EFFECT OF VARIOUS SALTS ON APPEARANCE OF MYOSIN AND α-ACTININ IN CENTRIFUGAL DRIP OF MEAT

B. GRZEŚ, E. POSPIECH, M.L. GREASER*, P. E. MOZDZIAK**,A.A.SOSNICKI***

Institute of Meat Technology, Agricultural University of Poznań, ul.Wojska Polskiego 31, 60-624 Poznań, Poland, *Meat Science and Muscle Biology Laboratory, University of Wisconsin-Madison, 1805 Linden Drive West, Madison, WI 53706 USA, ** Department of Anatomy, University of Wisconsin-Madison, 1300 University Avenue, Madison, WI 53706 USA, ***PIC 3033 Nashville Road, Franklin, KY 42134 USA

Keywords: water holding capacity of meat, centrifugal drip, myosin, α-actinin

INTRODUCTION

To the most common components of curing mixtures include sodium chloride, sodium nitrite, ascorbate and pyrophosphate. Recently carbonates have been used as well (Hammer 1993). The main effects of these salts on water holding capacity (WHC) of meat is connected with increasing the electrostatic forces, osmotic forces and the extraction/liberation of proteins from the myofibril. The experiments of Offer and Trinick (1983) have shown that water holding properties of meat are enhanced by using salts which cause the liberation of structural proteins from the A and I bands of the sarcomere. They hypothesized that this occured due to removal of myofibrillar constraints such C-protein, and M-proteins, and proteins in the Z-disk. The studies of Wang and Greaser (1985) revealed that selective extraction of myofibrillar proteins changed arrangement of cytoskeletal proteins. The solubilization of cytoskeletal proteins from muscle was confirmed by Paterson et al. (1988). In principle none of these studies observed the liberation or removal of α-actinin from the sarcomere, what occurs after prolonged storage of meat (Taylor et al. 1995). The aim of the current study was to analyse the effect of various salt mixtures on WHC of meat and to determine if WHC was correlated with the liberation of myosin and α-actinin from the myofibrillar structure in meat drip. Myosin is usually considered the main protein responsible for the WHC and α-actinin is considered an indicator of the integrity of myofibrillar structure (Taylor et al. 1995).

MATERIAL AND METHODS

Bovine m. quadriceps femoris was excised from six chilled carcasses 48 hours after the slaughter. The pH value of meat varied between 5.4 ÷5.7. After mincing it was divided into 4 portions. Either water or solutions containing curing salts were mixed with each portion (40% by weight). To the first portion water (W) was added. A solution containing 2% of sodium chloride, 0.0125% sodium nitrate and 0.03% sodium ascorbate was added to the second portion (S). To the third portion 0.125% sodium pyrophosphate (P) plus the components in (S) were added. Portion 4 was mixed with 0.2% sodium carbonate (Ca) plus the components in (S). Each portion was divided into 3 parts. Part 1 had no pH adjustment, part 2 was adjusted to pH 6.4, and part 3 was adjusted to pH 7.0. The pH adjustments were achieved using either 3N NaOH or 1N HCl solutions. Samples were held 1 hour after the salt mixing and further 0.5 hour after pH adjustment. Samples (about 8 g) were centrifugated at 30,000 x g for 20 minutes and the supernatant solutions (referred to subsequently as centrifugal drip) retained for electrophoretic analysis. Centrifugation was prolonged to 1 hour for samples with pH value adjusted to 7.0. The amount of centrifugal drip was used to estimate the WHC of meat. It was assumed that high WHC is inversely related to amount of centrifugal drip. Proteins from the drip were analyzed using the polyacrylamide gel electrophoresis on a Hoefer SE 250 Mighty Small unit according to procedure of Fritz and Greaser (1991). Western blotting was conducted (Fritz and Greaser, 1991) to confirm the presence of myosin and α-actinin in the centrifugal drip. Anti-myosin monoclonal MF-20 (Bader et. al., 1982) and anti-α-actinin monoclonal EA-53 (Sigma Chemical Company) were used as primary antibodies.

RESULTS AND DISCUSSION

Results presented in Fig. 1 showed that salts and differences in pH values influenced WHC of meat as measured by the amount of centrifugal drip (cd). The biggest differences among the samples in the amount of cd were observed when pH values of meat were not adjusted. The highest pH value was in the sample treated with carbonates (6.17). In two other samples treated with salts (S and P) the pH value were 5.67 and 5.78 respectively. Difference in pH values between the samples was probably the main factor for the low amount of centrifugal drip in the meat treated with carbonates. When the pH values were adjuested to 6.4 and 7.0 the differences among the samples treated with salts were smaller and statistically not significant ($P>0.05$).

The electrophoretic patterns of proteins from centrifugal drip (Fig. 2) showed bands in the positions of the myosin heavy chain and α-actinin. Their presence in drip was confirmed by western blotting. The amount of these proteins in the drip was dependent on type of sample. The highest amount of myosin was observed in the drip from meat treated with P and none was found in meat only treated with water (W). The α-actinin was found only in samples from meat treated with P independently to the pH value. These observations indicate that the enhanced WHC of cured meat could be caused through increased pH value and through the destruction of myofibrilar structural constraints. The removal of α-actinin from the Z-disk is one likely factor that weakens the myofibrillar network beside the pyrophosphate mediated dissociation of actomyosin (Highsmith, 1976). These structural changes may allow more space to form between the myofilaments and thus increase WHC.

CONCLUSIONS

1. Curing salts liberate/remove the myosin and α-actinin from myofibrils in meat. This process depends on the type of salts used.
2. Alpha-actinin was found only in drip from meat treated with a curing mixture containing pyrophosphate and was independent of pH value.
3. The release of α-actinin from myofibrils in meat may promote WHC and seems to be most important at low pH values.

ABSTRACT

The main effects of curing salts on water holding capacity (WHC) of meat is connected with increasing the electrostatic forces, osmotic forces and extraction/liberation of proteins from the myofibrils. The release of α-actinin from the sarcomere has been observed after prolonged storage of meat and has been used as an indicator of loss myofibrillar structural integrity. Its removal from the Z-disk by salts seems to influence the liberation of myosin from the myofibrilar structure and therefore increases the WHC of meat. The aim of this study was to analyse the effects of various salt mixtures on WHC of meat and the appearance of myosin and α-actinin in meat drip. The experiments were conducted on six chilled bovine m. quadriceps femoris. After mincing they were divided into 4 portions. Either water or solutions containing curing salts (sodium chloride, nitrite, ascorbate, pyrophoshate and sodium carbonate) were mixed with each portion (40% by weight). Additionally each portion was divided into 3 parts according to meat pH value (no correction, pH 6.4 and pH 7.0). The amount of centrifugal drip was used to estimate the WHC of meat. Proteins from the drip were analyzed using polyacrylamide gel electrophoresis. The presence of myosin and α-actinin in the centrifugal drip was confirmed by western blotting. Results of this study revealed that curing salts release some myosin and α-actinin from meat. This process depends on type of salts used. The α-actinin was found only in drip from meat treated with curing mixture containing pyrophosphate at all three pH values examined. The release of α-actinin from the Z-disks of meat may promote WHC and seems to be an especially important factor at low pH values.

REFERENCES

Bader D., Masaki T., & Fischman D. (1982): Immunochemical analysis of myosin heavy chain during avian myogenesis in vivo and in vitro. J. Cll Biol. 95, 763-770

Fritz J. & Greaser M.L. (1991): Changes in titin and nebulin in postmortem bovine muscle revealed by gel electrophoresis, western blotting and immunofluorescence microscopy. J. Food Sci., 56, 607-615

Hammer G. F. (1993): pH-Wert-Modifizierung bei Brühwurst durch Zusatzstoffe. Fleischwirtsch. 73, 10, 1122 - 1127

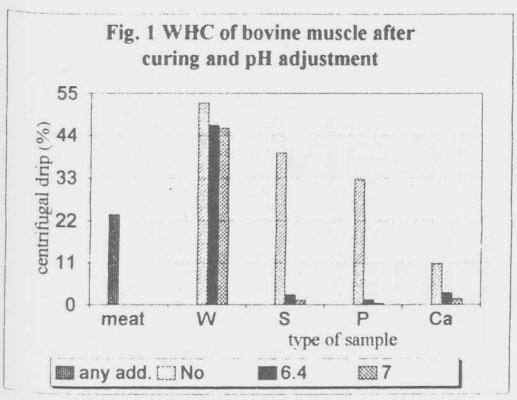
Offer G. & Trinick J. (1983): On the mechanism of water holding in meat: The swelling and shrinking of myofibrils. Meat Sci. 8, 245-281

Highsmith S. (1976): Interaction of the actin and nucleotide binding sites on myosin subfragment 1. J. Biol. Chem. 251, 6170- 6172

Paterson B.C., Parrish F.C.Jr., & Stromer M.H. (1988): Effects of salts and pyrophosphate on the physical and chemical properties of beef muscle. J. Food Sci. 53, 5, 1258 - 1265

Taylor R.G., Geesink G.H., Thompson V.F., Koohmaraie M. & Goll D.E.(1995): Is Z-disk degradation responsible for postmortem tenderization? J. Anim. Sci. 73, 1351-1367

Wang S-M. & Greaser M.L. (1985): Immunocytochemical studies using a monoclonal antibody to bovine cardiac titin on intact and extracted myofibrils. J. Muscle Res. & Cell Motility 6, 293 - 312



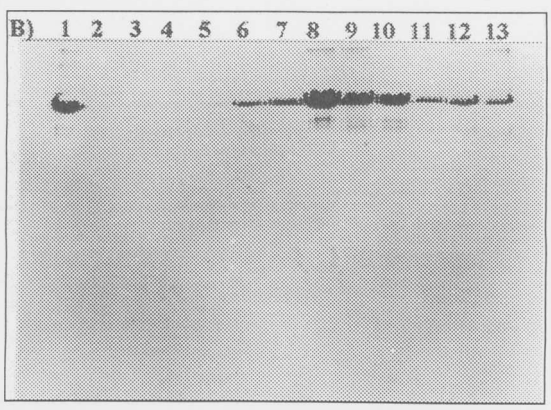
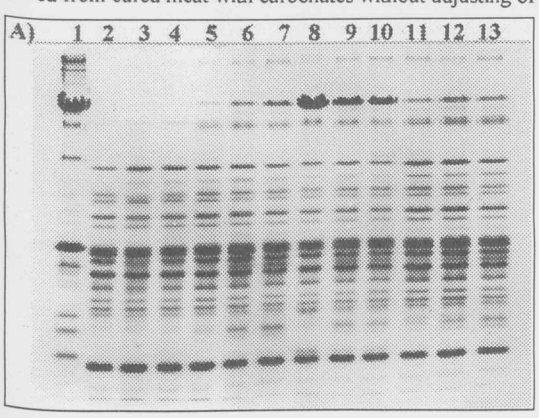


Fig. 2. Electrophoresis and immunoblots of bovine quadriceps femoris treated with various salts (panel A - Coomassie stained 10% SDS-polyacrylamide gel, B - western blot from identical gel reacted with anti-myosin MF-20 monoclonal antibody, C - western blot from identical gel reacted with anti-α-actinin AE-53 monoclonal antibody). Description of lines: 1. myofibrils from raw meat, 2 - centrifugal drip (cd) from meat without adjusting of pH value (cd/0), 3 - cd from meat with pH adjusted to 6.4 (cd/6.4), 4 - cd/7.0, 5 - cd from cured meat „S" without adjusting of pH value (cd-S/0), 6 - cd-S/6.4, 7 - cd-S7.0, 8 - cd from cured meat with pyrophoshate „P" without adjusting of pH value (cd-P/0), 9 - cd-P/6.4, 10 - cd-P/7.0, 11 - cd from cured meat with carbonates without adjusting of pH value (cd-Ca/0), 12 - cd-Ca/6.4, 13 - cd-Ca/7.0



C) 1 2 3 4 5 6 7 8 9 10 11 12 13