Protein Extraction from Pig Muscle in Concentrated Salt Solutions

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SUMMARY

Proteins extracted from pig **M. semitendinosus** into brine have been analysed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). This showed that when small meat blocks were immersed in one of a series of salt concentrations (0.1, 0.5, 1, 2, 3, 4 and 5 M NaCl) myosin was extracted only from those at 1 and 2 M NaCl. The addition of pyrophosphate enhanced myosin extraction at 1 and 2 M NaCl and myosin was also present in the extracts produced at 0.5, 3 and 4 M NaCl. In order to follow the influence of changing salt concentrations in the muscle tissue as it happens during meat curing, meat blocks were soaked for 24 h in either 0.1 or 5 M NaCl solution and were transferred each following day into the next solution of the series. Monotonic increase of salt resulted in higher myosin extraction in 1 and 2 M NaCl and the myosin band was also present in the 3 M brine sample. Monotonic decrease of salt showed poor myosin extraction even at 1 and 2 M NaCl.

INTRODUCTION

The curing process entails the treatment of meat with concentrated salt solutions which penetrate the tissues to give the desired final salt concentration (JOLLEY and PURSLOW 1988). In industrial practice, the requirements for obtaining a uniform salt distribution within the meat in a short time are met by using a multi-needle injector to inject the brine which is subsequently dispersed by tumbling and/or massaging. Injected brine forms pockets in the meat and salt is then absorbed into the adjacent tissue causing an abrupt rise in salt concentration followed by a decline towards equilibrium value. By contrast tissues far from brine pockets experience only an increasing salt concentration until the equilibrium value is reached. During this salt equilibration, proteins are solubilised and extracted to the surface where they contribute to the sticky exudate that influences the cooking properties of the product. Polyphosphates are sometimes included in the brine as they are found to improve the functionality of the sticky exudate and reduce the amount of NaCl required (OFFER and KNIGHT, 1988). Pyrophosphate (PPi) is thought to be the active species. It is therefore important to study the effect of salt and polyphosphate on protein extraction, including also the high concentrations of salt (up to 5 M NaCl) used in the initial step of the curing process. The effects of high salt on water uptake and protein extraction of small meat pieces were first observed by CALLOW (1931,1934), and KNIGHT and PARSONS (1988) studied the influence of concentrated salt on myofibrils. However the identity of proteins extracted when meat is treated with high salt has not been determined. The purpose of this study was to measure protein extraction when meat is submitted to a series of salt concentrations (from 0.1 to 5 M NaCl with and without PPi) or to increasing or decreasing salt concentrations as happens in large meat pieces during curing. Proteins extracted into the brine were analysed by SDS-PAGE to determine their identity.

MATERIALS & METHODS

Pig **semitendinosus** muscles were collected 24 h post mortem, freed of visible fat, cut into cubes of ~8 g and soaked in 20 volumes of brine at 1°C under orbital shaking (100 rev/min). Two types of experiment were carried out: in the first, each cube was soaked for 24 h in one of a series of salt concentrations (0.1, 0.5, 1, 2, 3, 4, 5 M NaCl) with or without 10 mM pyrophosphate pH 6.0. In the second, the meat cubes were soaked for 24 h either in 0.1 or 5 M NaCl solution and were transferred each following day into the next solution of the series to obtain monotonic increase or decrease of salt. Measurements of the sodium content of the meat cubes by using a sodium-specific electrode showed that soaking beyond 24 h did not change the salt content. Brine samples were analysed for protein content following the method of ITZHAKI and GILL (1964) and

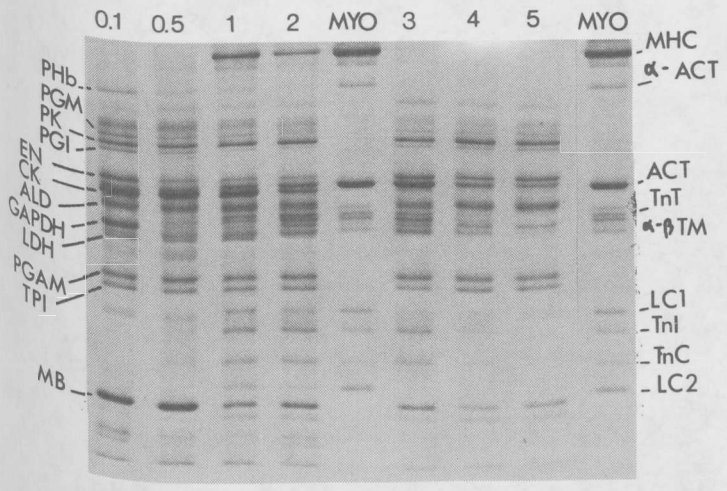
were prepared for SDS-PAGE by precipitating proteins with 3M trichloroacetic acid. After centrifugation the pellet was dissolved in 62.5 mM Tris/HCl pH 6.8, 0.4 % DTT (1,4-dithio-DL-threitol), 10 % glycerol, 2 % SDS and 0.01 % bromophenol blue, and heated for 5 min at 100°C. SDS-PAGE was performed with a Protean II (Bio-Rad) slab cell using a modified LAEMMLI (1970) discontinuous buffer system with a 12 % separating gel. The gels were stained with Coomassie Brillant Blue. Myofibrils prepared following the method of KNIGHT and TRINICK (1982) were also run on the gels as a standard.

RESULTS & DISCUSSION

Figure 1 shows the protein pattern obtained for brine samples collected after a 24 h incubation of small cubes of meat each in one of a series of salt solutions without phosphate. The proteins extracted at 0.1 M NaCl are as expected mainly sarcoplasmic as judged by comparison with the results of McCORMICK et al.(1988) and SAVAGE et al.(1990). With increasing NaCl concentration these proteins were generally extracted in diminishing amounts, especially CK and GAPDH, but the amounts of PK and ALD were less affected. The extraction of myosin showed a striking dependence on salt concentration. It was extracted in 1 and 2 M NaCl, with bands corresponding to MHC, LC1 and LC2 visible at these two concentrations. In all other brines myosin was not extracted. GRABOWSKA and HAMM (1979) also noticed a reduction in extraction of myosin of beef homogenates between 1 and 2 M NaCl, but their experiments did not extend to higher salt concentrations. KNIGHT and PARSONS (1988) irrigated rabbit myofibrils with 1, 2, 3, 4 and 5 M NaCl. Myofibrils exposed to 1 M showed maximum loss of contrast between A- and I-bands, whereas myofibrils exposed to high salt (4 or 5 M NaCl) showed no extraction of A-band. Low extraction of myosin in high salt is probably due to the salting out of the protein.

The actin band is situated between the strong enolase and creatine kinase bands which makes it difficult to see in the extracts at 0.1 and 0.5 M NaCl. However it could be clearly identified in higher salt extracts and was strongest in the 3 M NaCl extract. GRABOWSKA and HAMM (1978) also emphasized the difficulty of

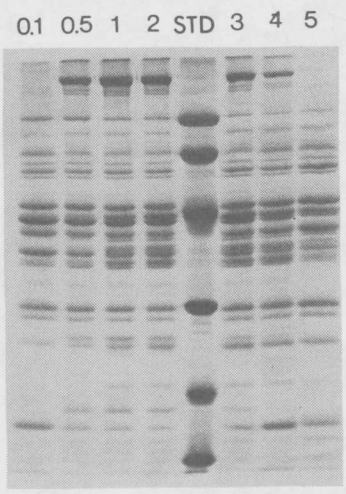
Figure 1 : Pattern of extracted proteins in different brines after 24 h



MYO = myofibrils

PHb, phosphorylase b
PGM, phosphoglucomutase
PK, pyruvate kinase
PGI, phosphoglucose isomerase
EN, enolase
CK, creatine kinase
ALD, aldolase
GAPDH, glyceraldehyde-3-phosphate dehydrogenase
LDH, lactate dehydrogenase
PGAM, phosphoglycerate mutase
TPI, triose phosphate isomerase
MB, myoglobin

Figure 2 : SDS-PAGE of brine samples with pyrophosphate added



STD = standard Pharmacia

MHC, myosin heavy chain
α-ACT, α-actinin
ACT, actin
TnT, Troponin T
α & ß TM, α & ß Tropomyosin
LC1, myosin light chain 1
TnI, Troponin T
TnC, Troponin C
LC2, myosin light chain 2

separating the actin band between enolase and creatine kinase in low salt (0.3 M NaCl) extracts of beef semitendinosus. Actin could easily be identified by these authors in 0.9 M NaCl extracts. The effect of high concentrations of NaCl on the solubility of actin is not known, but concentrations of KCl of 0.3 to 0.7 M cause partial depolymerisation of actin at 0°C (GUBA, 1950). Troponin I and C, and probably also T and tropomyosin, are extracted in 1, 2 and 3 M NaCl. In rabbit myofibrils extracted with 1 M NaCl OFFER & TRINICK (1983) noticed that nearly all the troponin was removed.

The addition of pyrophosphate (Figure 2) influenced mainly the extraction of myosin, which was enhanced. Strong MHC bands were visible in extracts at 0.5, 1, 2 and 3 M NaCl. Some myosin was also extracted in 4 M NaCl, but not in 0.1 or 5 M NaCl. It is well established that pyrophosphate enhances myosin extraction at intermediate NaCl concentration (OFFER and KNIGHT, 1988); our results indicate that it also promotes myosin extraction from meat at high NaCl concentrations, in accord with the observations on myofibrils by KNIGHT and PARSONS (1988).This probably originates in the ability of pyrophosphate to weaken the actin-myosin association, promoting the diffusion of dissolved myosin into the bathing solution.

Figure 3 shows the protein pattern obtained with brine samples produced in the experiment where meat cubes were moved through a series of solutions with monotonic increase of NaCl concentration. There is a greater extraction of myosin in 1 M after progressive increase of the concentration compared with the extraction obtained by soaking the meat cubes directly in 1 M NaCl. The extraction of myosin continues when the cubes are soaked in 2 M and 3 M NaCl. The myosin band in the 2 M sample is very strong. This might in part be due to extraction obtained in 2 M, but also because of myosin solubilised in the previous solution (1 M NaCl) which comes out into the solution. This latter effect can also explain the extraction observed in 3 M NaCl since direct soaking in 3 M NaCl did not extract myosin. Only little extraction of proteins occurs when meat blocks are transferred in high salt (4 and 5 M NaCl). Studies on myofibrils (KNIGHT & PARSONS, 1988) showed that extraction of the A-band is progressively inhibited in higher concentration of NaCl when the concentration is raised stepwise from 0.1 to 5 M NaCl. That sarcoplasmic proteins appear in most of the series of extracts shows that their extraction from the cubes is far from complete in the 24 h incubation period in each solution. Results obtained with the monotonic decrease of salt are shown in Figure 4. The

Figure 3 : Monotonic increase of NaCl.
SDS-PAGE of brine samples

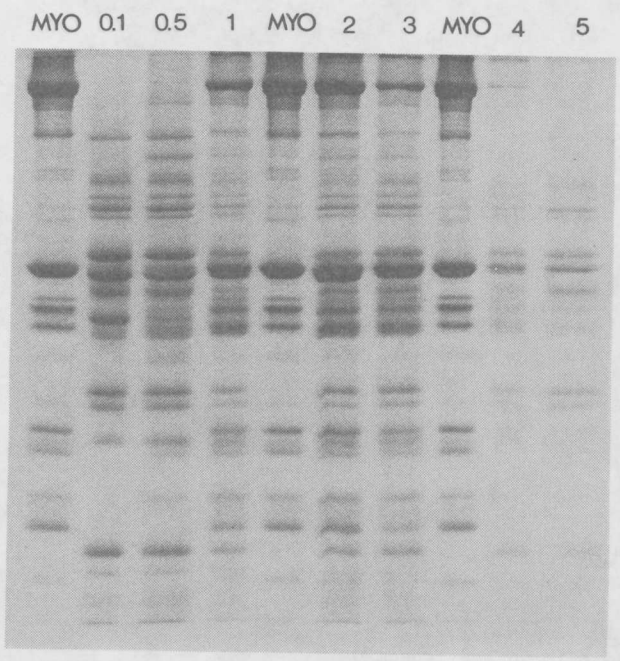
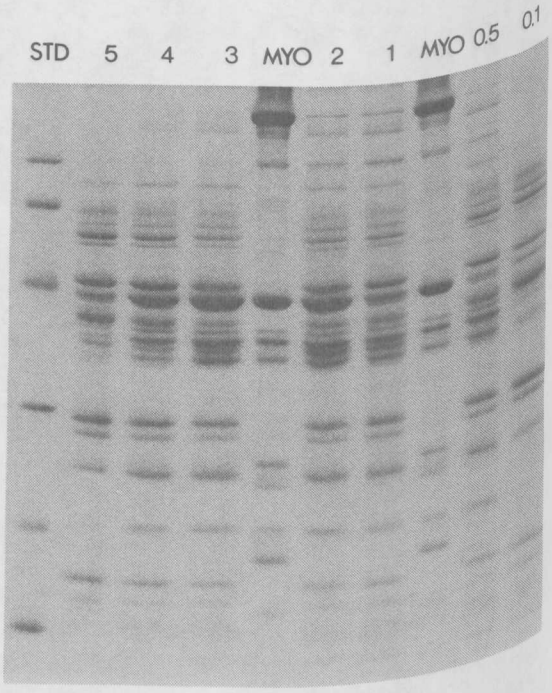


Figure 4 : Monotonic decrease of NaCl.
SDS-PAGE of brine samples



MYO = myofibrils STD = standard Pharmacia
Concentration (M) of salt solutions is given on the top of the gels

protein pattern of brine samples collected after soaking meat cubes in 5, 4 and 3 M NaCl did not differ markedly from the pattern of Figure 1. By contrast in 1 and 2 M NaCl only a little myosin is extracted. The most intense band in 2 M NaCl brine sample is in the area of actin and creatine kinase. Extraction of creatine kinase in lower salt (0.5 and 0.1 M NaCl) is also very low compared to the extraction obtained by placing directly meat blocks in 0.1 and 0.5 M NaCl (Fig.1). Myofibrils submitted to decreasing salt concentrations in steps of 1 M also show less marked extraction of A-band compared to the extraction observed by directly irrigating myofibrils with 1 M NaCl (KNIGHT and PARSONS, 1988). Our results suggest that myosin is almost irreversibely denatured by high salt. This observation might be important for industrial curing since by treating whole pieces with concentrated salt or by injecting, those tissues which have experienced a high salt concentration will not yield myosin during equilibration. This can also have important consequences when brine in meat is not rapidly and evenly distributed and it may underlie the appearance of "tiger stripe" in bacon (VOYLE et al., 1986).

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REFERENCES

CALLOW E.H. (1931): The Scientific Basis of Curing. Rep. Food Invest. Board for 1930, HMSO, London, 74-79.

CALLOW E.H. (1933): The Theory of Curing. Rep. Food Invest. Board for 1933, HMSO, London, 97-104.

GRABOWSKA J., HAMM R. (1978): Proteinlöslichkeit und Wasserbindung unter den in Brühwurstbräten gegebenen Bedingungen. I. Mitteilung : Methoden zur Untersuchung der Proteinlöslichkeit. Fleischwirtsch. 58, 1155-1161.

GRABOWSKA J., HAMM R. (1979): Proteinlöslichkeit und Wasserbindung unter den in Brühwurstbräten gegebenen Bedingungen. IV. Mitteilung : Einflüsse von NaCl-Konzentration, pH-Wert und Diphosphat. Fleischwirtsch. 59, 1166-1172.

ITZHAKI R.F., GILL D.M. (1964): A micro-biuret method for estimating proteins. Anal. Biochem., 9, 401-410.

JOLLEY P.D., PURSLOW P.P. (1988) : Reformed meat products - fundamental concepts and new developments. In : "Food Structure - its creation and evaluation". (J.Mitchell and J.M.V. Blanshard eds.), Butterworths, London, pp.731-764.

KNIGHT P.J., TRINICK J.A. (1982): Preparation of myofibrils. Methods in Enzymology, 85, 9-12.

KNIGHT P., PARSONS N. (1988): Action of NaCl and polyphosphates in meat processing: responses of myofibrils to concentrated salt solutions. Meat Sci., 24, 275-300.

LAEMMLI U.K. (1970): Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227, 680-685.

McCORMICK R.J., REECK G.R., KROPF D.H. (1988): Separation and identification of porcine sarcoplasmic proteins by reversed-phase high-performance liquid chromatography and polyacrylamide gel electrophoresis. J. Agr. Food Chem., 36, 1193-1196.

OFFER G., KNIGHT P. (1988): The structural basis of water-holding in meat. Part I : General principles and water uptake in meat processing. In: "Developments in Meat Science 4". (R.A. LAWRIE ed.), Elsevier Science Publishers, London. pp.63-171.

SAVAGE A.W.J., WARRIS P.D., JOLLEY P.D. (1990): The amount and composition of the proteins in drip from stored pig meat. Meat Sci., 27, 289-303.

VOYLE C.A., JOLLEY P.D., OFFER G.W. (1986) : Microscopical observations on the structure of bacon. Food Microstructure, 5, 63-70.