Studies of sarcoplasmic proteins by sodium dodecyl sulphate polyacrylamide gel electrophoresis.

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Introduction

Ground beef has been found to develop characteristic off-flavours during long term frozen storage. In an attempt to identify the source of these off-flavours a study of the changes taking place in sarcoplasmic proteins of meat stored under various conditions has been initiated.

Sarcoplasmic extracts of muscle contain up to one-third of the muscle protein (Scopes 1970). These proteins, the most labile of the muscle proteins, exist *in vivo* in a very concentrated solution which surrounds but does not permeate the myofibrils. At least 80% of the sarcoplasmic protein consists of the glycolytic enzymes. Of the several hundred enzymes thought to be present in the sarcoplasm, the six most abundant enzymes make up more than half the sarcoplasmic protein. (Scopes 1970).

Starch gels have previously been used for electrophoretic studies of sarcoplasmic proteins, when little difference was found between pre-rigor and post-rigor muscles of rabbit and pig. (Whitaker et al 1970). The present paper reports the use of sodium dodecyl sulphate - polyacrylamide gel electrophoresis (SDS-PAGE) of sarcoplasmic protein extracts of bovine psoas muscle identifying protein degradation occurring in the meat under different storage conditions.

Materials and Methods

Muscle: Whole psoas muscle was removed from a 2-year old heifer immediately following slaughter and evisceration. It was brought to the laboratory (30 min.) wrapped in several layers of sterile gauze and placed in a 17°Cincubator for 24 hrs. A sterile thermometer and sterile spear electrode (Russel pH) were inserted into the centre of the muscle from which readings were taken at intervals to confirm that the muscle went through rigor at the proper rate and achieved a normal ultimate pH (Henahan et al 1981). At 24 hr. the muscle was transferred to the refrigerator at 4°C.

Sampling: Samples were taken asceptically at 1, 4, 24, 96 and 168 hours post mortem and processed immediately. Samples were taken pre-rigor in pairs. One of each pair was ground. Whole and ground pairs were frozen and held at -20°C and -80°C. Similarly paired samples of post-rigor (24 hr.) muscle were taken and stored at 4°C, -20°C and -80°C. Grinding was carried out in a hand mincer (6 mm orifices).

Extraction: Five grams of whole or ground muscle were minced and extracted,with stirring in 20 ml distilled H_2O for six hours at 4°C. The resultant slurry was centrifuged at 1700g for 15 min. to yield a supernatant suspension of sarcoplasmic proteins.

Protein concentration: The protein concentration of each extract was determined by the buiret method (Gornall et al 1949) using bovine serum albumin as a standard.

Denaturation: An aliquot of each extract was added to 0.01M sodium phosphate buffer, 1% in SDS, 1% in β-mercaptoethanol and containing 8M urea to yield a protein concentration of 1 mg/ml. The protein was dissolved and denatured by heating in a boiling water bath for five minutes.

Electrophoresis: Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was carried out by the method of Weber and Osborn (Weber and Osborn 1969) using 10% gels containing 0.1% SDS. Approximately 25ug protein was applied to each gel. Molecular weights were estimated by comparison with concurrently run marker proteins purchased from Bio-Rad. (Richmond, California 94804, USA).

Results

Between twelve and twenty distinct protein bands can be found in the gels from beef stored under various conditions.

A high molecular weight component (<125,000 daltons) which appears in the gels of pre-rigor meat both at 1 hr. post mortem (p.m.) and at 4 hr. p.m., when the pH had fallen to 6.28, is not detectable in extracts of post-rigor meat extracted at 24 hr., 96 hrs. or 7 days p.m. (Fig.1).

Changes in intensity in specific bands suggest a complex progression of proteolytic events. For example, a band at 38,000 daltons (Fig.1) appears as a moderate band in pre-rigor and post-rigor (24 hr.) fresh meat and in meat frozen pre-rigor. The band is approximately doubled in intensity in gels of 4 day p.m. meat kept at 4°C and 4 day p.m. meat frozen post-rigor in one piece. The band has moderate intensity in meat kept to 7 days p.m. at 4°C and is very light in 4 day p.m. meat which was ground and frozen post-rigor, (i.e. at 24 hr. p.m.).

A possible interpretation of these band changes is as follows: at 4°C a high molecular weight protein is degraded between 24 and 96 hrs. to appear in the 38,000 dalton band. By seven days, it in turn has been degraded and the band returns to its original intensity. When post-rigor meat is frozen in a piece the same degradation of a high molecular weight protein occurs by 96 hrs. resulting in a strong band at 38,000. If the meat is ground before freezing, the protein is degraded further resulting in a very light band at 38,000.

No differences were found between meat stored at -20°C and meat stored at -80°C. (Fig.2).

Discussion

It has been suggested that the meat factory of the future will process beef pre-rigor. The small muscles of the fore-quarter, 'hot-deboned' would be put directly into the mincer, thus preserving the high water binding capacity of the pre-rigor state as well as offering great savings in energy and improvements in efficiency. (Joseph 1980).

The flavour precursors in lean meat are believed to be low molecular weight compounds present in the dialysable portion of the cold water extracts of raw lean meat. (Hornstein and Crowe 1960). Some of the precursors of beef flavour have been found to be a relatively simple mixture of glucose, inosinic acid and amino acids derived from a glycoprotein. (Batzer et al 1962). Parrish et al (1969) showed that the sarcoplasmic protein fraction yielded an increase in free amino acids and non-protein nitrogen in post mortem bovine muscle. Drabikowski et al (1977) showed that sarcoplasmic proteins can be degraded by acid cathepsins.

The aim of this study is to investigate the proteolytic degradation of sarcoplasmic proteins which would result in increases in the free amino acids and peptides in the meat. The amino acids may contribute to the development of a modified flavour by participating in Maillard type reactions during subsequent cooking.

Based on SDS-PAGE electrophoretograms changes have been observed in the components of sarcoplasmic protein in the transition from pre-rigor to post-rigor meat. Further changes were observed in the subsequent short term storage of meat. The changes may arise from progressive partial degradation of sarcoplasmic proteins and appeared to be more extensive in muscle ground prior to storage. Experiments are in progress to investigate these changes in detail with particular reference to low molecular weight products of protein degradation.

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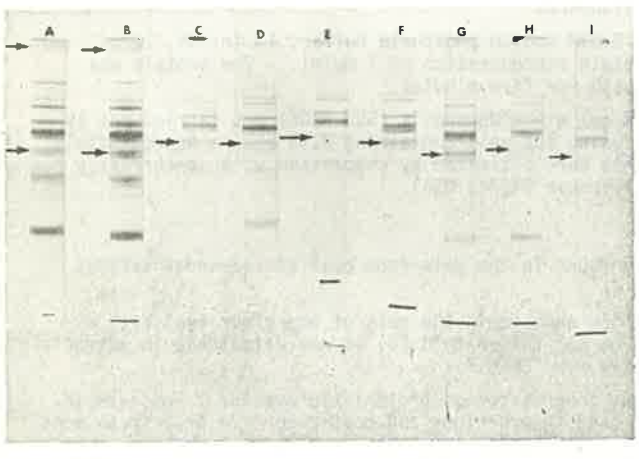

Fig.1. SDS-PAGE (10% gels) of sarcoplasmic extracts of bovine psoas muscle.
A: 1 hr. post mortem
B: 4 hr. p.m., 17°C
C: 24 hr. p.m., 17°C
D: 24 hr.p.m., pre-rigor frozen whole, -20°C
E: 24 hr. p.m., pre-rigor ground and frozen, -20°C
F: 96 hr. p.m., 17°C to 24 hr. then 4°C to 96 hr.
G: 96 hr. p.m., post rigor frozen whole, -20°C
H: 96 hr. p.m., post rigor ground and frozen, -20°C
I: 7 days p.m., 17°C to 24 hr. then 4°C to 7 days
Upper arrows indicate >125,000 dalton band
Lower arrows indicate 38,000 band (see text)

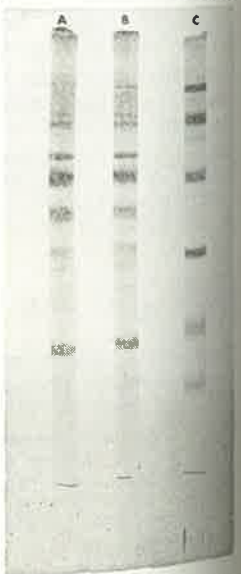

Fig.2. SDS-PAGE (10% gels) of sarcoplasmic extracts of bovine psoas muscle frozen pre-rigor and stored for three months at
A: -20°C
B: -80°C
C: contains marker proteins: Phosphorylase B, 92,500 daltons; BSA,66,200 daltons; Ovalbumin 45,000 daltons; Carbonic anhydrase 31,000 daltons; Soybean trypsin inhibitor, 21,500 daltons and Lysozyme 14,400 daltons.