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THE SEPARATION OF MYOFIBRILLAR PROTEINS USING ISOELECTRIC FOCUSING

McLellan, K.M. and Norton, F.

Procter Department of Food Science, The University of Leeds, Leeds LS2 9JT, West Yorkshire, U.K.

SUMMARY

The separation of myofibrillar proteins by electrophoretic techniques is hindered by the lack of solubility of the proteins in low ionic strength buffers. Thus, electrophoretic separation in polyacrylamide gels is generally achieved in the presence of the detergent sodium dodecyl sulphate (SDS). Isoelectric focusing (IF) is often considered to provide greater resolution of proteins than conventional polyacrylamide gel electrophoresis (PAGE) due to the concentrating nature of the technique. Isoelectric focusing would therefore represent an improvement in the techniques currently available for electrophoretic separation of salt soluble proteins from meat. However, problems are encountered in retaining solubility of the proteins during electrophoresis. IF is not compatible with the use of SDS which destroys the isoelectric characteristics of the protein. Furthermore, high salt concentrations cannot be used for protein solubilisation in samples to be subjected to IF. This paper reports a method for the separation of myofibrillar proteins using urea and mercaptoethanol to ensure the solubility of the proteins. Agarose was chosen as the support material for separation of the proteins as large pore gels can be prepared, which facilitates resolution of proteins of high molecular weight.

The method described allows the separation of several proteins from the meat extracts and differences can be observed in the number and isoelectric points of the proteins extracted from different meats and also following different periods of storage. In comparison to results reported using SDS PAGE, better protein resolution is obtained using IF. The technique described is likely to be particularly advantageous in achieving separation of large molecular weight proteins which migrate only a short distance into SDS polyacrylamide gels.

Present work is directed towards the separation of myofibrillar proteins from additional species and following different postmortem conditions. The improved separation of the myofibrillar proteins achieved using IF could have potential applications in meat species identification and in the investigation of changes in the structural proteins of meat during conditioning and processing.

INTRODUCTION

The separation of myofibrillar proteins by electrophoretic techniques is hindered by the lack of solubility of the proteins in low ionic strength buffers. Thus, electrophoresis of meat proteins in polyacrylamide gels is generally carried out in the presence of the detergent sodium dodecyl sulphate (SDS). Isoelectric focusing (IF) is often considered to provide greater resolution of proteins than conventional polyacrylamide gel electrophoresis (PAGE) due to the concentrating nature of the technique. IF would therefore represent an improvement in the techniques currently available for the separation of myofibrillar proteins. However, problems are encountered in retaining protein solubility. IF is not compatible with the use of SDS which destroys the isoelectric characteristics of the proteins. Similarly, high salt concentrations cannot be used for protein solubilisation in samples to be subjected to IF. This paper reports a method for the separation of myofibrillar proteins using urea and mercaptoethanol to ensure protein solubilisation. Agarose has been chosen as the solid support medium as large pore gels can be produced which facilitates resolution of proteins of high molecular weight.

EXPERIMENTAL METHODS

Myofibrillar proteins were extracted from fresh, conditioned meat as described by Liu et al (1982). The resulting solution of proteins in 40 mM sodium phosphate buffer, pH 6.8 containing 0.6 M NaCl was dialysed overnight at 4 °C against distilled water. The precipitated proteins were then resolubilised by the addition of urea and mercaptoethanol to final concentrations of 18 % (w/v) and 1 % (w/v) respectively.

Agarose gels were prepared by mixing 0.25 g Agarose IEF (Pharmacia Fine Chemicals, Sweden) with 10 mls 10% (w/v) sorbitol solution and boiling gently for 5-10 min to dissolve the agarose. 6 g urea, 2 mls prewarmed distilled water and 0.5 ml prewarmed Pharmalyte carrier ampholytes (pH 4-6.5 or pH 3.5-10) were added, and the solution injected into a prewarmed gel mould. The gel (0.8 mm thick) was allowed to set in the refrigerator for approximately 3 hours before removing from the mould.

Isoelectric focusing was carried out by applying 20 µl aliquots of the myofibrillar protein preparation to small filter paper applicators placed on the surface of the gel. A constant power of 4 W was applied for 3 hours using 1 M H_3PO_4 and 1 M NaOH as electrode solutions for the anode and cathode respectively. For further experimental details see Biorad Instruction Manual.

Following focusing, the gel was immediately placed in fixing solution (11.5 g trichloracetic acid and 3.5 g sulphosalicylic acid dissolved and diluted to 1 l with distilled water) for 10 min, rinsed in destaining solution (methanol:acetic acid:water 3.5:1:5.5) and stained in Coomassie Brilliant Blue R250 solution (methanol:acetic acid:water 4:0.7:5.3 containing 1.25 gl^{-1} Coomassie Blue). The gel was finally destained using several changes of destaining solution and proteins were then visualised as blue bands against a clear background.

RESULTS

Using the technique developed and described above, proteins were successfully separated from extracts of meat prepared in 0.6 M NaCl solution. Several well defined protein bands were observed in gels representing pH ranges of 3.5-10 (Fig.1) and 4-6.5 (Fig.2).

The results shown in Fig. 1 indicate the presence of some 13 proteins in the chicken extract, separated into well defined bands as opposed to the more diffuse bands observed after SDS PAGE of extracts of salt soluble proteins from chicken (Wolfe & Samejima, 1976). IF at pH 3.5-10 showed a group of proteins with pI values of approximately pH 4.5-6 and a second group with pI values in the region of pH 7. Fig. 2 shows the results of focusing over a narrow pH range in order to achieve improved separation of the proteins with pI values of 4.5-6.0. In this pH range, the proteins extracted from chicken, and those extracted from beef did not separate into the same patterns, indicating different isoelectric points. Furthermore, refrigerated storage of the chicken resulted in a larger number of protein bands being observed implying that proteolysis had occurred during storage.

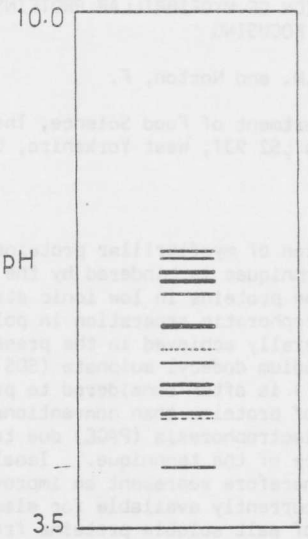

Fig. 1 Isoelectric focusing of salt soluble proteins from chicken at pH 3.5-10

CONCLUSIONS

Agarose was found to be a suitable support medium for isoelectric focusing and the myofibrillar proteins were successfully solubilised using urea and mercaptoethanol, reagents which do not adversely affect IF (Righetti, 1983).

The method described allows the separation of several proteins from the meat extracts and differences can be observed in the number and isoelectric points of the proteins extracted from different meats and also following different periods of storage. In comparison to results reported using SDS PAGE, better protein resolution is obtained using IF. The technique described is likely to be particularly advantageous in achieving separation of large molecular weight proteins which migrate only a short distance into SDS polyacrylamide gels.

Present work is directed towards the separation of myofibrillar proteins from additional species and following different postmortem conditions. The improved separation of the myofibrillar proteins achieved using IF could have potential applications in meat species identification and in the investigation of changes in the structural proteins of meat during conditioning and processing.

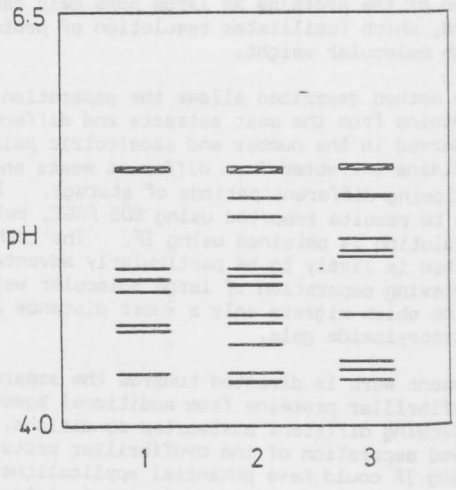

Fig.2 Isoelectric focusing of salt soluble proteins at pH 4-6.5

1. Extract prepared from fresh chicken
2. Extract prepared from stored chicken
3. Extract prepared from beef

REFERENCES

Biorad Instruction Manual. Analytical Electrofocusing in Horizontal Polyacrylamide Gel.

Liu, Y.M., Lin, T.S. and Lanier T.C. (1982). Thermal denaturation and aggregation of actomyosin from Atlantic Croaker. J. Food Science 47, 1916-1920.

Righetti, P.G. (1983) Laboratory Techniques in Biochemistry and Molecular Biology (Eds T.S. Work and E. Work) Volume 11 Isoelectric Focusing: Theory, Methodology and Applications, Elsevier Biomedical Press, The Netherlands.

Wolfe, F.H. and Samejima, K. (1976) Further studies on postmortem ageing effects on chicken actomyosin. J. Food Science 41, 244-249.