

## Changes in myofibrillar proteins during processing of pastirma (Turkish dry meat product) produced with commercial starter cultures

Nesimi Aktaş<sup>\*</sup>, Muhammet Irfan Aksu, Mükerrerem Kaya

Food Engineering Department, Agricultural Collage, Atatürk University, 25240 Erzurum, Turkey

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### Abstract

The effects of different commercial starter cultures (*Staphylococcus carnosus*, *S. carnosus* + *Lactobacillus pentosus* and *Staphylococcus xylosus* + *Lactobacillus sakei*), on myofibrillar proteins were investigated using differential scanning calorimetry (DSC) during the processing of pastirma. The stage of pastirma production significantly decreased the thermal stabilities of myosin and actin. Actin was less affected than myosin. The myofibrillar fraction of pastirma was hardly denaturated by *S. carnosus*, but more pronounced denaturation was obtained with *S. carnosus* + *L. pentosus*.

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### 1. Introduction

Pastirma, a traditional Turkish meat product, is the most popular dry-cured meat product produced in Turkey. It is categorized as an intermediate moisture food (Leistner, 1988). Pastirma is produced from whole muscle obtained from certain parts of beef and water buffalo carcasses. Muscles are cleaned from tendon and fat and then cured, dried, pressed and coated with garlic, paprika, red pepper and water-containing paste (cemen), and again dried. The production process of pastirma approximately extends over a month-period. In this period, significant structural changes take place in meat, including breakdown of the proteins by means of muscle proteinases (Anon., 1983; Gökalp, Kaya, & Zorba, 1999; Molina & Toldra, 1992; Toldra, Rico, & Flores, 1993).

Over the past decade, interest in the starter cultures in the meat products has greatly increased and numerous studies have been carried out. Various workers report that, using starter cultures in dry-cured raw meat

products, such as pastirma, improved quality properties of the end-product (Aksu & Kaya, 2001, 2002; Katsaras, Launtenschläger, & Bosckova, 1996a, 1996b). Some members of the *Micrococcaceae* family utilized as starter cultures in meat products are *Staphylococcus carnosus*, *Staphylococcus xylosus*, *Micrococcus varians* (Hammes & Hertel, 1998; Hammes & Knauf, 1994; Jessen, 1995; Lücke, 1985; Lücke, 2000). These microorganisms have proteolytic and lipolytic activity (Fadda, Sanz, Vignola, Conception, Aristoy & Toldra, 1999; Geisen, Lücke, & Kröckel, 1992; Johansson, Berdague, Larsson, & Borch, 1994; Lücke, 1985). Lactic acid bacteria used as starter cultures (*Lactobacillus plantarum*, *Lactobacillus sakei*, *Lactobacillus curvatus*, *Lactobacillus pentosus*) had weaker lipolytic and proteolytic activity than the *Micrococcaceae* (Johansson, Berdague, Larsson, & Borch, 1994; Kröckel, 1995; Lücke, 1985; Schiefer & Schöne, 1978).

It is well known that the quality of meat products depends on the extent and character of the denaturation changes in muscle proteins. At present, one of the most informative physicochemical methods used for studies of the thermally induced conformational changes in muscle proteins is differential scanning calorimetry

<sup>\*</sup> Corresponding author. Tel.: +90-442-2311625; fax: +90-442-2183647.

E-mail address: [aktasnesimi@hotmail.com](mailto:aktasnesimi@hotmail.com) (N. Aktas).

(DSC). The method allows the determination of the number of the structural transitions, their temperature intervals, and their enthalpies and measures the extent of denaturation under various processing conditions.

There is some research on pastirma produced with starter cultures (Aksu & Kaya, 2001, 2002). But, until recently, there is no information regarding the effects of commercial starter cultures on the myofibrillar proteins of pastirma. The aim of this research was to determine, by DSC, whether or not myofibrillar proteins are susceptible to degradation of pastirma produced with commercial starter culture.

## 2. Materials and methods

### 2.1. Production of pastirma

The production process of pastirma is given in Table 1. Three trials (treatment 1, treatment 2 and treatment 3) were carried out in this study. *Longissimus dorsi* muscles (24 h *post mortem*) from beef, carcasses were used as raw material. After removing fat and connective tissue from the surface, muscles were cut vertically into two pieces. Four pastirma were produced from a carcass. For each treatment one carcass were used. In treatments, commercial preparations of *S. carnosus* (Bactoform™ C-P-77), *S. carnosus*+*L. pentosus* (Bactoform™ C-P-77 S) and *S. xyloso*+*L. sakei* (Bactoform™ B-FM) were used as starter culture (CHR HANSEN, Rudolf Muller, Germany). Starter culture (25 g starter/100 kg meat) was introduced into muscle with curing compounds (47.250 g NaCl, 0.750 g KNO<sub>3</sub>, 1.0 g glucose, 1.0 g saccharose) and the curing process was employed in different trays for each group. As control group, pastirma samples made without starter cultures were also used. Paste mixture (cemen) used in this study contained 500 g flour, *Trigonella foenum graecum* seed, 350 g smashed fresh garlic, 75 g paprika, 75 g red pepper and 1200 ml water. After the second drying step, dried meat samples were mounted with paste (cemen) and stored for 4 days. Then

the surface of the meat was shaved to give 3–4 mm of paste mixture thickness on the surface of the meat.

### 2.2. Isolation of myofibrillar proteins

Myofibrillar proteins were isolated by using the procedure of Claeys, Uytterhaegen, Buts, and Demeyer (1995). 2.5 g minced meat were homogenized, using an Ultra-Turrax in 25 ml buffer solution (pH 7.6, 3 °C, 0.05 M sucrose, 0.05 M Tris, 1 mM EDTA). After centrifugation (1000g for 10 min) the supernatant was decanted. Myofibrils were resuspended in 25 ml of a buffer solution (pH 7.6, 3 °C, 0.05 M Tris, 1 mM EDTA) and again centrifuged at 1000g for 10 min. The supernatant was decanted and the treatment was repeated with 25 ml of KCl solution (3 °C, 0.15 M KCl). After isolation, the pellet was lyophilized and stored until required for analysis.

### 2.3. DSC analysis of myofibrillar proteins

The endothermal transitions of myofibrillar proteins were determined by using the DSC-50 (Shimadzu Corporation, Kyoto, Japan). Approximately 10 mg of sample from the myofibrillar proteins were weighed into an aluminium hermetic cell (Shimadzu, 201-53090) and sealed with a crimper. Samples were heated from 20 to 90 °C, at rate of 5 °C/min, using an empty cell as a reference. For temperature and heat flow calibration Indium was used ( $T$ , 156.4 °C;  $\Delta H$ , 28.47 J/g). The temperature at which the maximum rate of heat input occurred in an endothermic peak was expressed by  $T_p$ . For reproducibility, the temperature of the peak maximum was taken as the transition temperature. Denaturation enthalpies ( $\Delta H_d$ ) were estimated by measuring the area under the DSC transition curve.

### 2.4. Statistical analysis

This experiment was conducted according to a completely randomized block design, using three replicates.

Table 1  
The stages of pastirma production

Production stage	Time	Temperature (°C)	Relative humidity (%)
1 Curing <sup>a</sup>	2 d	6 ± 1	80–90
2 First Drying	4 d	15 ± 1	80–85
3 First Pressing <sup>b</sup>	17 h	7 ± 1	
4 Second Drying	3 d	20 ± 1	70 ± 2
5 Second Pressing <sup>b</sup>	7 h	25 ± 1	
6 Paste Cemening	4 d	7 ± 1	
7 Last Drying	2 d	15 ± 1	70 ± 2
	2 d	18 ± 1	65 ± 2
	6 d	20 ± 1	60 ± 2

<sup>a</sup>For 1 kg meat and fat, 50 g curing material.

<sup>b</sup>For 1 kg meat and fat, 25 kg weight.

Analysis of variance of all data was conducted using the general linear model (GLM) procedure (SAS, 1998).

### 3. Results and discussion

Differential scanning calorimetry curves of myofibrillar proteins showed two major endothermic peaks

(Figs. 1 and 2). It has been suggested that the first peak was due to myosin, and the second to actin. The  $T_p$  of these peaks shifted to lower temperatures and the peak areas decreased when production stages were carried out (Table 2). A greater effect of processing on  $T_p$  was observed for myosin, which has been shown to lower its  $T_p$  and to be less resistant to denaturation than actin (Table 3). The lower myosin  $T_p$  indicates reduced thermal

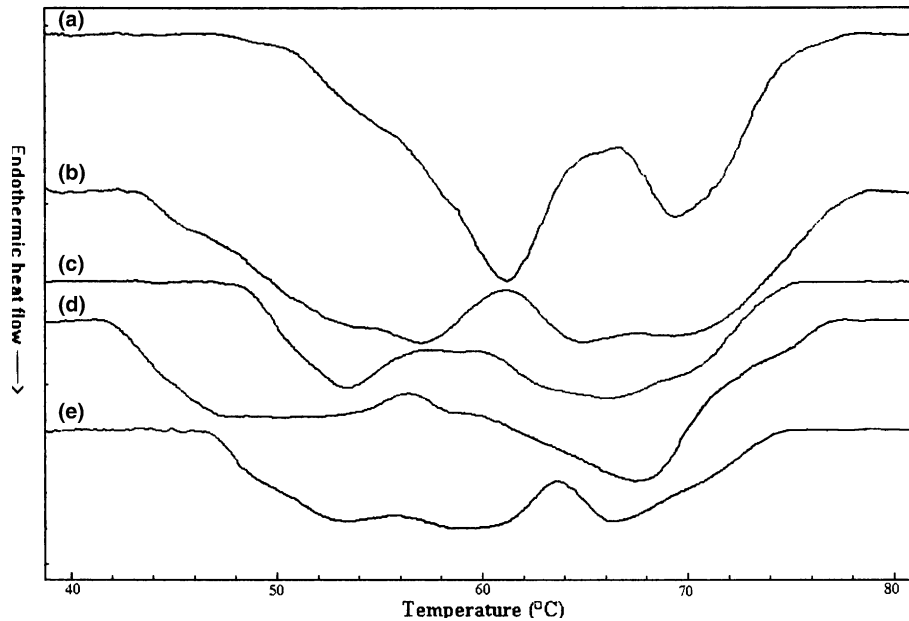


Fig. 1. Differential scanning calorimetry curves of myofibrillar proteins isolated from pastirma during the second drying stage: (a) raw material; (b) control at the end of second drying; (c) sample with *S. carnosus* at the end of second drying; (d) sample with *S. carnosus* + *L. pentosus* at the end of second drying; (e) sample with *S. xylosus* + *L. sakei* at the end of second drying.

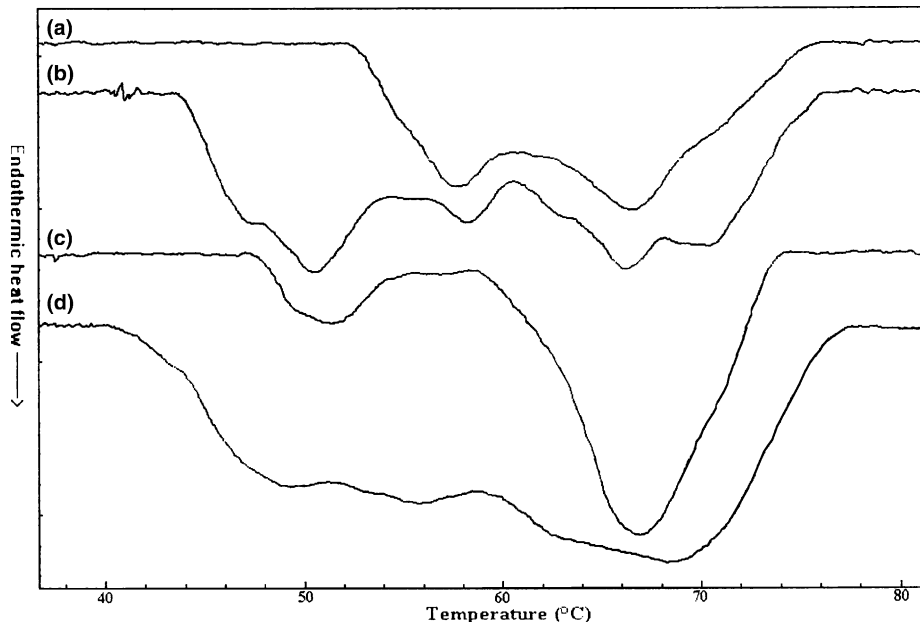


Fig. 2. Differential scanning calorimetry curves of myofibrillar proteins isolated from end-product (pastirma): (a) control; (b) sample with *S. carnosus*; (c) sample with *S. carnosus* + *L. pentosus*; (d) sample with *S. xylosus* + *L. sakei*.

Table 2  
Peak temperatures ( $T_p$ ) and denaturation enthalpies ( $\Delta H_d$ ) of myofibrillar proteins during pastirma processing

Starter culture	Myosin						Actin											
	First stage (raw material)			End of second drying			Pastirma			First stage (raw material)			End of second drying			Pastirma		
	$T_p$ (°C)	$\Delta H_d$ (J/g)		$T_p$ (°C)	$\Delta H_d$ (J/g)		$T_p$ (°C)	$\Delta H_d$ (J/g)		$T_p$ (°C)	$\Delta H_d$ (J/g)		$T_p$ (°C)	$\Delta H_d$ (J/g)		$T_p$ (°C)	$\Delta H_d$ (J/g)	
C	61.20 ± 0.52	4.76 ± 0.30	0.70 ± 0.62	54.50 ± 3.20	4.76 ± 0.30	0.65 ± 0.53	53.23 ± 4.27	0.65 ± 0.53	70.21 ± 0.61	1.49 ± 0.57	1.37 ± 0.43	65.46 ± 1.16	1.44 ± 0.38	70.21 ± 0.61	1.49 ± 0.57	1.37 ± 0.43	65.46 ± 1.16	1.44 ± 0.38
Sc	61.20 ± 0.52	4.76 ± 0.30	0.79 ± 0.38	55.94 ± 2.20	4.76 ± 0.30	0.90 ± 0.28	49.47 ± 2.10	0.90 ± 0.28	70.21 ± 0.61	1.49 ± 0.57	1.41 ± 0.48	65.44 ± 0.62	1.77 ± 0.52	70.21 ± 0.61	1.49 ± 0.57	1.41 ± 0.48	65.44 ± 0.62	1.77 ± 0.52
Sc + Lp	61.20 ± 0.52	4.76 ± 0.30	0.50 ± 0.39	53.45 ± 0.61	4.76 ± 0.30	0.31 ± 0.12	48.48 ± 3.40	0.31 ± 0.12	70.21 ± 0.61	1.49 ± 0.57	2.25 ± 0.70	67.89 ± 0.65	1.42 ± 0.59	70.21 ± 0.61	1.49 ± 0.57	2.25 ± 0.70	67.89 ± 0.65	1.42 ± 0.59
Sx + Ls	61.20 ± 0.52	4.76 ± 0.30	1.82 ± 0.47	58.25 ± 2.13	4.76 ± 0.30	1.28 ± 0.56	53.37 ± 3.87	1.28 ± 0.56	70.21 ± 0.61	1.49 ± 0.57	0.56 ± 0.04	67.79 ± 1.55	1.39 ± 0.09	70.21 ± 0.61	1.49 ± 0.57	0.56 ± 0.04	67.79 ± 1.55	1.39 ± 0.09

C, Control; Sc, *S. carnosus*; Sc + Lp, *S. carnosus* + *L. pentosus*; Sx + Ls, *S. xylosum* + *L. sakei*; ±, standard deviation of three replicates.

stability, possibly due to the liberation of subunits that previously behaved as part of the myosin transition. A greater effect of process on  $T_p$  of myosin was observed for *S. carnosus* + *L. pentosus*. At all of the stages, this mixed starter culture gave the lowest  $T_p$  and  $\Delta H_d$  values.

During processing of pastirma, with addition of salt, the moisture content of the muscle decreased and transition temperatures were decreased as compared to raw material (Table 2). There are three possible explanations for this observation: (1) irreversible denaturation had occurred during drying; (2) the structure of myofibrillar proteins was destabilized with the increase in ionic strength; (3) action of endogenous muscle proteinases, which can increase during the processing period.

Water suppresses thermal denaturation of myofibrillar proteins, perhaps by reducing the number of hydrogen bonds disrupted. Leuscher, Ruege, & Schindler (1974) reported that the enthalpy was progressively increased with increase in water content up to a critical level, and this was attributed to a rise in the number of hydrogen bonds between solvent and solute or nonuniform distribution of water throughout the sample, thereby restricting the amount of protein denatured.

The salt ions are believed to cause weakening of the interaction between oppositely charged side chains (Aktaş & Kaya, 2001; Thorarinsdottir, Arason, Geirsdottir, Bogason, & Kristbergson, 2002). At higher salt concentration in the muscle, proteins may denature, resulting in stronger protein-protein bonds, shrinkage of the muscle and dehydration. This has been attributed to contribution of hydrophobic interactions to the stabilization of the native conformation, probably via a modification of the water structure. Similarly, SDS-PAGE of myofibrillar protein extract showed that sodium chloride could greatly stimulate the bacterial proteolytic system in contrast with endogenous enzymes (Careri, & Mangia, 2003; Fadda, Vignola, Aristoy, Oliver, & Toldra, 2001).

As compared to raw material, processing of pastirma affected the stability of myosin and actin. This effect has been attributed mainly to endogenous proteinases. There is substantial evidence of the rate and extent of proteolysis. Some of this includes: (1) infusion of carcasses with zinc chloride, which inhibits *post-mortem* proteolysis (Koochmaraie, 1994a, 1994b); (2) differences in the extent of *post-mortem* proteolysis between breeds of cattle (Koochmaraie, 1994a), and; (3) differences in rates of *post-mortem* proteolysis between pigs, sheep and cattle (Koochmaraie, 1994b). Also, several experimental reports show that endogenous proteinases can degrade myofibrillar proteins. It is likely that the degradation causes decrease in  $T_p$  and  $\Delta H_d$  values of myofibrillar proteins.

Addition of starter cultures decreased the stability of myosin and actin as compared to control samples. A significant decrease in the peak area was observed, indicating breakdown or denaturation of the myofibrillar

Table 3  
The effects of processing of pastirma and treatment on  $T_p$  and  $\Delta H_d$  values of myofibrillar proteins

	Stage				Treatment		
	Myosin		Actin		Myosin		Actin
	$T_p$ (°C)	$\Delta H_d$ (J/g)	$T_p$ (°C)		$T_p$ (°C)	$\Delta H_d$ (J/g)	$\Delta H_d$ (J/g)
First stage (raw material)	61.20 <sup>a</sup>	4.76 <sup>a</sup>	70.21 <sup>a</sup>	Control	56.31 <sup>ab</sup>	2.04 <sup>b</sup>	1.44 <sup>ab</sup>
End of second drying	55.53 <sup>b</sup>	0.95 <sup>b</sup>	66.79 <sup>b</sup>	<i>S. carnosus</i>	55.53 <sup>ab</sup>	2.15 <sup>b</sup>	1.56 <sup>ab</sup>
Pastirma	51.14 <sup>c</sup>	0.79 <sup>b</sup>	66.65 <sup>b</sup>	<i>S. carnosus</i> + <i>L. pentosus</i>	54.38 <sup>b</sup>	1.85 <sup>b</sup>	1.72 <sup>a</sup>
				<i>S. xylosum</i> + <i>L. sakei</i>	57.61 <sup>a</sup>	2.62 <sup>a</sup>	1.15 <sup>b</sup>

<sup>a-c</sup>Values in a column with the same superscript are not significantly different by Duncan's multiple range test ( $p < 0.05$ ).

proteins. The myofibrillar fraction was hardly degraded by *S. carnosus* and *S. xylosum* + *L. sakei*. As can be seen from Table 1, *S. carnosus* + *L. pentosus* has greater effect than other starter cultures. Probably, this may be explained by the fact that *S. carnosus* + *L. pentosus* had higher proteolytic activity than other starter cultures. The *Staphylococcus* sp. is known to have proteolytic activity and lactic acid bacteria may possess weak proteolytic activity (Johansson, Berdague, Larsson, & Borch, 1994). Also, the difference in the myofibrillar protein degradation between pastirma samples made with either *S. carnosus* or *S. xylosum* starter cultures shows an interaction between the staphylococci starter culture and curing ingredients and/or pH, thus. These environmental factors directly influence staphylococci starter cultures catabolism, as reported by Arnau, Gou, and Comaposada (2003) and Olesen, Meyer, and Stahnke (2004). In other works (Benito, Rodriguez, Martin, Aranda, & Cordoba, 2004; Careri & Mangia, 2003), proteolytic changes obtained after inoculating a known proteolytic starter culture in fermented sausages were compared to those occurring in control sausages. The most evident difference was a lower concentration of some peaks in the sausages inoculated with the starter culture as ripening proceeded, indicating a further hydrolysis of the peptides by starter cultures (*S. carnosus*, *S. xylosum* and *Lactobacillus casei* CRL 705), especially those derived from amino acid catabolism. The electrophoretic studies revealed that proteolysis of high molecular weight myofibrillar and sarcoplasmic proteins was more prominent in protease-treated sausages and pastirma (Aksu, Aktaş, & Kaya, 2002; Hughes, Kerry, Arendt, Kenneally, McSweeney & O'Neill, 2002; Sanz, Fadda, Vignolo, Aristoy, Oliver & Toldra, 1999).

The average pH value of raw material used in pastirma production was 5.89. Treatment was statistically insignificant ( $p > 0.05$ ) on pH values at the end of the second drying, while the treatment had a significant effect on the pH values of pastirma ( $p < 0.05$ ; for control 5.97a, for *S. carnosus* 5.91ab, for *S. carnosus* + *L. pentosus* 5.86b, and for *S. xylosum* + *L. sakei* 5.69c). The lowest  $T_p$  value was obtained in pastirma having pH 5.86, produced with *S. carnosus* + *L. pentosus*, and the highest  $T_p$  value

was obtained from *S. xylosum* + *L. sakei* having pH 5.69. Even though *S. xylosum* + *L. sakei* has more acid production capacity than *S. carnosus* + *L. pentosus*, it is likely that the low level of the  $T_p$  obtained from *S. carnosus* + *L. pentosus* mixed cultures was because of the *S. carnosus* having proteolytic activity. In addition, *L. sakei* has limited proteolytic and lipolytic activity and produces a limited amount of pure lactic acid in support of the case mentioned above. It has also been reported that high pH caused production of branched-chain acids by *S. carnosus* while pH had the opposite effect for *S. xylosum* (Olesen et al., 2004). These results coincide well with the results in the present study.

#### 4. Conclusions

This study showed that commercial starter cultures contributed to breakdown of myofibrillar proteins, particularly myosin. The degree of protein degradation was highly dependent on the commercial starter cultures. Processing significantly decreased the thermal stability of myosin and actin. The protein denatured at low temperatures and with little energy input. The DSC method offers an alternative for the monitoring of protein degradation due to its appreciable time-saving, use of small samples with minimal preparation, and absence of toxic chemicals.

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