LOSS OF PROTEIN FUNCTIONALITY IN FROZEN BEEF MAY BE CAUSED BY INTERACTIONS INVOLVING FAT OXIDATION PRODUCTS AND FREE AMINO GROUPS

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Background

The functional properties of frozen manufacturing meat – water holding capacity, gelling, binding and emulsion abilities and cook yield – tend to diminish with storage time. Many hypotheses have been advanced to explain the reasons for protein denaturation and loss of functionality at low storage temperatures. Most of these hypotheses are based on the results of model studies done using fish muscle or isolated proteins and only one or two factors were investigated at a time. This laboratory previously reported that protein denaturation during storage is caused by the interaction of a number of factors – including free amino acids and lipid oxidation products – rather than by individual, isolated factors reported in many model studies (Farouk and Swan, 1998a,b). Because the results of model studies are difficult to repeat in a food system or in whole muscle, the present study was designed to determine whether the interacting factors previously identified in our model studies apply to the loss of functionality in whole meat.

Methods

Beef *semitendinosus* from 18 heifers (n = 36) was used. Muscles were held at 10°C until rigor, then stored for one year at –18°C. The functional properties of the meat were measured at 0, 1, 3, 6, 9 and 12 months of storage. These properties were measured as described in the following studies: aldehydes (Braggins, 1996), sausage batter physical and chemical properties (Farouk and Swan, 1997), hydrophobicity, sulphydryl content and emulsion activity (Li-Chan et al., 1985). Free amino acids and dipeptides were analysed using Picotag (Waters, USA) precolumn derivitisation HPLC method.

Results and discussion

The gelling ability of the meat proteins (cooked batter torsion stress and strain) increased ($P < 0.001$) during the first month of storage and decreased ($P < 0.001$) significantly thereafter (Figure 1a & b). The initial increase in the gelling ability at one month could be due to increased protein solubility that resulted from the release of peptides and amino acids by proteolysis. Batter cook yield and emulsion stability increased ($P< 0.001$) during the first three months of storage and then subsequently declined (Figure 1c & d). The increase in cook yield could be related to the increase in the concentration of free amino acids with storage time observed in the present study. Previous studies have shown that cook yield in patties were increased by increased concentration of added free amino acids (Farouk and Swan, 1998b). Emulsion activity index did not significantly change in the first 6 months but declined significantly thereafter ($P < 0.001$) (Figure 1e). Moisture loss in raw meat increased ($P < 0.001$) with storage and reached a maximum at 6 months, indicating an increase in protein denaturation and the attendant loss of ability to hold water (Figure 1f). The increase in moisture and fat retention in cooked batter during the first 3 months of storage and the loss of moisture in raw meat in the same period indicates the different effect the state of muscle protein denaturation has on raw meat and cooked batter. Loss of water holding capacity (increased moisture loss) in raw meat does not necessarily translate into loss of moisture in cooked batter made from the same meat.

Free amino acids (except arginine and taurine) tended to increase in the first three months and then decreased, whereas dipeptides (carnosine and anserine) increased throughout storage (data not shown). Volatiles of lipid oxidation (aldehydes) increased during storage except at three months, when their concentration decreased significantly ($P < 0.001$) (Figure 2a). The decrease in free amino acids coincided with a decrease in lipid oxidation products (Figure 2b), indicating the possibility of interaction between aldehydes and amino acids/peptides/proteins. These interactions are likely to result in an aggregation and reduced solubility of the muscle proteins and consequently a reduced gelling ability at storage times longer than 1 month. The aggregation could not be due to the formation of disulphide bonds, as the sulphydryl content (SH) of the extracted proteins increased significantly at 3 months and then decreased afterwards (Figure 1g). However, the aggregation could well be due to hydrophobic bonding, as surface hydrophobicity (S_o) of the extracted proteins decreased significantly at 3 and 9 months (Figure 1h). Changes in hydrophobicity at 6 and 12 months were statistically insignificant. Regardless of the forces involved in the reaction of aldehydes and amino groups, the data from this study indicates that the concentration of both free amino acids and lipid oxidation products must reach a certain critical level for the interactions of these components to significantly affect gelation of muscle proteins. The critical level seems to have been reached around the three-month storage time under the conditions of the present study.

Conclusions

It is concluded that the decrease in the gelling ability of the meat, as measured by torsion test, which began sometime after 1 month's storage at –18°C, was due to complexes formed between free amino acids, products of lipid oxidation and muscle proteins, as evidenced by a decrease in protein surface hydrophobicity and lipid oxidation volatiles at three months and in free amino acids after three months storage. This result partially explains why muscle proteins lose functionality during frozen storage.

References

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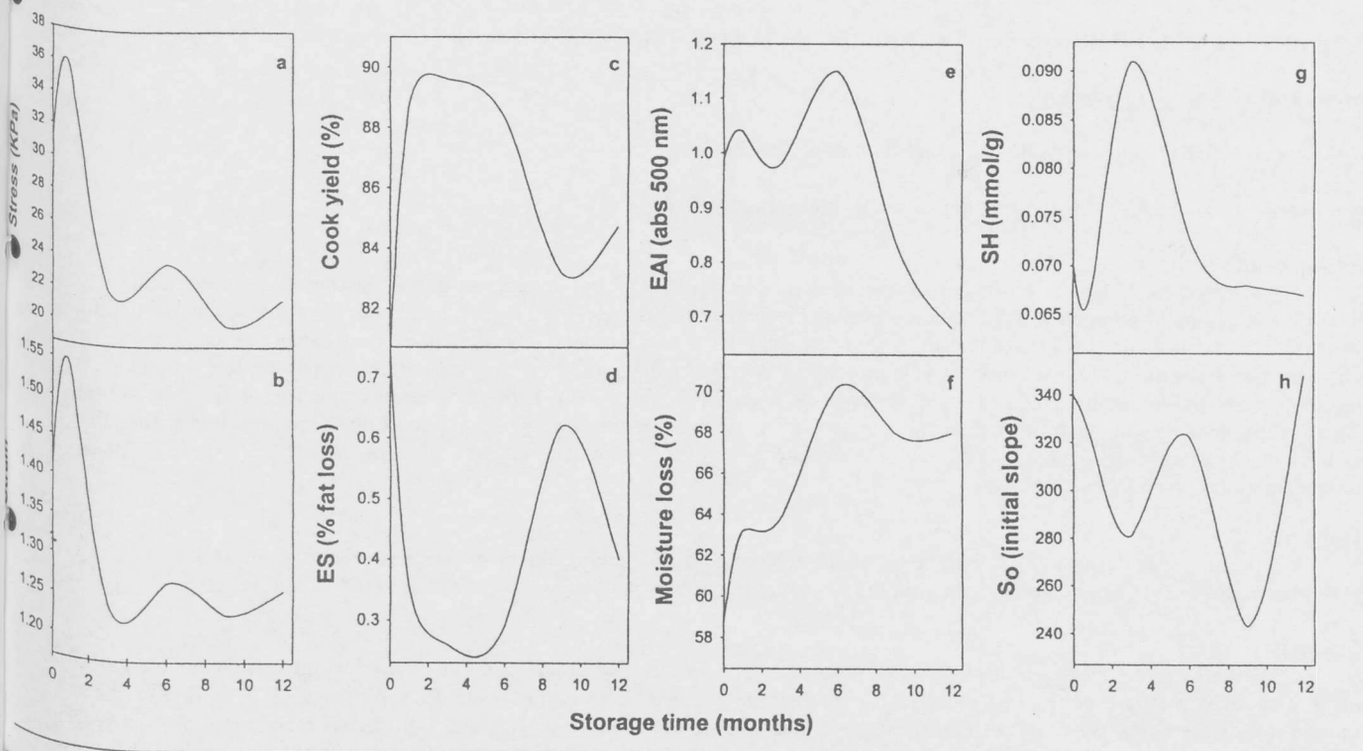

Figure 1 (a-h). Changes in the functional properties of beef stored frozen for one year at -18° C. ES, EAI, SH and S_o are emulsion stability, emulsion activity index, sulphydryl content and surface hydrophobicity respectively.

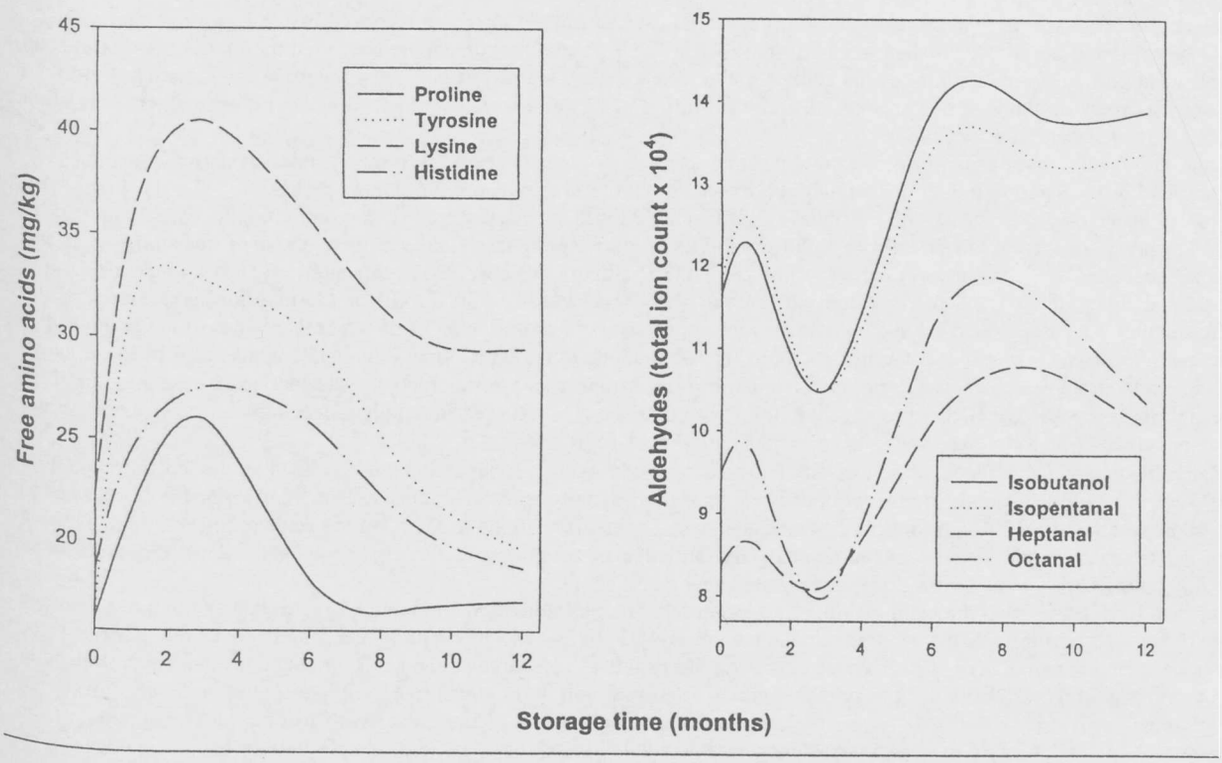

Figure 2 (a & b). Changes in free amino acids and aldehydes concentration in beef stored frozen for one year at -18°C The amino acids and aldehydes graphed are representative of the ones measured in this study