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Traditional cured meat-making process degrades the proteins of *M. latissimus dorsi* of bovine

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Abstract

This study investigated the changes in some properties of protein in cured meat product (Pastirma) made from the *M. latissimus dorsi* (LAT) muscle of cattle as a result of traditional process. As an effect of the salting and curing process, protein extractabilities were significantly increased in Pastirma muscles when compared to the fresh cuts ($P < 0.01$). The analysis of fluorescent intensity showed that values of processed samples were higher than those of the control samples at all guanidine-Hydrochloride (Gu-HCl) concentrations. The increase in the hydrophobicity is a vital key of indication that new peptides were created during the traditional pastirma-making process. The metmyoglobin percentage was greatly increased to a great extent (by as much as 55%) in pastirma samples compared to the fresh samples. The coloured images of histological sections also exhibit the pastirma processing had no negative influence on the structure of the muscle but results in a firmer construction of the processed meat muscle. The SDS-PAGE patterns suggest that this traditionally type of process catalyzed indispensable enzymatic digestion on muscle proteins. Results also imply that during processing of pastirma products myosin heavy chain (MHC) degraded into polypeptides with a molecular weight in the range of lower than 20- kDa. The differences in the protein structure between the control and pastirma samples were thus likely to be contributable to protein digestion in which make it palatable and the most famous meat product throughout Turkey. Compounds identified in this study are important to pastirma flavor, and some compounds have been linked to specific flavors such as the fruity and spicy taste.

Introduction

The Anatolian region has a long, outstanding history and a splendid eating and nutritive dietary culture. Some traditional meat products have been consumed in Turkey, among which Kayserian Pastirma is the most famous (Ahhmed *et al*., 2013a). Although the taste and palatability perception is a subjective issue, pastirma is still appreciated by the majority of people at different ages. Pastirma is a popular dry-cured beef product made from whole muscle (Gok *et al*., 2008). Pastirma is a sort of cured meat has an attractive exterior and interior appearance, delicious taste, unique smell and muscle-like shape. It is a popular meat product coming from the region of Kayseri in central Turkey, and consumers have recently shown considerable interest in related sliced meat products (Aksu *et al*., 2005). Although pastirma used to be produced and consumed only in the Kayseri region, it is now available in retail markets throughout Turkey (Aksu *et al.*, 2001).

The first stage of pastırma processing is dry curing, during which the meat strips are rubbed and covered with a curing mixture (NaCl + KNO_3), with several incisions made in the meat to facilitate penetration of the salt and nitrate mixture (Kaban, 2009). The beef cuts then undergo a series of processes and treatments lasting about a month. Because of the length of the process, the muscle structure and proteins undergo many physicochemical changes. During this period, muscle proteins and fat are hydrolyzed mainly by endogenous enzymes, which results increased amounts of peptides, free amino acids and free fatty acids (Zhou and Zhao, 2007). The salting and drying procedure affects the structure of the proteins and the enzyme mechanisms, potentially increasing the nutritional and sensory values of pastirma.

During the long pastirma-making process, muscle protein and fat are hydrolyzed to some extent by internal enzymes, which eventually produce many small peptides, aromatic amino acids, free fatty acids and volatiles. Those newly developed compounds, directly contribute to the unique flavor of Turkish Pastirma. The pastirma product encompasses many flavors including spicy, fruity, acidic and roasted-meat and is typically perceived as a positive attribute by consumers. The flavor of pastirma is a key attribute for consumers' acceptance and marketing. Using

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instrumental and sensory analysis, many aspects of pastirma flavor would have to be characterized. The enzyme-mediated transamination can synthesize by degrading proteins some aromatic amino acids that are the source of flavor.

This article emphasized on the changes occurred to the water-soluble proteins and myofibrillar proteins. These changes occur especially during the ripening period, contributing to the development of an adequate texture and the characteristic flavour (Flores and Toldra, 1998). 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 (Ahhmed *et al*., 2013a). 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 and aromatic peptides.

The aim of this work was to estimate the degradation capacity of proteins and value the generation of new compounds as a result of the pastirma-making process. Also because of local consumers want to know why the product is so delicate and many people are more interested in the functionality and health benefits of pastirma. Therefore, some physicochemical properties of pastirma made from the *M. latissimus dorsi* (LAT) muscle of cattle were examined in order to evaluate protein degradation capacity that might contribute to palatability of meat. Additionally, some aromatic compounds were determined using gas chromatography.

Materials and methods

Meat cuts and pastırma-manufacturing process

Cuts manufactured about 1 month prior to the experiment were obtained from a local retailer. The pastırma was produced in a factory in Kayseri city using the traditional process. Top (inside) round muscles (*M. latissimus dorsi* (LAT)) were sourced from male cows at 30 months old. The pH values of the muscles before processing ranged from 5.6–6.0. LAT muscles were used 48 h post-mortem. Two groups of muscles were prepared for each experiment: one group was analyzed as fresh meat, the other group was processed into pastirma. Fresh and processed meat samples were sourced from the same animals, and all fresh samples were kept at -30°C until the experiment.

Meat cuts were processed by removing most of the subcutaneous fat layers and visible connective tissue from the surface (Aktas *et al*., 2005; Kaban, 2009), followed by cutting into rod-shapes, as preferred by consumers. Muscles were placed on a curing mixture (crystallised salt 1 kg + 0.015 kg nitrate/kg of meat) at room temperature. Muscles were salted on each side for about 24 h. After curing, the muscles were washed thoroughly using fresh water to remove excess salt from the surface. The cuts were then dried in the open air for a period of 10 days, depending on the weather. Muscles were further processed by squeezing at room temperature for 24 h. Furthermore, the cuts were hanged for extra drying process in the shade for 4-6 days at 15-20°C. The cuts were subsequently covered with a paste of ground spices known as çemen (chemen), as described by Kaban (2009). The cured and dried strips were put in a bowl of seasoning mixture containing 12, 20, 13 and 55% of milled fenugreek seeds, crushed garlic, red pepper and water, respectively. Later the cuts were left to cure for 12-24 h in hot weather, and an extra 1-2 days in cooler weather. The excess çemen was removed to leave a thin layer (about 3 mm), and the product was dried again at a moderate temperature for 2 days (Ahhmed *et al*., 2013a). Finally the pastirma was wrapped in a paper sheet ready to be sent to the market for consumers. The çemen paste covering the pastirma is an important element in terms of both flavour and preservation. The mixture known as çemen in Turkey is reported to improve the appearance, colour, texture, taste, and flavour of pastirma, and is also effective against microbial contamination and helps to prevent excessive drying of the pastirma (Isıklı and Karababa, 2005).

Protein extraction

Proteins were extracted from the fresh and processed (pastirma) samples by adding 28 ml of solution to 2 g of the samples. Proteins were extracted using three different solutions. Distilled water was used to examine the changes in most proteins (H_2O P-ex), enzymes and small peptides, which can be extracted in water. The second solution was a low-ionic-strength solution, which extracted proteins defined as water-soluble proteins (WSP) (50 mM imidazole-HCl pH 6.0, 2 mM EDTA). The third solution was a high-ionic-strength solution, Guba-Straub-adenosine triphosphate solution (GS-ATP) (0.09 M KH_2PO_4, 0.06 M K_2HPO_4, 0.3 M KCl, 1 mM ATP pH 6.5) (Ahhmed *et al.*, 2007). The solutions along with the samples were homogenized three times for 30 s, at 10 s intervals, using a polytron homogenizer (Kinematica Co., Littau, Switzerland) at setting 4. The mixtures were then centrifuged at

12,000 rpm for 30 min at 4°C in a Himac CR 20E centrifuge (Hitachi, Tokyo, Japan). The supernatants were removed and filtered using filter paper No. 5A (Advantec Toyo K. Ltd., Tokyo, Japan), and the final solution was used as the extracted-protein solution. The protein concentration of the extracted solution was determined using the biuret method (Gornall *et al.*, 1949), with bovine serum albumin as a standard. The absorbance of the samples was measured at 540 nm using a spectrophotometer (Model Bio Spec 1600; Shimadzu Co., Kyoto, Japan), with each sample evaluated in triplicate.

pH values

The pH values of the aqueous solutions of extracted proteins and meat cuts were measured to determine the qualities of the meat and protein solutions. pH values were measured using a pH meter (HM-30R Model HM-30R pH Meter).

Fluorescence intensity

Surface hydrophobicities of the actual peptides and proteins before curing (fresh) and after processing (pastirma product) were determined by the 8-anilino-1-naphthalenesulfonic acids (ANS) method (Hayakawa *et al.*, 1985; Ahhmed *et al*., 2009b). ANS 50 mL (8 in 10 mM phosphate buffer, pH 7.0) was added to 4 mL of protein solution. The fluorescence intensity of the ANS-protein conjugate was measured in a spectrofluorometer (Model RF-1500, Shimadzu Co., Kyoto, Japan) at an excitation wavelength of 365 nm and an emission wavelength of 470 nm. Quinine sulphate (1 mg/L) was used as the standard solution (Tomita, 1963).

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS–PAGE)

SDS–PAGE was used to separate the proteins according to their size after extraction in the different ionic-strength solutions (Kawahara *et al*., 2007; Ahhmed *et al.*, 2009a). Electrophoresis was carried out using two different acrylamide gel gradients: 7.5% (to visualize high-MW proteins), and 7.5–17.5% (to visualize all proteins and peptides), with 2-mercaptoethanol at 20 mA/gel, according to the discontinuous buffer system Laemmli (1970).

Metmyoglobin measurements

Metmyoglobin concentrations were evaluated using a modification of the procedures described by Krzywicki (1979) and Saito (2007). Samples were blended with five volumes of cold 0.04 M phosphate buffer at pH 6.8 for 10 sec in a homogenizer (Kinematica Co., Littau, Switzerland). After standing at 1°C for 20 h, the mixture was centrifuged at 2,700 rpm for 30 min. The supernatants were then removed and filtered using filter paper No. 5A (Advantec Toyo K. Ltd.). The filtrate was measured at absorbance of 525, 572, 700 nm using a spectrophotometer (Model Bio Spec 1600; Shimadzu Co., Kyoto, Japan). The percent of metmyoglobin was calculated using the following formula:

$$\text{Metmyoglobin \%} = 1.395 - (A572 - A700) / (A525 - A700) \times 100$$

Where $A\lambda$ = absorbance at λ nm

Histology

Histological imaging was carried out essentially as described earlier (Astruc *et al*., 2008), with slight modifications as we described in earlier publication (Ahhmed *et al*., 2009c). A cut (10 × 12 × 12 mm) was removed from each pastirma and fresh muscle sample (positive and/or control) and soaked for 14 days in 10% (v/v) formalin buffer. The fixation process was designed to preserve the shapes of the cells and tissues, and involved successive immersions in different concentrations of ethanol, followed by three different concentrations of toluene for 8 h each. Finally, the samples were incubated in three different paraffin solutions for 8 h each and refrigerated. The stained slices (hematoxylin and eosin-safran) were mounted on glass microscope slides, visualised and photographed at ×20 magnification under a transmission electron microscope (Nikon microphot-FXA) connected to a computer with the appropriate software (Nikon-ACT-2u), to obtain the clearest images.

Flavour and odor compounds analysis

The application of analytical qualitative aromatic techniques such as gas chromatograph analysis provides a useful and comprehensive indication to identify and characterize flavor and flavor sources. The objective using this technology was to characterize the compound(s) responsible for causing a unique flavor in pastirma. Pastirma cut of 2 mm^2 was extracted for 10 min using SPME (Pastirma 1g with 0.8% NaCl 5 ml). The samples was loaded into a column Agilent technologies (DB-WAX, 30 mm x 250 mm x 0.25 µm) while the spectrometer was Agilent technologies (5975C inert XL NSD) linked with Gestel multipurpose sampler. The samples were treated in an oven: 40°C for 2 min, and then 10°C / min up 250°C which was eventually held for 1 min at 250°C. The analysis was carried out in Ito ham laboratories (Ito Ham Ltd. Ibaraki, Japan).

Statistical analysis

All statistical calculations were carried out

using the SAS statistical software (SAS). Two-way analysis of variance (ANOVA) to show the effect of independent variables on the dependent variables was conducted using the general linear model procedure. Duncan Multiple Range Test was used to determine differences among mean values with the significance level of 0.05 and 0.01.

Results and Discussion

Protein extractability and concentration

Samples from pastirma muscles showed significantly increased concentrations of extracted proteins in the three solutions ($P < 0.01$), as a result of the salting process (Figure 1). The salting treatment and curing process was possibly to have a potential effect on the extractability of muscle proteins, probably as a result of releasing some proteins from each other and cleaving the structures between certain proteins such as myofibrils.

In both fresh (control) and processed muscles (pastirma), the extractability of WSPs was lower than that of proteins extracted in H_2O P-ex and GS-ATP solution. 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, especially those connecting myosin and actin (Ahhmed *et al*., 2013a). Interestingly, more proteins were extracted from pastirma in H_2O P-ex compared to fresh cuts, probably because of the activities of certain enzymes in the course of processing ($P < 0.01$). Because the process lasts for a month, degradation may have occurred through the activation of some unidentified enzymes. We suggest that the difference in extracted protein concentrations between the two types of muscles is because of the initiation of a reaction activated by certain proteases, such as aminopeptidase or by the cemen addition. It is well known that cemen contains fenugreek which might involve in the protein degradation. In addition to the fenugreek antimicrobial activities, possibly cemen ingredients had an impact on the proteins during storage of pastirma. Based on the results of a study carried out by Yetim *et al.* (2006) cemen paste and garlic significantly inhibited the growth of three pathogenic bacteria. The protein concentration change is likely to be the result of endogenous enzyme activity since low numbers of micro-organisms are found inside dry-cured ham (Toldra and Etherington, 1988; Zhen *et al*., 2004). In general, the inhibitory effect of cemen paste was higher than that of garlic. This might have resulted from the synergistic effect of cemen ingredients.

In general, the pastirma-making process increased the amounts of proteins extracted by all three solutions, implying that the salting and curing process had a beneficial effect (Fig. 1). Enzymatic production of bioactive and antioxidative protein hydrolysates and peptides most likely occurred during Pastirma-making process. This process might lead to the generation of new biologically-active peptides that could provide multifunctional remedies for some cultural diet-related diseases.

Table 1. pH values of fresh and pastirma cuts made from the LAT muscle of cattle, also pH values of their proteins extracts (dissolved in GS-ATP, WSP buffer, and distilled water). Values of those fresh and pastirma cuts the pH meter probe was inserted directly into the meat cut in contact with the meat tissues.

Status	pH of meat flesh	Extract type					
		GS-ATP		WSP		H2O P-ex	
		means	SDV	means	SDV	means	SDV
Fresh	5.40	6.21	0.08	5.57	0.06	5.66	0.06
Pastirma	5.55	6.37	0.05	5.84	0.03	5.83	0.07

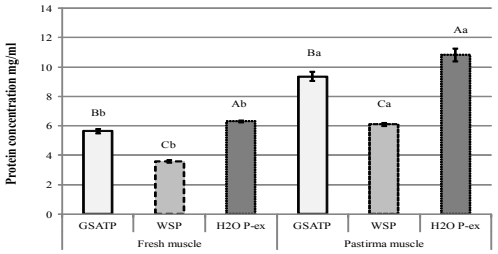

Figure 1. Extractabilities of proteins from fresh LAT muscle in GS-ATP, WSP buffer, and distilled water (mg/ml). Different capital letters in each muscle group show the statistically significance ($P < 0.01$), different small letter in each extract type show the statistically significance between the muscle types ($P < 0.01$).

pH

The curing of beef meat must be done very cautiously under strict conditions, to avoid unfavorable reactions such as lipid auto-oxidation, rancidity, color deterioration, and pH changes. Table 1 shows the pH values of proteins extracted in different buffers. The pH values of fresh samples showed normal readings, except that those extracted in GS-ATP solution were higher than those extracted in WSP and H_2O P-ex. 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 (Ahhmed *et al*., 2013b). The increase might have been associated with the degradation of some proteins and enzymes, as indicated by SDS-PAGE. Table 1 also shows the pH values for 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. Since

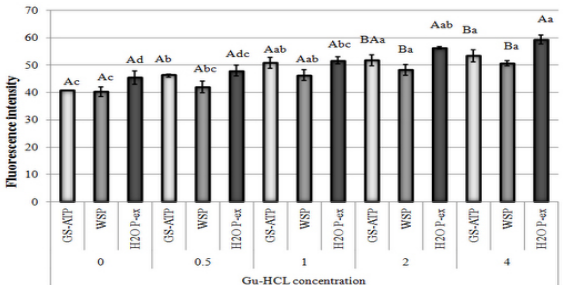

Figure 2. Effect of Gu-HCl concentration on fluorescence intensity (surface hydrophobicity) of native proteins (extracted in GS-ATP, WSP and H2O P-ex solutions) from fresh LAT muscle. Different capital letters in each muscle type show the statistically significance ($P < 0.01$), different small letter in each extract type show the statistically significance between the GU-HCl concentrations ($P < 0.01$).

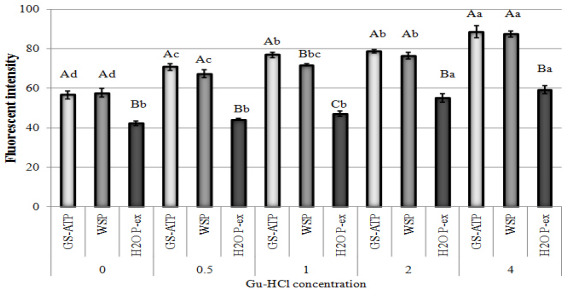

Figure 3. Effect of Gu-HCl concentration on fluorescence intensity (surface hydrophobicity) of degraded proteins (extracted in GS-ATP, WSP and H2O P-ex solutions) from pastirma of LAT muscle. Different capital letters in each muscle type show the statistically significance ($P < 0.01$), different small letter in each extract type show the statistically significance between the Gu-HCl concentrations ($P < 0.01$).

the pH of pastirma samples ranged from 5.55-6.4. We suggest that the following enzymes were coexisted with myofibrillar cells in pastrima muscles: cysteine peptidases (cathepsin B, H, C and L and dipeptidyl-peptidase I) and serine peptidase (dipeptidyl-peptidase II) as they are active at a range of pH 5.5-6.5 and 5.0-6.0, respectively. Cathepsin B and L may play key roles in hydrolyzing insoluble proteins to soluble proteins, and soluble proteins to peptides as a results of *in vitro* conditions and the long duration of Jinhua ham processing (Zhao *et al.*, 2005). Also the same authors reported that the activity of muscle dipeptidyl-peptidase I decreased gradually before the beginning of ripening and then increased gradually until the end of post-ripening. As we believe that there is a relative similarity in processing method between Pastirma and Jinhua hams, the results of this study thus in accordance with the results demonstrated by a study conducted by Zhou and Zhao (2007).

Fluorescence intensity

The polar and hydrophobic amino acid residues were examined in proteins extracted from fresh and pastirma cuts of *M. latissimus dorsi* using all three solutions. Fluorescence intensity measurements indicated that the surface hydrophobicity and the relative proportions of amino acids in the proteins extracted from fresh muscle increased with increasing guanidine hydrochloride (Gu-HCl) concentration (Figure 2). In general, the hydrophobicity of the control-sample proteins extracted in H_2O P-ex at all Gu-HCL concentrations was higher than those of proteins extracted in GS-ATP and WSP (Figure 2). The slight lower fluorescence intensity values were associated with proteins extracted in WSP, which might reflect the myoglobin concentration or refers to the EDTA addition, as this reagent inhibits enzyme activity during preparation of samples for tests. The high fluorescence intensity values of proteins extracted in distilled water may be contributable to the fact that fresh distilled water is chemical free, as well as to their extractability.

In relation to the changes of physicochemical properties, we suggest the increase in disassociated proteins was associated with an increase in protein hydrophobicity in pastirma cuts. The fluorescence intensities of the processed samples were higher than those of the control samples at all Gu-HCl concentrations (Figure 3). An increase in the surface hydrophobicity of the treated samples indicates an increase in the polarity of the amino acids. The fluorescence intensity values of the samples extracted in distilled water were significantly lower than those in samples extracted in GS-ATP and WSP ($P < 0.01$), which might be attributable to oxidation occurring during the processing time. Oxidation of myoglobin would convert it to oxymyoglobin, while further oxygenation would further convert it to metmyoglobin. This could explain why the hydrophobicity of the distilled water samples was lower than that of the other extractions, and also suggests that a considerable number of amino acids disassociated from each other as a result of the salting and curing process (Ahhmed *et al*., 2013b). The creation of new small compounds such as peptides and free amino acids by the traditional pastirma-making process is associated with an increase in hydrophobicity and changes in the physical properties. As expected, protein denaturation by Gu-HCl increased the surface exposure of hydrophobic residues, as measured by ANS fluorescence (Muguruma *et al*., 2003; Ahhmed *et al*., 2009b), in a concentration-dependent manner. The differences in fluorescence intensity between the control and pastirma samples were probably associated with the protein degradation and creation of free amino acids.

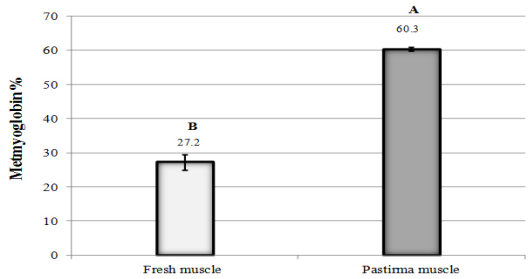

Figure 4. Metmyoglobin percentage in fresh beef cuts and pastirma, both from the LAT muscle of cattle. Different letters show statistically significance between cut types ($P < 0.01$).

The surface hydrophobicity of the degraded 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. This indicates that changes in the protein nature because of processing resulted in increased protein content (namely newly generated peptides) and alterations in surface hydrophobicity.

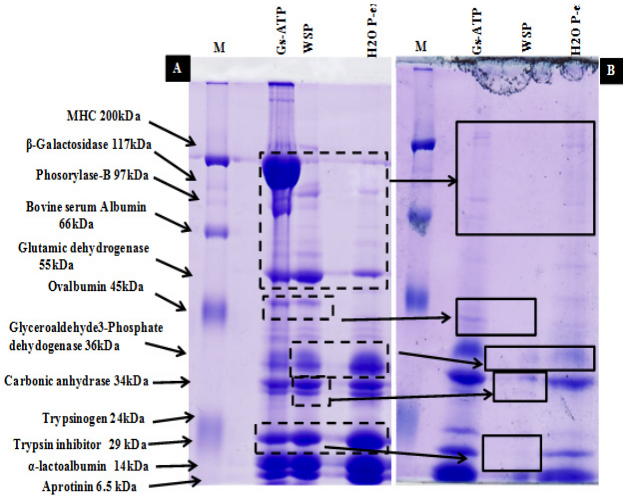

Figure 5. SDS–PAGE showing effects of traditional pastirma-making process on MHC and other low molecular mass proteins in the LAT muscle of cattle. Samples were extracted in GS-ATP solution. (A) Bands of proteins extracted from fresh muscle; (B) bands of proteins extracted from pastirma. Gradient slab gel (7.5%). Dotted boxes indicate the original bands in (A) that are undetectable in (B).

Metmyoglobin

Changes in myoglobin in meat cuts are one of the fastest chemical reactions occurring during food processing. The mechanism is rather complex, as myoglobin is converted to oxymyoglobin, which is subsequently converted to metmyoglobin within a short time (Ahhmed *et al.*, 2013a). Scientists also consider that metmyoglobin may revert to oxymyoglobin under certain circumstances. Essentially, color depends not only on the measured material, but also on the light source and the detector, be it human or instrumental, making different measurement response as s consequence of different experimental conditions (Castigliego *et al*., 2012). The discoloration of fresh meat is an important process, which is determined by the relative concentration of the three redox forms of myoglobin (deoxymyoglobin, oxymyoglobin, and metmyoglobin) (Faustman and Phillips, 2001). Food quality is the sum of three principal components, nutritional value, safety and consumer acceptability (Clydesdale, 1978). Consumer acceptability includes a large array of attributes such as visual appeal, aroma, flavour, texture, mouth feel, convenience and cultural relevance (Bekhit and Faustman, 2005). Consumers are more likely to purchase beef products that have an acceptable color, such as bright red or pink, as this is regarded to be an indication of freshness (Carpenter *et al*., 2001). Turkish consumers prefer pastirma with a bright-red colour, because they consider that the darker the colour, the inferior the pastirma (Ahhmed *et al.*, 2013a). Over time, the pastirma turns browner and darker, and becomes firmer in texture. Thus, in an oxidizing and acidic environment, meats with high myoglobin and iron contents and rich in unsaturated fatty acid may show a tendency toward browning (Guidi *et al*., 2006).

The results of the current study indicated that the metmyoglobin percentage in pastirma samples was significantly increased ($P < 0.01$), by as much as 55% compared to fresh samples (Figure 4). The pastirma-making process lasts about 30 days, which is long enough to allow most of the myoglobin to be converted to oxymetmyoglobin and then to metmyoglobin. However, we suggest that the metmyoglobin reverted to oxymetmyoglobin as a result of wrapping the pastirma in a spice paste (çemen). This process provided an anaerobic environment that isolated the meat cut from further contact with oxygen. The characteristic flavour and aroma of pastirma can be attributed to the ingredients used in its processing, namely fresh mashed garlic, red pepper, and ground fenugreek (*Trigonella foenum graecum*) seeds (Tekinsen *et al.*, 1999). The paste increases the aromatic properties of the meat and protects it from drying and spoiling through contact with the open air, which would otherwise cause the fat in the pastirma to oxidise and give a rancid flavour. Visually, pastirma is aesthetically unique but darker than the equivalent fresh cuts of meat. That can be explained by the hypothesis of Swatland (1994) as reported that after prolonged exposure to the atmosphere, however, the iron atoms in Mb may be converted to the ferric form, resulting in the formation of brown Met-Mb. The Met-Mb% is not only an indicator of oxidation but

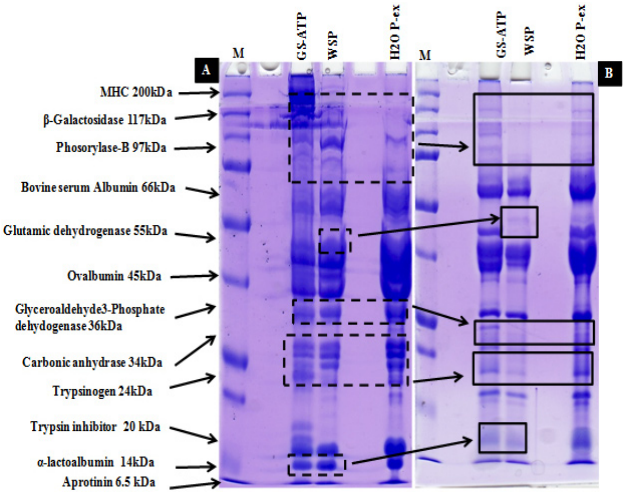

Figure 6. SDS–PAGE showing effects of traditional pastirma-making process on MHC and other low molecular mass proteins in the LAT muscle of cattle. (A) Bands of proteins extracted from fresh muscle; (B) bands of proteins extracted from pastirma. Samples were extracted in GS-ATP solution. Gradient slab gels (7.5–17.5%). Dotted boxes indicate the original bands in (A) that are undetectable in (B).

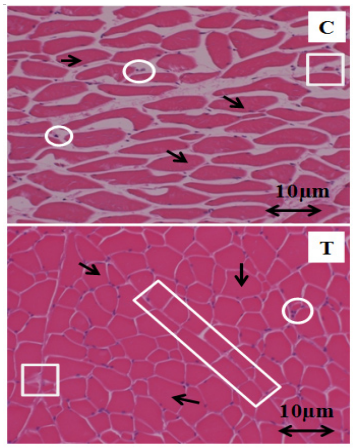

Figure 7. Histological images showing the effects of the traditional pastirma-making process on the structure of the LAT muscle of cattle. C: fresh cut sample and T: treated cut sample (pastirma). Box, arteries; circles, nucleus; arrows, fibres or filaments; rectangle, endomysin or connective tissue. Images were obtained at the magnifications indicated.

plays an important role in the coloration of the meat (Saito *et al.*, 2009) and that can be avoided by using natural wrapping materials. Cemen is considered one of those excellent natural wrapping materials, which composed of ground classical fenugreek seeds, local garlic, and chilli pepper, mixed to a paste with a little tomato and plenty of water.

Electrophoresis

The extracted proteins (GS-ATP, WSP, and distilled water) from the fresh and pastirma cuts were separated by SDS-PAGE to evaluate their molecular weights and to detect changes in the native proteins (Figure 5-A and B). In general, the SDS-PAGE pattern indicated that most muscle proteins were metabolized to new, smaller molecules, including peptides (Figure 5-B). In the fresh meat cuts, the MHC protein band in the GS-ATP sample was clear, with another band representing a subunit of MHC. There are likely to be several bands of different molecular weights. In contrast, the MHC bands were absent in pastirma samples, suggesting degradation of the muscle proteins during the pastirma-making process. Other bands, possibly representing β-galactosidase with a molecular weight of 117-kDa and carbonic anhydrase with a molecular weight 34-kDa, 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 peptides and possibly functional compounds.

Furthermore, separation of the same samples on different gradients (7.5-17.5%) showed less degradation of low-molecular-weight proteins (Figure 6-A). Most major small proteins retained their native structure, but some changes occurred: the WSP 36-kDa Glyceroaldehyde3-Phosphatedehydogenase band disappeared in the pastirma samples, as did carbonic anhydrase, with a molecular weight 34-kDa bands in the three extracts (GS-ATP, WSP and H_2O P-ex), all of which were apparently degraded into smaller peptides. Additionally, many small peptides are absent from Figure 6-B such as the 29-kDa trypsin inhibitor band in GS-ATP samples. These results thus demonstrate that certain proteins were degraded by enzymes activated during or after the processing. This suggestion of enzyme action is supported by the increases in protein content and hydrophobicity of the extracts, as well as the increased concentration of metmyoglobin. Thus the traditional pastirma-making process results in the degradation of many proteins into peptides, which might then be obtainable to treat some diet-related diseases (Ahhmed *et al.*, 2013). However, further biochemical and physiological studies are needed to confirm this hypothesis (Ahhmed *et al.*, 2013a). It is important to study techno-functional properties of active peptide fractions and how these peptides can retain their antioxidant activities in different targeted food matrices (Samaranayaka and Li-Chan, 2011).

Histology

Histological study was used in this work to examine the changes in myofibrillar proteins. Figure 7-C shows the structure of the muscle protein filaments, as well as the nucleus, intracellular structure, and tubular arteries. The structure of the

Table 2. Some of the aromatic compounds available in pastirma samples that were determined by GC

No.	Compound name	Odor	Agreement rate %	Mw (Da)	Chemical formula
1	Cyclotrisiloxane, hexamethyl-	Paprika	94	22	C6H18O3S3
2	Sulfide, allyl methyl	Fruit	91	88	C4H8S
3	Butanoic acid, 2-methyl-, ethyl ester	Fruit and sweet	46	130	C7H14O2
4	Butanoic acid, 3-methyl-, ethyl ester	Plastic and spicy	93	130	C7H14O2
5	Hexanal	Grass	95	100	C6H12O
6	Diallyl sulfide	Roast and organic	96	114	C6H10S
7	1-Propene, 3,3'-thiobis-	Roast and organic	95	114	C6H10S
8	N HeptanaL	Plastic, sour and sweet	97	114	C7H14O
9	Nonanal	Fruit and lemon	96	142	C9H18O
10	1-Octen-3-OL	Grass and woody	53	128	C8H16O
11	Acetic acid, aminooxo-, hydrazide	Grass and plant	47	103	C2H5N3O2
12	1,1,1-Trichloro-2-hydroxy-2-[2-acetamidoethylthio]ethane	Spicy	53	265	C6H10Cl3NO2S
13	Pentadeuterio-2-acetyl-1-pyrroline	Meat and organic	64	116	C6H4D5NO
14	Naphthalene, 2-methyl-	Roasted meat	86	142	C11H10
15	12-Crown-4	Sweet and syrup	64	176	C8H16O4

muscle protein filaments in the pastirma samples had changed after the course of processing. The most recognizable change was occurred within the intracellular and tubular gaps were smaller than in fresh muscles. Figure 7-T shows that the muscle protein filaments (arrows) were 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 in muscles maximizes the firmness of cured-meat cuts. From the preference point of view, however, the traditional pastirma making process had no negative impact on the structure of the muscle (Ahhmed *et al*., 2013a). 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. However, Huff-Lonergan and Lonergan (2005) have reported that fresh meat that retains its moisture and tenderness is arguably one of the most important quality characteristics of raw products. It is not the case for cured meat products such as pastrima (Ahhmed *et al*., 2013a), this sort of product unrestrained most of the moisture that later it becomes slightly harder in texture and had a drastic change in the colour as it turned to dark brown.

Aromatic analysis

These results are an important clue into pastirma flavor because many flavor compounds are derived from amino acids that formed during protein degradation. However, it is likely that additional compounds may also contribute to the overall flavor quality of pastirma. Table 2 showed some of those compounds that we suggest have a crucial role in consumers' acceptance. Pastirma also exhibit a strong and unclean flavor that is typically detected after sweating which originated from fenugreek in the covering materials. That stinky-spice flavor type is considered as undesirable odor.

Fifty odor-active compounds were identified in the GC analysis of pastirma samples. Fifteen compounds were typically identified as partially responsible compounds for the pastirma flavor. The compounds associated with the flavor they produce, their agreement rate, molecular weight, and chemical formula (Table 2). Agreement rate means the similarity percentage of the characterized compound to the original standard compound of the same chemical. Using this method, we determined which compounds had responsible aroma activities in the Turkish pastirma. We suggest that these compounds along with other 35 (data not shown) were potentially responsible for causing the unique flavor based on their aroma character. This study systematically confirms that a combination of some aromatic compounds contributes to the unique flavor in Turkish pastirma. These results also provide us with a chemical formula for each compound that advised to be attributing to the overall flavor. Compounds identified in this study and others are important to pastirma flavor, and some compounds have been linked to specific flavors such as the fruity and spicy taste.

Conclusions

Changes in physicochemical properties between fresh samples and pastirma products sourced from the same muscles shoulder rose muscle *M. latissimus dorsi* (LAT) of cattle were significantly different. The salting and curing process had no negative impact on the firmness of the cured-meat cuts, only the filaments

and myofibrillar proteins are magnetized due to the hydration process. These results demonstrate that certain proteins were degraded by enzymes activated during or after the processing. Traditional cured meat-making process degrades the muscle proteins and then release some aromatic amino acids in which improve palatability and delicacy of bovine meat. These results suggest that pastirma may contain newly-generated peptides that can serve as nutraceuticals suitable for reducing some chronic diseases. Furthermore, this study systematically confirms that a combination of some aromatic compounds contributes to the unique flavor in Turkish pastirma. However, further biochemical biological assays, and bioavailability and metabolism studies are needed to confirm this hypothesis.

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