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CHANGES IN EXTRACTION CHARACTERISTICS OF MUSCLE PROTEINS DURING COOKING OF MEAT.

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SUMMARY

Consecutive extracts of fresh muscle with 0.015 M and 0.230 M phosphate buffer pH 7.4 gave SDS-PAGE profiles characteristic of sarcoplasmic and myofibrillar proteins respectively.

Similar extracts from muscle cooked at 80°C for up to 60 min contained significantly less protein (only 20-25% of that of fresh muscle extracts).

Extracts isolated from fresh muscle, when heated at 80°C up to 60 min showed extensive precipitation. Treatment of these suspensions with SDS in preparation for electrophoretic separation caused solubilisation of the precipitate and gave SDS-PAGE profiles indistinguishable from those of unheated controls.

It was concluded that cooking alters the extractability but not the electrophoretic mobility of muscle proteins.

Modified extraction procedures will therefore be required to identify products of proteolytic degradation of muscle proteins during cooking.

INTRODUCTION

Proteolytic degradation of muscle proteins is believed to contribute to tenderisation and flavour development in meat. Alterations in muscle proteins may be detected using SDS-PAGE separations of muscle protein extracts. However, during cooking of meat, proteins undergo changes in extractability by the conventional procedures. The present study was undertaken to characterise these changes.

METHODS

Bovine sternomandibularis muscle was removed 20 min after slaughter of a 24 months old heifer, rinsed with 1 mM NaH_3 to prevent bacterial growth, vacuum packed in 30 g portions, held at room temperature up to 24 h post-mortem and thereafter at 0-4°C to 96 h. Pre-rigor muscle (3 h post-mortem) and post-rigor muscle (96 h post-mortem) were studied.

Diced muscle was extracted sequentially in 4 vol. 0.015 M and 4 vol 0.23 M. Na_2HPO/NaH_2PO_4 buffer pH 7.4 with mechanical stirring for 3 h at 0-4°C, centrifuged at 1700 g for 15 min to extract sarcoplasmic and myofibrillar protein fractions respectively. The residual pellet was dissolved in 8 vol 4% SDS-4% NH_4OH.

Minced muscle was homogenised in 12 vol 4% SDS-4% NH_4OH to extract total muscle protein.

Extracts were made of control muscle and of muscle cooked at 80°C for 7.5, 15, 30 and 60 min periods.

'Drip' formed during cooking was studied and compared with control 'drip' isolated from fresh muscle by the method of Tarrant et al 1977.

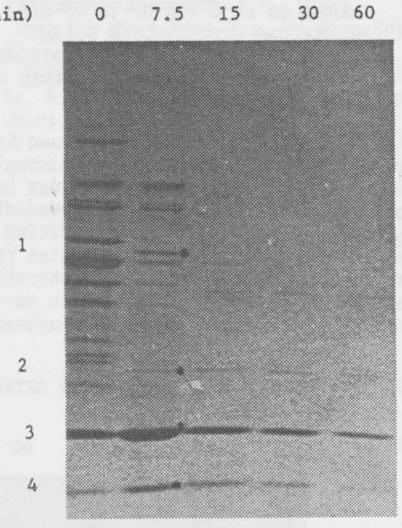
Protein content of extracts was determined by the Biuret method (Gornall et al 1949) using bovine serum albumin as standard.

Water content was determined by drying at 105°C to constant weight.

RESULTS

SDS-PAGE profiles of proteins extracted in 0.015 M phosphate buffer from fresh bovine muscle samples cooked at 80°C for periods of 7.5, 15, 30 and 60 min are compared with those extracted from fresh uncooked muscle as shown in Fig.1. After 7.5 min cooking the quantity of most proteins extracted was considerably decreased; after 15, 30 and 60 min cooking most proteins were not extracted. Exceptions to this general trend were four proteins labelled 1, 2, 3 and 4 in Fig.1. Protein labelled 1, M_r approx. 45,800 and 2, M_r approx. 25,000 were not found in fresh muscle, were present at 7.5 min and decreased in amount during subsequent cooking. Protein bands labelled 3 and 4 (approx. M_r 18500 and 14,500 respectively) represent proteins present in fresh muscle appear to increase in band density after 7.5 min cooking and on further cooking decrease in band density to 60 min. The behaviour of these protein bands is believed to represent proteolytic degradation initially of higher molecular proteins which undergo further proteolytic degradation with continued cooking. Similar results were obtained with extracts of aged muscle.

FIG. 1 SDS-PAGE PROFILES OF PROTEINS EXTRACTED IN 0.015M BUFFER pH 7.4 FROM FRESH AND COOKED BOVINE MUSCLE

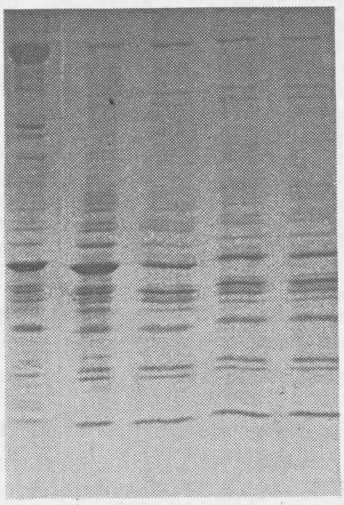


FRESH BOVINE STERNOMANDIBULARIS MUSCLE WAS COOKED AT 80°C FOR UP TO 60 MIN. DICED MUSCLE WAS EXTRACTED IN 0.015M Na_2HPO_4/NaH_2PO_4 BUFFER. PROTEINS WERE SEPARATED ON SDS-PAGE. STAINED WITH COOMASSIE BLUE B.

SDS-PAGE profiles of proteins extracted in 0.230 M phosphate buffer from fresh bovine muscle samples cooked at 80°C for periods of 7.5, 15, 30, 60 min are compared with those extracted from uncooked muscle as shown in Fig.2. The behaviour of myofibrillar proteins was extremely complex with several protein bands showing increased density whereas other protein bands show decreasing density as cooking proceeded. While several of these changes may reflect the occurrence of proteolytic degradation during cooking, the results with sarcoplasmic and with myofibrillar proteins together suggested that cooking altered the extractability of muscle proteins.

FIG. 2 SDS-PAGE PROFILES OF PROTEINS EXTRACTED IN 0.23M BUFFER pH 7.4 FROM FRESH AND COOKED BOVINE MUSCLE

TIME (min) 0 7.5 15 30 60

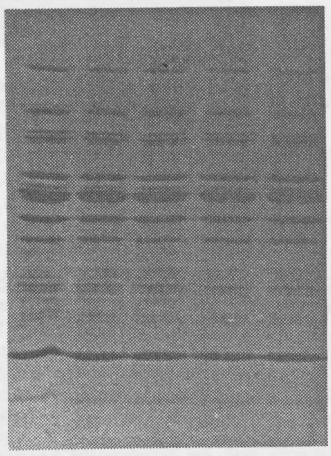


FRESH BOVINE STERNOMANDIBULARIS MUSCLE WAS COOKED AT 80°C FOR UP TO 60 MIN. DICED MUSCLE WAS EXTRACTED IN 0.23M Na_2HPO_4/NaH_2PO_4 BUFFER. PROTEINS WERE SEPARATED ON SDS-PAGE. STAINED WITH COOMASSIE BLUE B.

That this in fact occurred was confirmed in two experiments. Firstly, an extract of sarcoplasmic proteins in 0.015 M phosphate buffer was heated at 80°C for up to 60 min. Samples removed at 7. 5, 15, 30 and 60 min all showed precipitation due to protein denaturation. The precipitates redissolved on treatment with SDS as for electrophoretic separation and gave SDS-PAGE profiles which were indistinguishable from the profile of the unheated sample Fig.3.

FIG. 3 SDS-PAGE PROFILES OF HEATED EXTRACTS OF BOVINE MUSCLE.

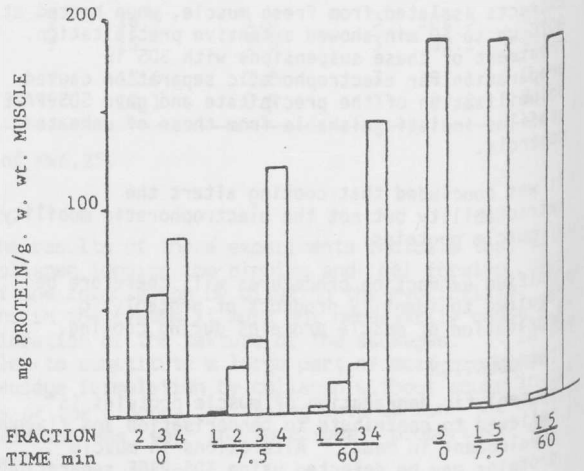
TIME (min) 0 7.5 15 30 60



FRESH BOVINE STERNOMANDIBULARIS MUSCLE WAS EXTRACTED IN 0.015M Na_2HPO_4/NaH_2PO_4 BUFFER pH 7.4
ISOLATED EXTRACT WAS HEATED AT 80°C FOR PERIODS UP TO 60 MIN. AND SEPARATED BY SDS-PAGE. STAINED WITH COOMASSIE BLUE B.

Secondly, protein determinations on all muscle fractions isolated from fresh uncooked muscle samples cooked for 7.5 min and 60 min were carried out and are shown in Fig.4. Analysis for the protein content of 'drip' formed during the cooking and for the total SDS-NH_4OH soluble protein of unfractionated fresh and cooked muscle are also shown. As cooking progressed the amount of protein extracted by 0.015 M phosphate buffer and subsequently by 0.230 M phosphate buffer decreased with a corresponding increase in the quantity of protein extracted by SDS-NH_4OH.

FIG. 4 PROTEIN CONTENT OF BOVINE MUSCLE FRACTIONS ISOLATED BEFORE AND AFTER COOKING AT 80°C FOR 7.5 AND 60 MIN.



PROTEIN DETERMINED BY BIURET METHOD (GORNALL ET AL. 1949)

FRACTION 1. PROTEINS IN COOKING DRIP
2. PROTEINS EXTRACTED IN 0.015M BUFFER
3. PROTEINS EXTRACTED IN 0.23M BUFFER
4. RESIDUAL PROTEINS
5. WHOLE MUSCLE PROTEINS

PROTEIN CONTENT EXPRESSED AS mg/g. WET WT. INITIAL MUSCLE TISSUE (MEAN OF 3 MUSCLES)

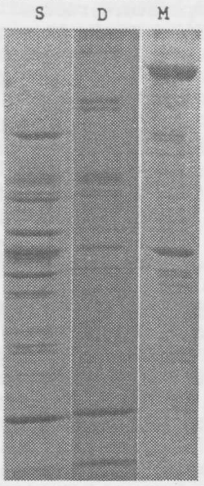
SDS-PAGE separation of 'drip' proteins showed protein bands corresponding to proteins found in the sarcoplasmic fraction; not all sarcoplasmic proteins were extracted as 'drip'. In addition several protein bands were not identifiable with bands in either sarcoplasmic or myofibrillar separations (Fig.5). The nature of these proteins is currently under investigation.

DISCUSSION

The work presented here was initiated with a view to following by SDS-PAGE separation of muscle protein fractions the extent to which sarcoplasmic proteins in particular, but also myofibrillar proteins, underwent proteolytic degradation during cooking. The classical techniques of low and high ionic strength buffers to extract sarcoplasmic and myofibrillar proteins respectively were used. SDS-PAGE separations and protein determinations of the isolated fractions showed that the extractability of muscle proteins was altered significantly during the cooking process. The observation that proteins not found in extracts from uncooked muscle were detected both in extracts from cooked muscle and in 'drip' formed during cooking strongly points to the occurrence of limited proteolysis of muscle proteins during cooking. Characterisation of the nature and extent of this proteolysis will require the development of modified extraction procedures and of methods of identi-

fication of substrates and products based on both enzymatic and immunological procedures.

FIG. 5 SDS-PAGE PROFILES OF PROTEINS IN COOKING "DRIP", AND IN SARCOPLASM AND MYOFIBRILS OF UNCOOKED MUSCLE.



S: SARCOPLASMIC PROTEINS EXTRACTED IN 0.015M PHOSPHATE BUFFER FROM UNCOOKED MUSCLE

D: PROTEINS IN "DRIP" FORMED DURING COOKING AT 80°C FOR 7.5 MIN.

M: MYOFIBRILLAR PROTEINS EXTRACTED IN 0.230M PHOSPHATE BUFFER FROM UNCOOKED MUSCLE FOLLOWING EXTRACTION OF SARCOPLASMIC PROTEINS.

PROTEINS WERE SEPARATED ON SDS-PAGE AND STAINED WITH COOMASSIE BLUE B.

Degradation of muscle proteins by endogenous muscle proteinases contributes to metabolic turnover in live animals and to post-mortem conditioning of muscle as meat. Studies on proteolytic activities in muscle have mainly focused on degradation of myofibrillar proteins and its role in tenderization (e.g. Penny, 1980). Sarcoplasmic proteins which constitute up to 30% of muscle proteins have so far received little attention (Etherington, 1981). Sarcoplasmic proteins, occurring in the aqueous cytosol may provide more ready substrates for proteolytic enzymes and may thereby have priority in degradation over myofibrillar proteins. Hydrolysis of sarcoplasmic proteins would not affect the tenderness of meat, but the products of their hydrolysis may contribute to development of flavour.

Much attention has been paid to proteolytic degradation during post-mortem ageing of muscle, when meat is held at room temperature and subsequently at 0-4°C. This paper presents the results of preliminary investigation of the action of muscle proteinases during cooking when as the temperature of the muscle is raised these enzymes would become progressively more active until denaturation occurs.

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