ELSEVIER

Food Chemistry 69 (2000) 461–465

Food Chemistry

www.elsevier.com/locate/foodchem

Preliminary analysis of amino acids at various locations along the *M. longissimus dorsi* in aged beef

A.M. Mullen [a,*], S. Stoeva [b], K. Laib [b], G. Gruebler [b], W. Voelter [b], D.J. Troy [a]

[a] *The National Food Centre, Teagasc, Castleknock, Dunsinea, Dublin 15, Ireland*
[b] *University of Tuebingen, Germany*

Received 20 March 1999; accepted 13 January 2000

Abstract

Proteolysis of myofibrillar proteins results in an increase in substrate made available for the release of free amino acids. Levels of these amino acids have previously been determined in different muscles over the post mortem ageing period in beef. However, there is a shortage of information available regarding variations within a muscle over this ageing period. The objective of this study was to carry out preliminary investigations into the free amino acid levels in eight locations along the *M. longissimus dorsi* (LD) over the post mortem ageing period. Free amino acids were analysed in the LD of three Hereford cross heifers at 1 h, 1, 3 and 15 day post mortem. In general most amino acids increased in all locations over the ageing period while a 100% increase was observed in total amino acids averaged over the eight locations. More variations occurred between locations in the earlier post mortem period, however, there were no consistent differences during ageing which might suggest variations in proteolytic activity along the LD. As these analyses were carried out on three animals a larger number of animals would need to be sampled to verify these results.

1. Introduction

The precise biochemical reactions which occur during the post mortem ageing of meat remain unclear. However, it is thought that endogenous proteolytic enzyme activity and myofibrillar protein degradation are involved in the tenderisation process during ageing (Dransfield, 1994; Penny & Dransfield, 1979; Takahashi, 1996; Taylor, Geesink, Thompson, Koohmaraie & Goll, 1995). Much research in the area of meat science has focused on these two aspects. Polypeptide fragments from myofibrillar proteins are further susceptible to attack by peptidyl peptidases and aminopeptidases which will lead to the production of smaller peptides and release of individual amino acids. Measurement of free amino acid can give some indication of the proteolytic activity within a tissue. While protein degradation and polypeptide production occur within muscle, only a few studies have dealt with the final products of protein degradation in aged beef. Due to the differences in the composition of different muscles, levels of amino acids vary depending on muscle type (Cournet & Bousset, 1999; Feidt, Petit, Bruas-Reignier & Brun-Bellut, 1996). The levels of free amino acids in various bovine muscles has been reported (Ma, Matlack & Hiner, 1961) as have changes over the post mortem ageing period (Feidt et al., 1996; Feidt, Riley & Chang, 1971; Parrish, Goll, Newcomb, de Lumen, Chaudhry & Kline, 1969). While these authors reported on amino acid levels in various muscles none have been concerned with variations over the length of the muscle. It has previously been reported that apart from tenderness there was little variation in quality parameters from different parts of the bovine *M. longissimus dorsi* (LD) (Gariepy, Jones & Robertson, 1990; Martin, Freeden & Weiss, 1970). These authors monitored attributes such as shear force, colour, drip loss, and pH. However no biochemical parameters were investigated. In general there has been very little work carried out in the area of variations of quality or biochemistry within a muscle. The release of certain amino acids can have an influence on meat quality attributes such as drip loss, water holding capacity (Lawrie, 1991)

* Corresponding author. Tel.: +353-1-838-3222; fax: +353-1-838-3684.
E-mail address: a.mullen@nfc.teagasc.ie (A.M. Mullen).

0308-8146/00/$ - see front matter
PII: S0308-8146(00)00066-2

and the development of flavour (Nishimura et al., 1988; Rossi, Denoyer & Berdague, 1995; Spanier, McMillin & Miller, 1990). Proteolytic degradation of myofibrillar proteins gives rise to increased substrate for enzymatic degradative systems with the concomitant release of free amino acids. It is possible therefore, that amino acid levels may also contain some information regarding the tenderness or flavour of meat.

The present study was conducted to carry out preliminary investigations into the release of free amino acids over the ageing profile from bovine LD. In order to determine if variations occur throughout the muscle, amino acid levels were assessed at eight separate sites along the LD.

2. Methods

Three Hereford Cross heifers were slaughtered conventionally. The LD was excised from the carcass at 1 h post mortem, vacuum packed and stored at 4°C for 15 days. At 1 h, 1, 3 and 15 days post mortem samples of approximately 40 g weight were excised from each of eight locations along the LD. The eight locations were labelled A–H with location A at the cranial end of the muscle and H at the caudal. Extractions of these samples, were carried out immediately after sampling, on fresh tissue using a procedure adapted from Nakai, Nishimura, Shimizu and Arai(1995). Following homogenisation of 25 g of muscle in 50 ml dH_2O, TCA (50%) was added to bring the final concentration to 5%. After centrifugation at 3600 rpm for 30 min, the supernatant was filtered through cheese cloth (X8) and stored at −20°C until analysis.

2.1. Amino acid analysis

Free amino acids were determined directly using 20 μl of the whole supernatant. Amino acid analyses were performed with an amino acid analyser LC3000 (Eppendorf, Hamburg, Germany). Samples were applied to the ion exchange column (a spherical resin 10% DVB cross-linked polystyrol, 4×125 mm) in citrate buffer at pH 2.2. Ninhydrin was used for post-column derivatisation of the amino acids (Moore, Spackman & Stein, 1958; Moore & Stein, 1948, 1951, 1954a, 1954b, 1963; Spackman, Stein & Moore, 1958). Separation was achieved with a multi-step pH and ionic strength gradient.

2.2. Supernatant yield

Supernatant yields were calculated for each sample analysed. These were calculated by recording the weight difference between the centrifuge tube after centrifugation and the centrifuge tube without the supernatant. Individual amino acids were first expressed as nmol/g tissue. This was calculated by using the supernatant yield from each sample.

2.3. Statistical analysis

Analysis of variance of the amino acids was performed using the SAS statistical package. Analysis of variation both between locations and over the time post mortem was calculated for both individual and total amino acids.

3. Results and discussion

Seventeen free amino acids were measured in each of the samples analysed. The results obtained over the post mortem ageing period for locations A–H of the three animals are presented in Fig. 1. The patterns of increase, in each location, of the individual amino acids over the ageing process are represented graphically in this figure. While readings were obtained for most of the amino acids both cysteine and methionine were non-detectable in a number of samples. Supernatants were obtained from every sample. The average volume of supernatant obtained varied over the ageing process from 68.2 to 71.7 ml with a standard deviation from the mean of between 2.4 and 3.5 ml. Using the respective supernatant volumes the total amino acids were calculated as nmol/g tissue (Fig. 2). Variations in the individual amino acids over the ageing period are presented in Fig. 1.

Analysis of variance was carried out on the results to determine if amino acid levels in the regions along the LD varied significantly. No statistically significant results were observed. However, this may be due to the small number of animals assessed ($n = 3$) and that each location was treated as a repeated measure of the respective animal. While statistical significance was not attained some notable variations between locations were apparent. During the earlier post mortem period the amino acid levels displayed some variations between locations. This was most notable at 1 h post mortem (Figs. 1 and 2). The variations, in both individual and total amino acids, between locations were less obvious at 3 and 15 days post mortem. Although the sample size was small and significant differences were difficult to obtain it is possible to view the patterns of increase of the individual amino acids. When these are monitored in all locations over the ageing period (Fig. 1) similar patterns of increase were evident for the majority of the amino acids. As there was no consistent variations between locations it is possible that there was no significant variations in proteolytic activity along the length of the LD. However, in order to verify this statistically a larger number of animals would need to be assessed.

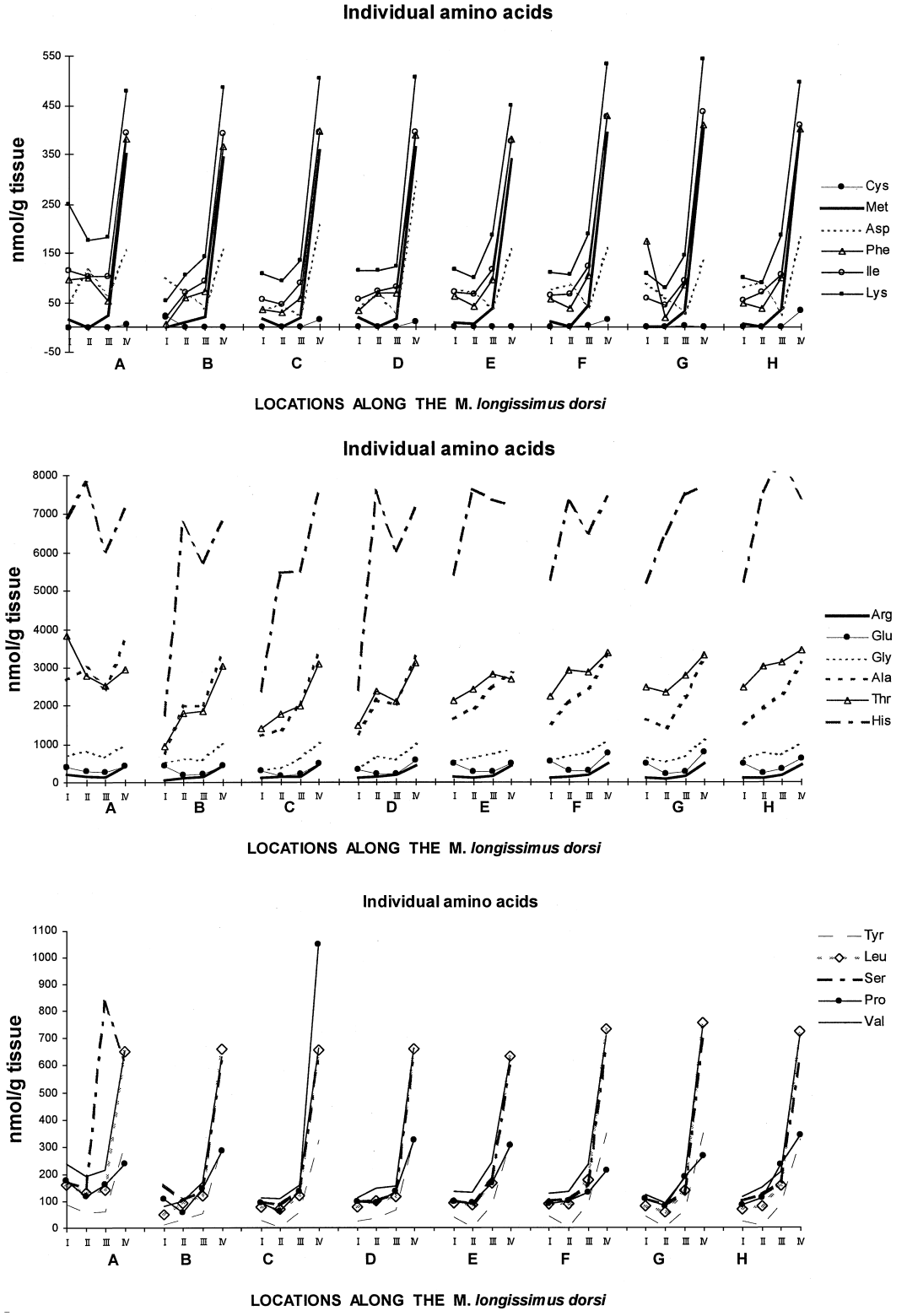

Fig. 1. Variations in individual amino acids over the ageing process (I = 1 h, II = 1 day, III = 3 days, IV = 15 days post mortem). Eight locations along the length of the *M. longissimus dorsi* (A–H) are presented.

Increases in total amino acids are evident in all locations over the ageing period (Fig. 2). The most dramatic increase between 1 h and 15 days post mortem is evident in total amino acids in location B. In general all individual amino acids increased over the 15 day conditioning period. The smallest variation in individual amino acids were observed in alanine, threonine, glutamine and glycine while cysteine displayed very

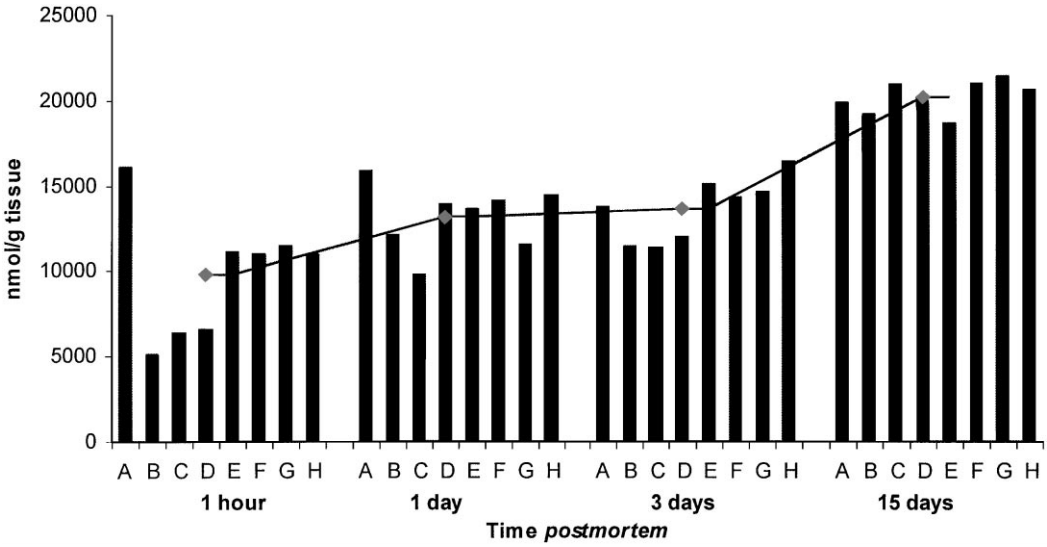

Fig. 2. Total free amino acids (nmol/g tissue) over eight locations (A–H) of bovine *M. longissimus dorsi*. Analysis was carried out at 1 h, 1, 3 and 15 days postmortem on samples from three animals. The average of the totals from all locations at each time point is represented by the solid line.

minor increases in most locations. Approximately 2–6-fold increases were evident in many amino acids — asparagine, phenylalanine, isoleucine, serine, proline, arginine, valine, lysine, between 1 h and 15 days post mortem. Larger increases (7–15-fold) were observed in methionine, tyrosine and leucine. Marked increased in tyrosine, aspartic acid-phenylalanine, threonine-leucine, and isoleucine have also been reported in aged beef and rabbit muscle by both Parrish et al. (1969) and Feidt et al. (1971), respectively. Feidt et al. (1996) also reported increases in all detected amino acid from 3 to 15 days post mortem. Over a 100% increase in total amino acids was observed in the current investigation when the average of all eight locations was plotted over the ageing period (Fig. 2).

Although enzymes were not monitored in this study, it is likely that the increased release of amino acids over the ageing process is due to the action of the various proteolytic systems existent in muscle. It is established that proteolytic degradation of muscle proteins occurs over the ageing process. While there are various opinions as to what enzyme systems are involved the role of the calpains has been extensively reviewed (Koohmaraie, 1996) particularly with respect to the initial proteolytic events. The action of both calpains and cathepsins (Feidt, Brun-Bellut & Dransfield, 1998; Nishimura, Rhyu, Tajuma & Kato, 1996; Toldra, Flores & Aristoy, 1995) and possibly high molecular weight proteases such as the multicatalytic proteinase (Orlowski & Wilk, 1988) results in the production of small polypeptides from myofibrillar and sarcoplasmic proteins. Other enzymes such as the peptidyl-peptidases further reduce these polypeptides to smaller peptides which serve as substrates for aminopeptidase. Nishimura et al. (1996) concluded that the increase in free amino acids during post mortem storage of meat was caused by the actions of aminopeptidases C, H and P.

To conclude, 17 individual amino acids were measured in eight discreet locations along the LD of three heifers. While variations were apparent between locations along the LD these differences did not reach statistical significance. A larger scale experiment would be beneficial to obtain more definitive results on variations between locations. Increases in most individual amino acids were evident over a 15 day ageing period with approximately a 100% increase evident in total amino acids. The usefulness of measurements of amino acids as well as their increases over time in determining various aspects of meat quality needs further investigation.

Acknowledgements

This project is funded by the EU FAIR programme, project number PL96-1107.

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