Other bands, possibly representing phosphorylase with a molecular weight of 97kDa and bovine albumin dehydrogenase with a MW 66kDA and carbonic anhydrase with a molecular weight 29kDa, were present in the GS-ATP, WSP, and distilled-water extracts of fresh meat, but absent from the pastirma samples. Furthermore, trypsinogen and trypsin inhibitor were also disappeared in Pastirma samples. Probably this was occurred as a result of degradation into smaller peptides and possibly functional compounds. In general, the SDS-PAGE pattern indicated that most muscle proteins were metabolized to new, smaller molecules, including peptides (Fig. 4-B). Furthermore, separation of the same samples on different gradients (7.5–17.5%) showed further degradation of low-molecular-weight proteins (Fig. 5-A). Most major small proteins retained their native structure, but some changes

occurred: the GS-ATP and WSP 14kDa α-lactoalbumin band disappeared in the Pastirma samples, as did carbonic anhydrase, with a molecular weight 29kDa, and the WSP 24kDa trypsinogen band, all of which were clearly degraded into smaller peptides. Additionally, many small peptides are absent in Figure 5-B. These results thus explain that certain proteins were degraded by enzymes activated during or after the processing. Thus the traditional Pastirma-making process results in the degradation of many proteins into peptides, which might then be available for nutritional aims.

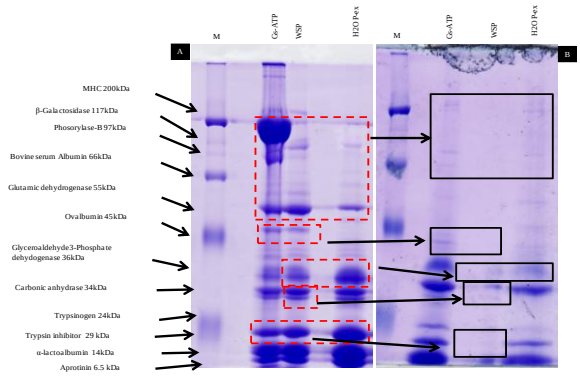

Figure 4. SDS–PAGE pattern shows muscle proteins bands of samples extracted in GS-ATP solution. A: fresh; B: Pastirma. Gradient slab gels (7.5-17.5%).

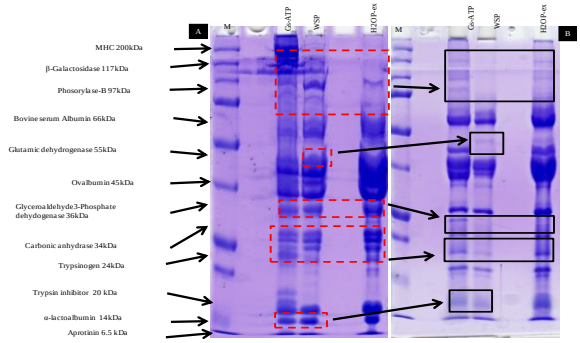

Figure 5. SDS–PAGE pattern shows muscle proteins bands of samples extracted in GS-ATP solution. A: fresh; B: Pastirma. Gradient slab gels (7.5-17.5%).

Histology: Changes in myofibrillar proteins were examined histologically. Figure 6-A shows the structure of the muscle protein filaments, as well as the nucleus and intracellular structure, and tubular arteries. The structure of the muscle protein filaments in the Pastirma samples was slightly changed; the intracellular and intra-filament gaps were smaller than in fresh muscles. Figure 6-B shows that the muscle protein filaments were tightly attracted to each other and were closer than in fresh samples. The filaments and myofibrillar proteins are magnetized due to the hydration process. Minimizing the gaps size in muscles maximizes the firmness of cured-meat cuts. However, the traditional Pastirma making process had no a slight impact on the structure of the muscle. This indicates that this method of processing meat products is not detrimental to the texture, and results in a firmer texture of the processed meat products.

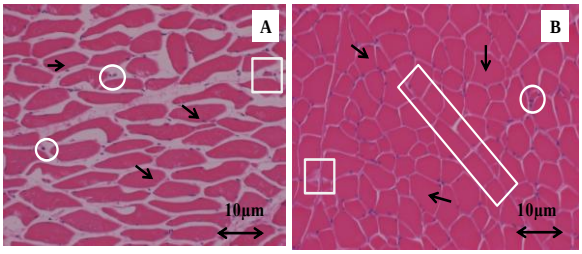

Figure 6. Histological analyses images show the structure of *M. latissimus dorsi* muscle. A: fresh and B; Pastirma. Box: arteries; circles: nucleus; arrows: fibers or filaments; rectangle: endomysin.

IV. CONCLUSION

In this study, we examined changes in the physicochemical parameters of muscle proteins as a result of beef processing to produce cured Turkish meat product (Pastirma). The traditional Pastirma-making process thus changes many of the physicochemical parameters of fresh muscle. We observed that the muscle proteins in Pastirma were degraded to smaller compounds, such as peptides. These results may provide evidence regarding the chemical changes in proteins in the course of processing, including its effects on protein degradation and the generation of new bioactive peptides.

REFERENCES

1. 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.
2. Krzywicki, K. (1979). Assessment of relative content of myoglobin, oxymyoglobin and Metmyoglobin at the surface of beef. Meat Science 3:1-10.