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CHANGES IN pH AND FREE AMINO ACIDS IN SHEEPMEAT DURING EXTENDED CHILLED STORAGE

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Background:

Braggins and Frost (1997), studying lamb legs stored chilled (-1.5°C) in a CO_2 packaging or frozen (-35°C) in a vacuum pack for 14 weeks, found meat pH increased significantly during chilled, but not frozen, storage (max. average change 0.48 pH unit). At the same time, chilled-stored legs, but not frozen-stored, underwent a flavour deterioration: 'livery/offaly' flavour intensity increased and 'sweet' and 'roasty' flavour intensities decreased. This present study aims to identify what causes meat pH to rise in lamb legs stored chilled in carbon dioxide atmosphere by monitoring changes in meat pH and in meat proteins and free amino acids during storage.

Methods:

Unstimulated carcasses from 50 Coopworth lambs were held at 5±1°C for 6 hours then at 3°C for 22 h. Both hind legs of each carcass were removed and the ultimate pH of each *semimembranosus* muscle was measured with a pH probe. Pairs of legs were randomly sorted into five groups of ten. Left and right legs in each of the groups were then divided into two subgroups with equal numbers of left and right legs (10 all told) with the contralateral leg in the other subgroup. The mean pH of each of the two groups was not significantly different. One subgroup of lamb legs was packed with 1.5 litres of CO_2 (containing less than 500 ppb oxygen) per kg of meat in aluminium foil-lined gas-impermeable polypropylene bags and stored at -1.5°C (chilled treatment). The other subgroup was placed in the same type of bag, vacuum packed, and stored at -35°C (frozen control). Replicate legs were assigned to groups and stored for 0, 4, 8, 12 and 16 weeks at the respective treatment temperatures. At the end of each storage period legs were tempered to 10°C, the pH of the *semimembranosus* muscle was remeasured using a pH probe, and legs were swabbed for determination of total aerobic plate count, then were deboned and minced. The pH of minces was measured by homogenising duplicate 1-g samples of mince in 10 mL of distilled water. A subsample of mince was also set aside for a repeat pH measurement 2 hours after mincing, to allow time for any dissolved CO_2 to evolve from the minced meat and not affect the true meat pH value.

Free amino acids were analysed using a Picotag (Waters) precolumn-derivitization method to quantify 28 individual amino acids and the peptide carnosine. Total soluble protein and myofibrillar and sarcoplasmic proteins were determined on homogenised mince samples. Non protein nitrogen and total protein were measured using standard methods (AOAC, 1994). Aerobic plate count, pH, and all metabolite assay data were analysed for source of variance by standard methods (Genstat).

Results and Discussion

At each storage time, storage treatment had no effect on aerobic plate count [range: 1.5 to 2.5 CFU cm^{-2} (log_{10})]. These low counts indicate good processing and storage practices and are too low to have affected meat pH or cooked meat flavour or odour (Gill *et al.*,1979). *Semimembranosus* muscle pH, measured by direct probe insertion, decreased ($P<0.001$) in samples held at -1.5°C overnight (zero weeks) and then stored for 4 weeks in CO_2 atmosphere packs. The mean maximum decrease was -0.15 pH unit (from 5.62 to 5.47) at 4 weeks of storage. This effect progressively diminished, and disappeared by 16 weeks storage (Fig. 1). This decrease in pH could be the result of dissolved carbon dioxide lowering the pH of the meat surface at least to the depth (~ 1.5 cm) the pH probe was inserted. After 4 weeks storage the metabolic changes that cause an increase in meat pH could have begun to reduce the effect of the dissolved carbon dioxide. This is also shown in Figure 1, where the slope of the probe curve (■) increases in parallel to the curves for meat pH measured on homogenates immediately and 2 hours after mincing. The pH of minced tissue (measured on homogenates) from lamb legs stored in carbon dioxide atmosphere increased almost linearly during the storage period to a maximum increase of 0.3 unit at 16 weeks compared to minced meat from legs stored vacuum packed and frozen for a similar period. Even after only 4 weeks storage the mince pH had increased by 0.12 units (Fig. 1). Other studies have also found a pH increase during chilled storage of beef (Boakye and Mittal, 1993) and lamb (Doherty *et al.*, 1996, Moore and Gill, 1987) in a variety of packagings.

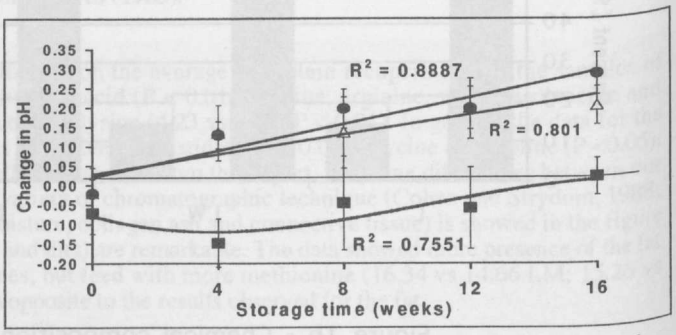

Figure 1. *Difference in meat pH (chilled-frozen) for semimembranosus muscles of paired lamb during storage measured by a pH probe inserted directly into the muscle (■), and difference in meat pH for minced whole deboned lamb legs. ▲ = pH immediately after mincing; ● = pH 2 hours after mincing.*

Total protein concentration was unaffected by storage regime and storage time. However, non-protein nitrogen increased in chilled-stored samples (concentration increase of 0.05% at 16 weeks, $P<0.001$), with the difference between treatments increasing linearly by 0.01% at each storage time. This increase in non-protein nitrogen was probably due to free amino acids, peptides and nucleic acids. Total soluble protein and myofibrillar protein also increased ($P<0.001$) during storage in chilled-stored meat compared with frozen controls. There was a slight but significant ($P<0.05$) increase in sarcoplasmic protein for both treatments over time. Total free amino acid concentrations had increased about two-fold in the chilled legs after 16 weeks. Of the 29 free amino acids measured, only eight (hydroxyproline, β-alanine, citrulline, 3-methyl histidine, α-amino butyric acid, cystine, taurine and ornithine) either remained the same

or decreased in concentration with storage time. The amino acid hydroxyproline is found only in collagen. Therefore, it appears that collagen was not broken down under these conditions. The dipeptide carnosine, and its degradation product β-alanine, also did not change significantly during either chilled or frozen storage. Carnosine and anserine (not measured) are reported to be main contributors to maintaining the buffering capacity of meat.

The remaining 21 amino acids increased in concentration with storage time in chilled-stored legs. Toldrá *et al.* (1995) attribute such an increase to proteolytic degradation of sarcoplasmic and myofibrillar muscle proteins by an aminopeptidase enzyme complex. The pool of amino acids produced by this complex contributes to the formation of flavour compounds during cooking. The concentration or ratio of particular free amino acids or peptides formed by excessive proteolysis over prolonged periods may therefore contribute to the development of 'livery/offaly' flavour notes and decrease the intensity of 'sweet' and 'sheepmeat' flavours. When considering changes in the free amino acid concentrations over the 16 weeks of storage, the increase over time was greater for basic amino acids (pI>5.6) than acidic amino acids (pI<5.6). This was true no matter how data were expressed, μmoles g^{-1} 16 $weeks^{-1}$ or as a relative percent of total free amino acids content over the same period.

The overall isoelectric point (pI) of meat protein is about pH 5.5 to 5.6. Therefore, pH 5.6 would be a logical point to consider the relative contribution of free amino acids to the final pH of meat stored for extended periods. If the majority of free amino acids that increased in concentration or relative concentration over time were amino acids of a more basic nature (relative to acidic amino acids) than the overall meat pI, then one would expect the meat pH to increase, although relative changes in concentration would also play a role (e.g. 1).

To assess the relative contribution of basic and acidic amino acids to the final pH of the stored meat, the following calculation was performed:

$$\text{Relative contribution}_{\mu moles} = \Delta pI_{amino\ acid} \times \Delta\mu mole_{amino\ acid} \quad (1)$$

where:

$\Delta pI_{amino\ acid}$ is the difference between the isoelectric point of the amino acid and the isoelectric point of meat (5.6).

$\Delta\mu mole_{amino\ acid}$ is the change the free amino acid concentration in μmoles 100 g^{-1}.

This calculation gives an indication of the relative influence that each amino acid could have on the final pH of the meat. The sum of the relative contribution of all free amino acids with isoelectric points greater than 5.6 was 3102, five times greater than the sum for the free amino acids with isoelectric points less than 5.6 (695). Therefore, the rise in pH of meat stored chilled for extended periods is probably due to an increase in basic free amino acids relative to acidic free amino acids. The more acidic [aspartic acid (pI = 2.97), glutamic (pI = 3.22)] and basic amino acids [arginine (pI = 10.76) and lysine (pI = 9.74)] had the greatest effect. This increase would affect the overall buffering capacity of the remaining meat protein, mainly governed by carnosine and anserine.

Conclusions

The main conclusions that can be drawn from this study are: pH increased by up to 0.3 pH units, in chilled lamb stored in CO_2 for 16 weeks, but not in frozen controls; total and most of the individual free amino acids increased in concentration in the chilled lamb but not in frozen lamb. A greater proportion of these were basic free amino acids a change that probably contributes to the observed rise in meat pH on extended chilled storage.

The results of this study and those of a more recent study (Braggins, unpublished data) suggest that the rise in pH in sheepmeat during extended chilled storage probably does not have the same effect on meat quality attributes as high ultimate pH (dark firm and dry appearance, propensity to microbial spoilage, and reduced flavour intensity).

Acknowledgements

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