EFFECTS OF CALCIUM CHLORIDE AND POTASSIUM CHLORIDE ON THE ALTERATIONS IN PROTEINS DURING PROCESSING OF REDUCED-SALT PASTIRMA, A DRY CURED BEEF

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Abstract – This study was conducted to evaluate the effects of adding selected salt mixtures as partial replacers for NaCl on proteolytic changes during processing of reduced-salt pastırma. Beef cuts were cured using 4 different salt combinations: 1) NaCl standard formulation (SC), 2) 50% NaCl (RS) 3) 50% NaCl + 50% KCl (PC), and 4) 50% NaCl + 50% CaCl (CC). Proteolytic changes were measured by assaying sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) profiles of sarcoplasmic and myofibrillar proteins, non-protein nitrogen (NPN), α-amino nitrogen (α-AN) and salt soluble protein (SSP) contents at selected processing stages, namely, initial, curing, first drying, third drying and finished product. NPN and α-AN contents showed significant increases ($p<0.05$) in all groups during processing with respect to the initial value. It was observed that CC resulted in higher NPN content as compared to the other groups, and PC had the highest α-AN content ($p<0.05$). SSP content decreased in all groups during processing due to denaturation of proteins, where the most noticeable effects were detected in CC. Divalent $CaCl_2$ (CC formulation) also had a strong denaturation effect on both sarcoplasmic and myofibrillar proteins as determined by SDS-PAGE.

I. INTRODUCTION

Dietary intake of sodium, from all sources, has been associated with hypertension, gastric cancer, osteoporosis, cataracts, kidney stones and diabetes, and should be limited for a healthy life [1, 2]. Salt reduction in processed meat products is one of the new approaches to produce functional products since there have been great interest of reducing sodium in food products to minimize health risks related to excessive intake of dietary sodium. Processed meat products constitute an important part of dietary sodium intake [3, 4]. Pastırma, a traditional Turkish dry cured meat product, is one of the most popular types of meat products in Turkey in which sodium chloride content might reach 9% [5].

Proteolysis is one of the main reactions affecting final product characteristics of dried cured meats such as texture and flavor [4, 6]. Salt in meat products not only contributes to salty taste and product stability, it also imparts an important role in proteolytic reactions, resulting in changes in texture, as well as generation of typical flavor of aged products due to accumulation of low molecular weight peptides and free amino acid [4]. The objective of this study was to evaluate the effects of partial replacement of CC or PC for NaCl on changes in proteins during processing of reduced-salt pastırma.

II. MATERIALS AND METHODS

Pastırmas were manufactured using beef *M. longissimus dorsi* muscles with an average weight of 6 kg each, obtained from two different animals (for the two replications) slaughtered in a local slaughterhouse in the area of Ankara 24 h postmortem. The beef parts were randomly divided into 4 groups: 1) Traditional NaCl dry curing (SC), 2) Dry curing with 50% reduced NaCl (RS), 3) Dry curing with partial replacement of NaCl with KCl as 50% NaCl + 50% KCl (PC), and 4) Dry curing with partial replacement of NaCl with $CaCl_2$ as 50% NaCl + 50% $CaCl_2$ (CC). The muscles were subjected to curing by manually rubbing the meat with the standard curing salt mixture of 6% for SC, PC and CC, and 3% for RS of the meat weight.

Proximate composition (protein, ash, fat, moisture) and pH were determined by standard AOAC methods [7].

Alterations in sarcoplasmic and myofibrillar protein fractions during processing were monitored with SDS-PAGE according to the procedures reported by Toldra et al. (1992) and Toldra (1993) (for protein extraction) [8, 9], and Laemmli (1970) (for SDS-PAGE) [10]. Water soluble proteins extracted by the method of Wang (2001) [11] were used for determination of non-protein nitrogen (NPN) and α-amino nitrogen (α-AN) contents [12]. NPN measurement was performed with trichloroacetic acid precipitation method. NPN filtrate was analyzed for nitrogen with Kjeldahl method [7] and expressed as mg N/100 g dry matter (DM). For α-AN measurement, the water soluble protein extract was assayed by reacting primary amines (free amino acids and acyl-peptides) as determined spectrophotometrically from absorption at 340 nm following reaction of the amines with o-phtaldehyde [12]. Data from two replications were analyzed by analysis of variance with the general linear models procedure of SAS. Means were separated ($p<0.05$) using least significant difference procedure.

III. RESULTS AND DISCUSSION

Moisture, protein and fat contents of pastırma samples were 53.7-55.0%, 28.8-30.3%, 9.7-10.4%, respectively, which did not differ ($p>0.05$) between the groups. RS had lower ($p<0.05$) ash content (4.7%) than the other three groups (5.8-6.3%) due to the reduction of salt without any replacement. $CaCl_2$ added group (CC) possessed significantly lower ($p<0.05$) pH value (pH=5.1) as compared to SC, RS and PC which had pH values of around 5.6 in the finished pastırma.

In the SDS PAGE profiles of sarcoplasmic proteins, differences were observed due to the processing and treatment (Fig. 1). For instance, the band at 107 kDa increased in SC and RS groups; however, the intensity of this band decreased through the finished product in group PC, and totally disappeared in group CC after the first drying process. The band at 48 kDa, despite the decrease in the intensity in groups PC and CC due to the proteolysis, increased in intensity in SC and RS. In all groups, towards the end of the processing, new fractions were formed in the ranges 32-25 kDa. Fig. 2 shows SDS-PAGE profiles of myofibrillar proteins of the pastırma samples during processing. The intensity of the band at 202 kDa, estimated as heavy myosin band, decreased in all groups, but it increased markedly in group CC. While the intensity of the band at 102 kDa decreased through processing only in SC, there were slight decreases in the intensities of the bands at 65 kDa and the one estimated as actin (42 kDa) in group CC. With the exception of RS, the bands in the ranges 30-22 kDa also showed decreases.

The NPN content in all groups increased significantly ($p<0.05$) during processing due to the breakdown of proteins (data not shown), with the highest value at the end of processing. In the finished products, sodium replacement with PC did not result in a difference in NPN content ($p>0.05$) as compared to the SC group (Fig. 3). The lowest NPN content was detected in RS and the highest in CC ($p<0.05$).

α-amino nitrogen (α-AN) contents showed significant increases ($p<0.05$) during processing, particularly after the third drying period in all groups. The highest α-AN ($p<0.05$) was observed in PC in the finished product (Fig. 4).

Salt soluble proteins (SSP) decreased ($p<0.05$) in all groups after the curing process as compared with the initial value (Fig. 5). In the finished product, the most remarkable change in SSP was detected in CC, which could be attributed to the strong denaturing ability of divalent salts on myofibrillar proteins. On the contrary, the highest SSP determined in RS was likely due to the lower salt content.

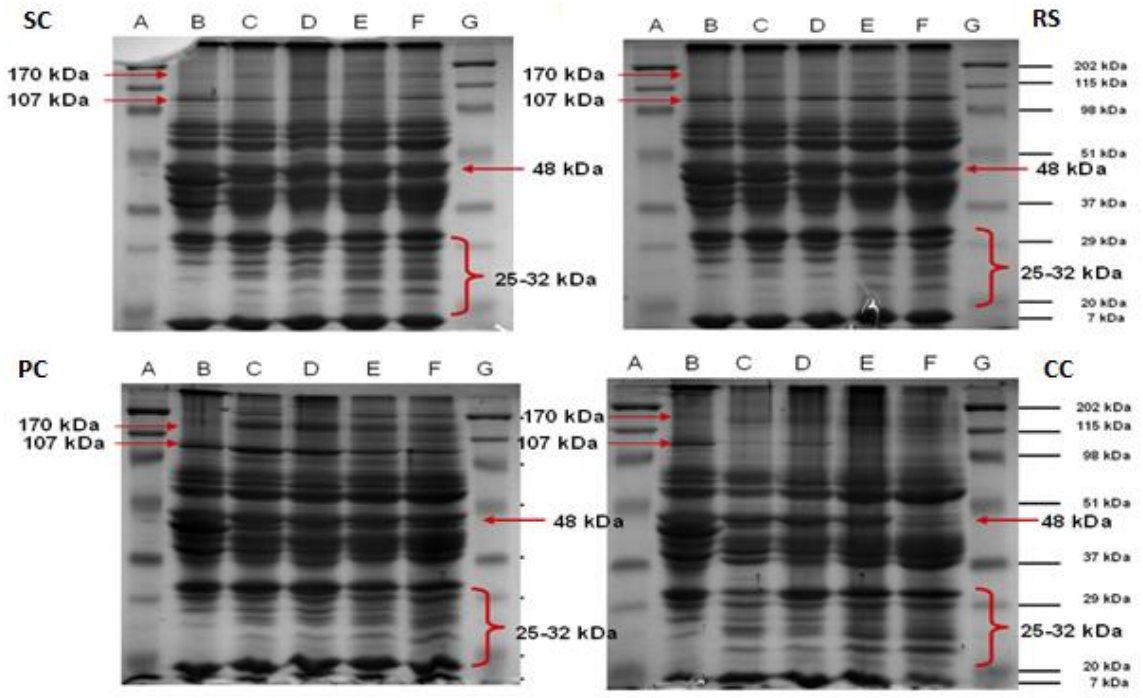

Fig. 1. SDS–Polyacrylamide Gel Electrophoretic patterns of sarcoplasmic proteins during pastırma processing. A,G: Molecular weight markers, B: Initial, C: Curing, D:1st Drying, E: 3rd Drying, and F: Finished pastırma.

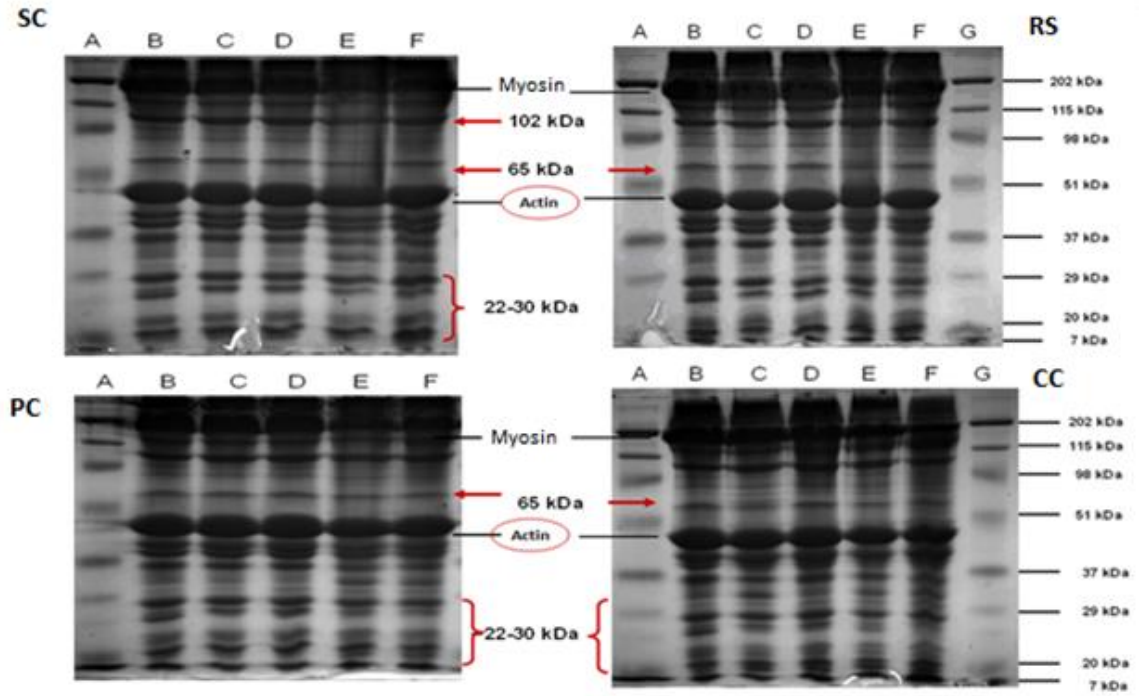

Fig. 2. SDS–Polyacrylamide Gel Electrophoretic patterns of sarcoplasmic proteins during pastırma processing. A,G: Molecular weight markers, B: Initial, C: Curing, D:1st Drying, E: 3rd Drying, and F:* Finished pastırma.

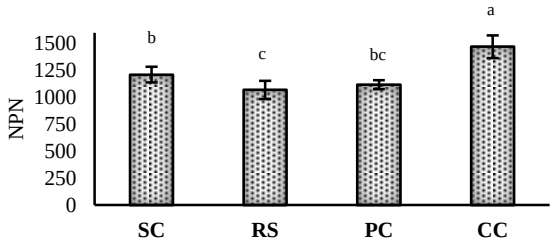

Fig. 3. NPN contents (mg /100 g DM) of finished pastırma. Mean ± standard deviation. ($^{a-c}$): Bars having different letters are different ($p<0.05$).

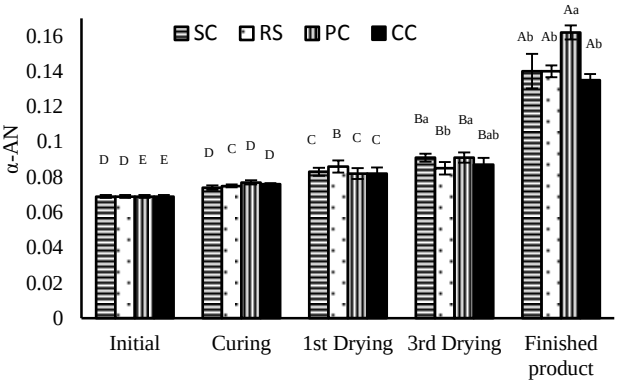

Fig. 4. α-AN (absorbance at 340 nm) during pastırma processing. Mean ± standard deviation. ($^{a-b}$): within a processing stage between groups, and ($^{A-E}$): within a group between processing stages, bars having different letters are different ($p<0.05$).

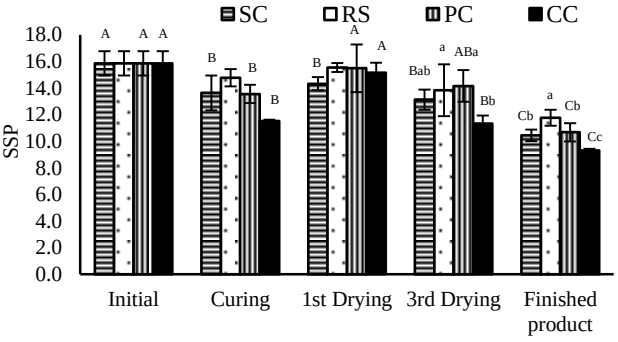

Fig. 5. SSP contents (g/100 g DM) during pastırma processing. Mean ± standard deviation. ($^{a-c}$): within a processing stage between groups, and ($^{A-C}$): within a group between processing stages, bars having different letters are different ($p<0.05$).

IV. CONCLUSIONS

Reducing salt in meat products without negatively affecting ultimate product characteristics is an important issue. Excessive proteolysis might impart undesirable sensory and physical characteristics. In the current study, the most noticeable changes in proteins were observed in the pastırma with partial replacement of salt with divalent $CaCl_2$, particularly in sarcoplasmic and myofibrillar proteins, which might impact undesirable sensory and physical properties. Partial replacement of salt with KCl did not generally result in significant changes as compared to the salt control, and would be a more preferable treatment in manufacturing reduced salt pastırma.

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