Effect of Commercial Starter Cultures on the Myofibrillar Proteins of Pastirma, a Turkish Dry Meat Product

M.I. AKSU, N. AKTAS, M. KAYA

ABSTRACT: The effect of 3 different commercial starter cultures (*Staphylococcus carnosus*, *Staphylococcus carnosus* + *Lactobocillus pentosus* and *Staphylococcus xylosus* + *Lactobacillus sakei*) on the myofibrillar proteins of pastirma was investigated. It was observed that using commercial starter cultures in pastirma manufacture had a significant effect on myofibrillar fragmentation. However, the effect of each starter culture on myofibrillar fragmentation in pastirma was different. Most of the changes in myofibrillar proteins were produced with *S. carnosus* + *L. pentosus*.

Keywords: pastirma, myofibrillar proteins, starter culture, proteoliytic activity, NPN, meat product

Introduction

PASTIRMA IS THE MOST POPULAR DRY-CURED MEAT PRODUCT produced in Turkey. It is produced from whole muscle obtained from certain parts of beef and water buffalo carcasses. After removing fat and connective tissue from surfaces, muscles are cured, dried, pressed, and coated with a paste (cement) of garlic, paprika, red pepper, and water, and again dried. In this period (approximately 1 mo), significant structural changes in the meat take place. Pastirma, a traditional Turkish meat product, is categorized as an intermediate-moisture food (IMF) (Leistner 1988; Kaya and others 1996; Gökalp and others 1999; Aksu and Kaya 2001a).

Intense proteolysis has been observed during the dry-curing process in meat products (Molina and Toldra 1992; Buscailhon and Monin 1994; Toldra 1994). Dry-cured hams develop a characteristic texture and flavor as a result of biochemical changes (Bellati and others 1983). Toldra and others (1993) reported that the muscle proteinases remain active during the ripening process in this type of meat product, and these enzymes are broken down into peptides. The amounts of nonprotein nitrogen are increased in the ripening process of pastirma and dry-cured ham. This increase confirmed the breakdown of proteins in the ripening process (Martin and others 1998; Aksu and Kaya 2001 b, Aksu and Kaya 2002a). Not only the formation of aroma, but also tenderization in meat occur in the dry-cured meat products during ripening. Price and Schweigert (1987) reported that the tenderization is increased because of myofibrillar protein fragmentation during ripening of meat products.

Various researchers have stated that using starter cultures in dry-cured raw meat products like pastirma have improved the quality properties of the end-product (Katsaras and others 1996; Aksu and Kaya 2001 b, Aksu and Kaya 2002 a, b). Some members of *Micrococcaceae* (*Staphylococcus carnosus, S. xylosus, Micrococcus varians)* can be used as starter cultures in similar meat products (Jessen 1995; Hammes and Hertel 1998). These microorganisms generally have nitrate reductase activity (Kröckel 1995). These microorganisms also have proteolytic and lipolytic activities, which cause increases in flavor compounds (Johansson and others 1994; Fadda and others 1999). The microorganisms used for meat fermentation consist of a group of lactic acid bacteria (*Lactobacillus plantarum, L. sakei, L. curvatus, L. pentosus*) in the starter culture, plus the *Micrococcaceae* (Schelifer and Schöne 1978; Kröckel 1995). However; the lactic acid bacteria possess weaker proteolytic activity than the *Micrococcaceae* (Lücke and Hechelmann 1985; Johanssonn and others 1994).

There has been some research on pastirma produced with starter cultures in Turkey (Aksu and Kaya 2001 a, b; 2002 a, b). However, there has been no research on the effects of commercial starter cultures on the myofibrillar proteins of pastirma. The purpose of this study was to determine the effect of commercial starter cultures on the myofibrillar proteins of pastirma by using SDS–PAGE.

Materials and Methods

Production pastirma

Replicates (Replicate 1, Replicate 2, and Replicate 3) were carried out in this study. *Longissimus dorsi* muscle (24 h post mortem) from beef carcasses was used as the raw material. After removing fat and connective tissue from surfaces, muscle tissue was cut vertically into 2 pieces. Four pastirma lots were produced from the carcass coming from a 3 y old cow. One carcass was used for each replicate (total of 3 carcasses). In a treatment, commercial preparations of *Staphylococcus carnosus* (Bactoferm™ C-P–77), *Staphylococcus carnosus* + *Lactobocillus pentosus* (Bactoferm™ C-P–77 S), and *Staphylococcus xylosus* + *Lactobacillus sakei* (Bactoferm™ B–FM) were used as starter cultures (Chr Hansen's, Pohlheim, Germany). Starter culture (25 g starter / 100 kg meat) was introduced into muscle with the curing mixture (47.0 g NaCl, 0.75 g KNO_3, 1.0 g glucose, 1.0 g sucrose; for 1 kg meat: 50 g curing mixture, dry curing), and the curing process was carried out in different trays for each group (dry curing). Pastirma samples made without starter cultures were used as control group. The production process of pastirma is given in Table 1 (Aksu 1999; Gökalp and others 1999; Aksu and Kaya 2001b). The paste mixture (cement) used in this study contained 500 g flour of *Trigonella foenum graecum* seed, 350 g smashed fresh garlic, 75 g paprika, 75 g red pepper, and 1200 mL water. After the 2nd drying step, dried meat samples were mounted with paste (cement) and stored for 4 d. Then the surface of the meat was coated with the paste mixture (3 to 4 mm thickness) (Aksu 1999; Aksu and Kaya 2001b).

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Isolation of myofibrillar proteins

Isolation of myofibrillar proteins were according to the method of Parrish and others (1973) with some modifications (Claeys and others 1995). Minced meat (2.5 g) was homogenized using an Ultra-Turrax and 25 mL of buffer solution (pH 7.6, 3 °C, 0.05 M sucrose, 0.05 M tris, 1 mM EDTA). After centrifugation (1000 × *g* 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 at 1000 × *g* for 10 min, again centrifuged. The supernatant was decanted and the treatment was repeated with 25 mL of KCl solution (3 °C, 0.l5 M KCl). After the isolation, the pellets were transferred into volumetric flasks and frozen (–40 °C), and then lyophilized.

Sample preparation for SDS–PAGE

Lyophilized myofibrillar protein (10 mg) was dissolved, at room temperature, in 2 mL sample buffer (pH 6.8, Tris, glycerin, SDS) and 10 µL 2–mercaptoethanol. Then the sample solutions were filtered to remove connective tissue (Claeys and others 1995).

Analysis of SDS–PAGE

Characterization of myofibrillar proteins was carried out using SDS–PAGE (Laemmli 1971; Aktas and Kaya 2001). A vertical slab gel system (SE 600 Series; Hoefer Scientific Instruments (HSI), San Francisco, Calif., U.S.A.) was used to prepare a 1.5 mm thick gel. A 5% stacking gel and 12.5% separating gel were used to separate the protein bands. Electrophoresis was conducted at 80 and 130 volt, respectively. The sample (40 µL) was injected in each samples slot.

Analysis of moisture, salt, NPN and pH

Nonprotein nitrogen values (Anonymous 1989), moisture and pH values (Ockerman 1985; Gökalp and others 2001), salt values (Tauchman 1987) were determined.

Statistical analysis

Completely random block design was used in the statistical analysis. Results were analyzed by ANOVA. Comparisons of mean values were made using the Duncan test (SAS 1990).

Table 1—The stages in the pastirma production process.

Production stage	Time	Temperature (°C)	Relative humidity (%)
Dry-curing*	2 d	6 ± 1	80 to 90
First drying	4 d	15 ± 1	80 to 85
First pressing**	17 h	7 ± 1	
Second drying	3d	20 ± 1	70 ± 2
Second pressing**	7 h	25 ± 1	
Paste cemening	4 d	7 ± 1	
The last drying	2 d	15 ± 1	70 ± 2
	2 d	18 ± 1	65 ± 2
	6 d	20 ± 1	60 ± 2

*For 1 kg meat, 50 g curing mixture.
**For 1 kg meat, 25 kg weight.

Results and Discussion

ELECTROPHORETIC PATTERNS OF MYOFIBRILLAR PROTEINS OF RAW material, the sample from the end phase of 2nd drying, and the end product are shown in Figures 1, 2, and 3. As can be seen, changes in myofibrillar proteins of the raw material (line a), the samples from the end of 2nd drying (line b), and the control pastirma samples (line c, no starter culture) were determined. The myosin heavy chain, which was especially seen in the raw material, was relatively weak in the control group pastirma (line c), indicating a fragmentation of this chain as a result of processing. This fragmentation may be explained by the actions of some bacteria with high proteolytic activity, which can grow during the ripening period and also by the endogenous muscle proteinases. Gehlen (1998) reported that active muscle proteinases mainly contribute to proteolysis. Starter cultures used in the treatments have a significant effect on the myosin chain. The most apparent difference in myosin chain was recorded in pastirma produced with *S. carnosus* + *L. pentosus* (line g). *S. carnosus* and *S. xylosus* + *L. sakei* (line e, i) had no effect on myosin chain as compared with the control pastirma (line c). However, the myosin band was weaker in pastirma with *S. carnosus* (line e) and *S. xylosus* + *L. sakei* (line i) than the raw material (line a).

As in myosin chain, changes in the actin in pastirma samples were also found. The density of actin bands decreased in control pastirma samples produced in replicate 1 (Figure 1) and 3 (Figure 3), but it had no changes in replicate 2 (Figure 2). The greatest change among the pastirma samples occurred in pastirma with *S. carnosus* + *L. pentosus*. The actin band was weakened in pastirma with *S. carnosus* + *L. pentosus*, as compared to control and other pastirma groups. This may be explained by the fact that *S. carnosus* + *L. pentosus* had higher proteolytic activity than the other starter cultures (*S. carnosus, S. xylosus* + *L. sakei*). *S. xylosus* + *L. sakei* had no effect on actin bands. Changes in NPN values were similar to the above-mentioned results. In replicates 1, 2, and 3, NPN values of raw material were determined as 1.97%, 2.30%, and 2.29%, respectively. NPN values at the end of the 2nd drying were increased because of protein degradation. The end of 2nd drying, NPN values in control samples were as 3.70%, 3.60%, and 3.38%, respectively. NPN values of samples with *S. carnosus, S. carnosus* + *L. pentosus*, and *S. xylosus* + *L. sakei* were 3.48%, 3.66%, and 3.68%; 3.84%, 4.07%, and 4.07%; and 3.27%, 3.45%, and 3.60%, respectively. The greatest NPN value was observed in pastirma samples produced with *S. carnosus* + *L. pentosus*, with an average of 5.05% (Table 2). The pH values of raw materials used in pastirma production were

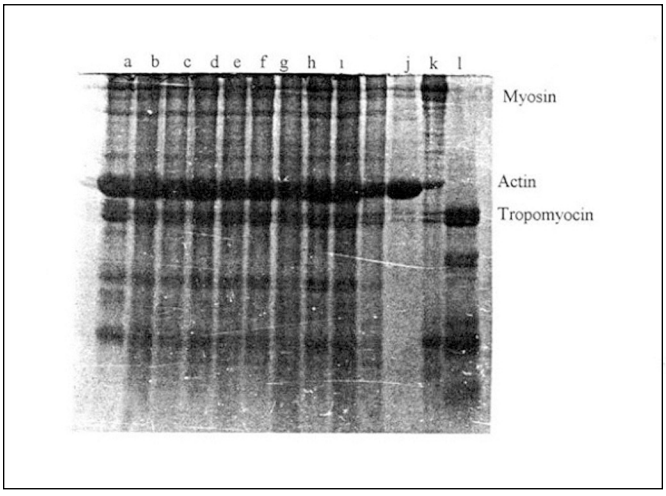

Figure 1—Electrophoretogram (SDS–PAGE) of myofibrillar proteins in replicate 1. (a): raw material (meat), (b): control sample (2nd drying), (c): control pastirma (without starter cultures), (d): sample with *S. carnosus* (2nd drying), (e): pastirma (with *S. carnosus*), (f): sample with *S. carnosus* + *L. pentosus* (2nd drying), (g): pastirma (*S. carnosus* + *L. pentosus*), (h): sample with *S. xylosus* + *L. sakei* (2nd drying), (i): pastirma (*S. xylosus* + *L. sakei*), (j): standard actin, (k): standard myosin, (l): standard tropomyosin.

Table 2—Moisture, salt, NPN and pH values in pastirma samples and results of the Duncan test.

Starter	Moisture	Salt	pH	NPN(%) 2nd drying	NPN(%) Pastirma
Control	44.31 a	5.31 a	3.56	4.84 a	5.97 a
S. carnosus	44.52 a	5.35 a	3.61	4.78 a	5.91 ab
S. carnosus + L. pentosus	42.55 a	5.49 a	3.99	5.05 a	5.86 b
S. xylosus + L. sakei	44.52 a	5.06 a	3.44	4.,74 a	5.69 c

a, b, c, Any 2 means in the same column having the same letters are not significantly different at P < 0.05.

determined in treatment 1, 2, and 3 as 6.00, 5.92, and 5.70. The lowest pH was obtained in pastirma produced with *S. xylosus + L. sakei* (5.67), while the highest value was obtained in control pastirma (5.98) (Table 2). The pH values of pastirma with *S. xylosus + L. sakei* were close to the isoelectric point of myofibrillar protein, which prevented proteolytic activity of *S. xylosus* in this culture. Johansson and others (1994) recorded similar results, indicating that the solubility of myofibrillar proteins decreased as the pH dropped from 5.7 to 4.9. Aksu and Kaya (2002b,c) also reported that *S. carnosus* has an effect on protein degradation of pastirma, and more so than *S. xylosus*. Furthermore, Molina and Toldra (1992) reported that in dry-cured hams *S. xylosus* showed weaker proteolytic activity than *P. pentosaceus*.

The density of tropomyosin band in the raw material was clear (line a), while this band was relatively weaker in the control pastirma samples. In myosin and actin, the most apparent change in tropomyosin was determined in pastirma samples with *S. carnosus + L. pentosus*. The density of tropomyosin band was weaker in pastirma with *S. carnosus + L. pentosus* than that with *S. carnosus*. *S. xylosus + L. sakei* mixed culture had no effect on the tropomyosin bands, as compared to control pastirma samples (Figure 1, 2, and 3).

Some of the weak bands seen on actin in the raw material and standart myosin disappeared during the processing stages in pastirma with starter culture (Figure 1, 2, and 3).

Starter cultures also had an effect on proteins with lower molecular weight than tropomyosin (Figure 1,2, and 3). The mixed cultures of *S. carnosus + L. pentosus* had especially more effect on these myofibrillar proteins than other commercial starter cultures.

Conclusions

THE MAJOR EFFECTS ON MYOFIBRILLAR PROTEINS OF PASTIRMA WERE with *S. carnosus + L. pentosus*, as compared to control pastirma. The myofibrillar fragmentation was higher in samples with *S. carnosus* than that with *S. xylosus + L. sakei*. Results of this study indicated that the effects of each starter culture on myofibrillar proteins were different.

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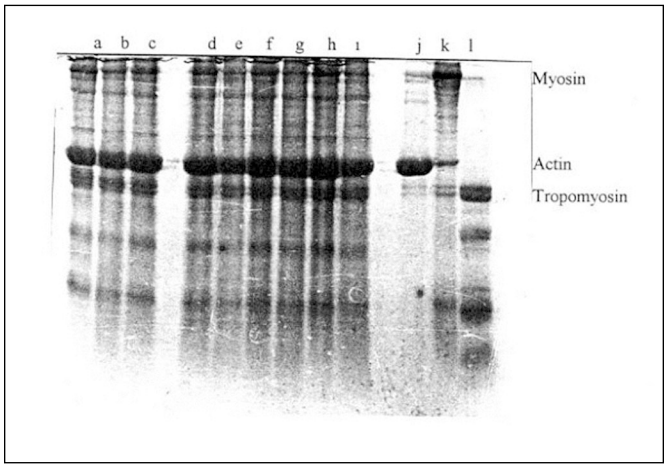

Figure 2—Electrophoretogram (SDS–PAGE) of myofibrillar proteins in replicate 2. (a): raw material (meat), (b): control sample (2nd drying), (c): control pastirma (without starter cultures), (d): sample with *S. carnosus* (2nd drying), (e): pastirma (with *S. carnosus*), (f): sample with *S. carnosus + L. pentosus* (2nd drying), (g): pastirma (*S. carnosus + L. pentosus*), (h): sample with *S. xylosus + L. sakei* (2nd drying), (i): pastirma (*S. xylosus + L. sakei*), (j): standard actin, (k): standard myosin, (l): standard tropomyosin.

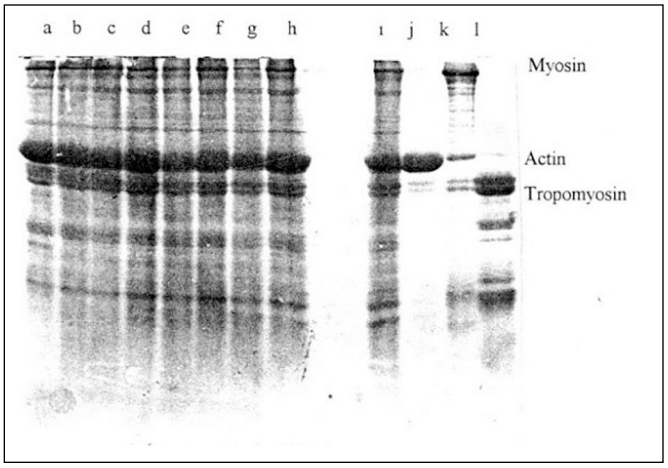

Figure 3—Electrophoretogram (SDS–PAGE) of myofibrillar proteins in replicate 3. (a): raw material (meat), (b): control sample (2nd drying), (c): control pastirma (without starter cultures), (d): sample with *S. carnosus* (2nd drying), (e): pastirma (with *S. carnosus*), (f): sample with *S. carnosus + L. pentosus* (2nd drying), (g): pastirma (*S. carnosus + L. pentosus*), (h): sample with *S. xylosus + L. sakei* (2nd drying), (i): pastirma (*S. xylosus + L. sakei*), (j): standard actin, (k): standard myosin, l: standard tropomyosin.

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The authors are with the Food Engineering Dept., Agricultural College, Atatürk Univ., 25240, Erzurum, Turkey. Direct inquiries author Aksu (E-mail: miaksu@atauni.edu.tr or miaksu@hotmail.com).