ISOLATION AND CHARACTERISATION LOW MOLECULAR WEIGHT NITROGEN FRACTION OF BEEF

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Introduction

Flavour is an important aspect of meat quality. Characteristic odours and flavours are produced from precursors in the lean and fat when meat is cooked (Ford and Park, 1980). Meat flavour development during cooking arises from complex interactions involving amino acids, peptides, sugars, thiamine, metabolises of nucleotides, lipids and products of lipid oxidation. (Imafidon and Spanier 1994). In general the flavour of cooked meat is due to a mixture of compounds, including, (1) non-volatiles or watersoluble compounds and (2) volatiles which give rise to odour properties (Reineccius, 1994).

A lyophilized water extract of beef was found to possess the flavour of cooked beef, thus it was concluded that some of the precursors of meat flavour were indeed water soluble (Hornstein and Crowe, 1960). Batzer *et al.,* (1962) observed a typical beefy odour from a heat dialysed water extract from uncooked beef. This fraction was found to contain peptides, carbohydrates, phosphates and free amino acids (Batzer et al., 1960 & 1962). The objective of the this study was to isolate the low molecular weight, nitrogen containing fraction of beef and characterise this fraction.

Materials & Methods

Meat Samples

M. Iongissmus dorsi was obtained from a 2 year old Friesian steer 24 hours post-mortem. Fat was trimmed from the muscle. One portion (the control sample) was minced and stored in vacuum packed bags at -20°C. The other portion (the aged sample) was stored at 2°C for 20 days before mincing, vacuum packing and freezing.

Preparation' of water soluble nitrogen' extract

Water soluble nitrogen (WSN) was prepared by homogenising 15 g of meat in 75 ml water for 5 min using a Colworth stomacher (A.J. Seward & Co. Ltd., Stanford, London, UK). The homogenate was centrifuged at 3,000 g for 30 min at 4°C and the supernatant filtered through glass wool. An aliquot of supernatant was Iyophilised for further analysis by gel electrophoresis. The remainder was used for further fractionation by ultrafilation (UF) (molecular weight cut-off of 10,000 Da) or treated with protein precipitants, 12% trichloroacetic acid (TCA) and 70% ethanol. Free amino acid analysis was also carried out on the TCA soluble fractions using a Beckman model 6300 amino acid analyser (Beckman Instruments Ltd. High Wycombe UK) at the National Dairy Research Centre, Moorepark, Co. Cork, Ireland.

Sodium DodecylSulphafe PolyacrylamideGelElectrophoresis(SDS-PAGE)

Electrophoresis in polyacrylaminde (12% separation, 4% stacking) gels was performed in a Protean II (Biorad Laboratories Ltd., Watford, Herts, UK) vertical slab cell according to the method of Laemmli (1970).

Nitrogen determination

The nitrogen content of the water soluble extracts and various fractions were determined by the Kjeldahl method (AOAC, 1990).

HPLC

Samples for HPLC were prepared by homogenising 5 g of meat (in triplicate) with 25 ml of 0.1 M HCl in a stomacher homogeniser for 8 min and centrifuged at 10,000 g for 20 min. The supernatant was filtered through glass wool and deproteinised by adding 2.5 volumes of acetonitrile and centrifuging at 10,000 g for 5 min. Samples were then evaporated at 30°C under vacuum to dryness and resuspended in a volume of 0.1 M HCl equal to the volume of the original supernatant.

The deproteinised meat extracts were filtered through a Millipore membrane filter (0.45 ~m) and assayed (80 p1) directly by Waters HPLC (Waters, Millipore Corporation, Waters Chromatography Division, Milford, MA 0177757 USA) at a wavelength of 214 nm using a RP-18 column and a temperature of 35 ~ 1 °C. The eluant system consisted of Solvent A, water with 0.1% (v/v) trifluoroacetic acid (TFA) and solvent B. acetonitrile with 0.07% TFA. Separation was performed at 0.8 ml/min with a linear gradient 0-25% B (25 min) and 25-100% B (25 min) (Rodriguez-Nunez et al., 1995).

RESULTS

The aged beef samples had a higher content of WSN, 12% TCA soluble nitrogen, 70% ethanol soluble and UF permeable nitrogen than the control beef samples (Table 1). The electrophoretic pattern of the WSN, ethanol insoluble and UF retentate fractions of the aged and control samples were similar and were dominated by the sarcoplasmic proteins. The UF permeate and ethanol soluble fractions from the aged and control samples did not stain on the SDS-gel (Fig.1). Free amino acid analysis showed an increase in concentration of thirteen amino acids during the post-mortem ageing period (Fig. 2).

The HPLC profiles (Fig. 3) showed that the aged samples contained more peptides, especially hydrophobic peptides, than the control sample.

Table 1 Nitrogen content of the various fractions.

Sample	% Total Soluble Nitrogen	% TCA Soluble Nitrogen	70% Ethanol Soluble Nitrogen	UF Permeable Nitrogen
Control	18.90 ± 0.34	8.35 ± 0.61	10.33 ± 0.58	8.21 ± 0.37
Aged	20.60 ± 0.21	10.20 ± 0.25	11.73 ± 1.01	9.82 ± 0.60

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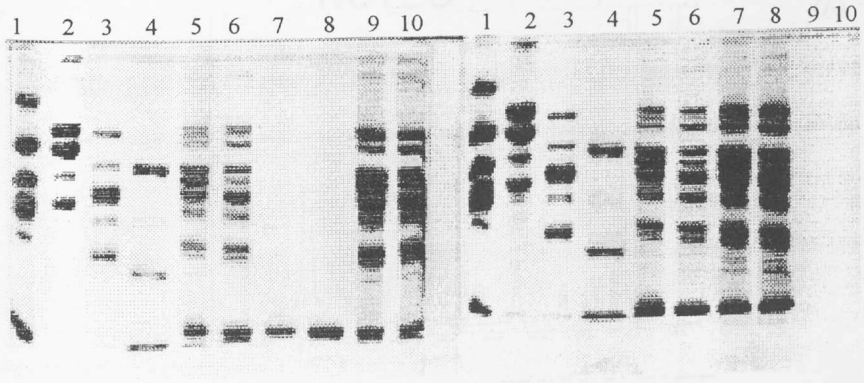


Gel 1 Gel 2

Fig. 1. SDS polyacrylamide gel electrophoretograms. In gel 1 and gel 2; lane 1; mixture 1: phosphorylase b, Phosphofructokinase, Creatine kinase, Lactate dehydrogenase, Myoglobin; lane 2; mixture 2 : Bovine serum albumin, Phosphoglucose isomerase, phosphoglycerol kinase, Glyceraldehyde 3 phosphate dehydrogenase; lane 3; mixture 3 : Phosphoglucomutase, Aldolase, α-glycerol dehydrogenase, Triose phosphate isomerase; lane 4; mixture 4 : Enolase, Myokinase, Cytochrome C. Lanes 5-6 control and aged WSN fractions. Gel 1 lanes 7-10; control ethanol soluble nitrogen (ESN), aged ESN, control ethanol insoluble nitrogen (EISN) and aged EISN. Gel 2 lanes 7-10; control UF retentate, aged UF retentate, control UF permeate and aged UF permeate.

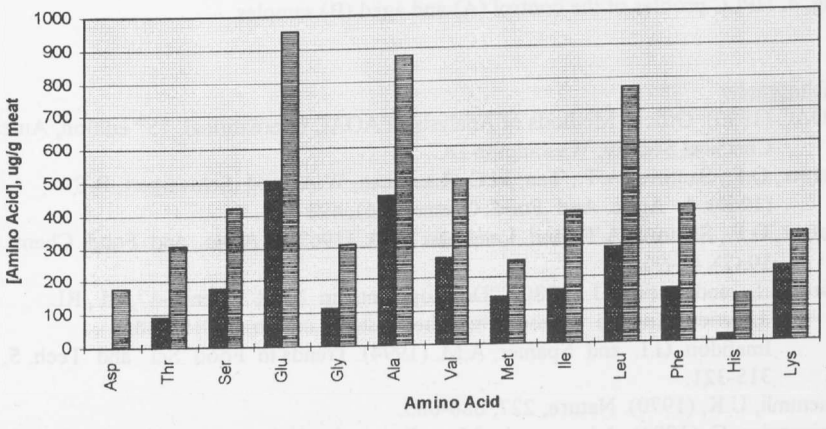
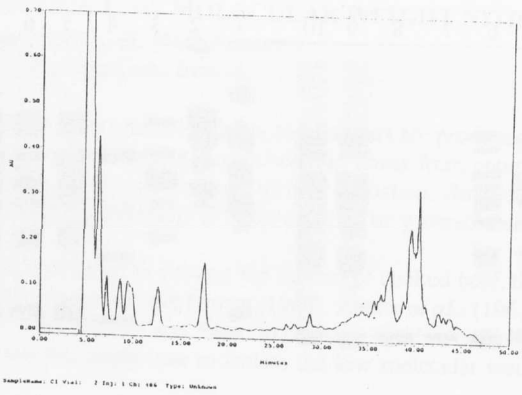


Fig. 2. Profile of amino acid concentration and type of the control (solid bar) and the aged (striped bar) samples.

(A)



(B)

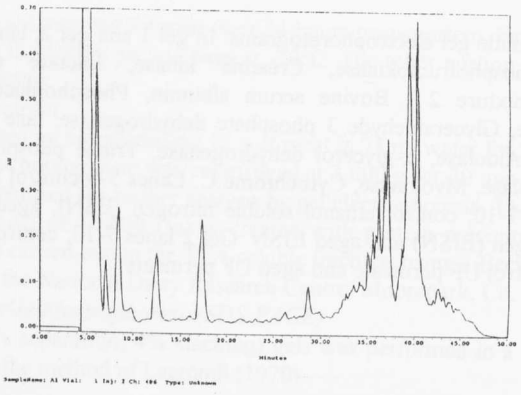


Fig. 4. HPLC profiles of the control (A) and aged (B) samples.

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