## Factors Affecting Protein Functionality In Frozen Beef

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### Introduction

Protein functionality in frozen meat may be affected by: 1) ice crystal formation due to freezing, 2) dehydration due to freezing, 3) an increase solute concentration, 4) fat hydrolysis and/or oxidation, 5) gases, particularly oxygen, 6) protein oxidation and proteolysis, 7) free amino acids and 8) rigor temperature (Matsumoto, 1979; Shenouda, 1980; Farouk and Swan, 1997). Most of the studies on the effect of frozen storage protein functionality were done on fish muscles and involved only one or a few of the factors listed above. An increasing amount of beef is boned, then held frozen for a long period for export purposes; yet there is a dearth of information on the extend to which some of the factors listed above interact and affect protein functionality in such meat. The present study was therefore designed to investigate the effect of some of the chemical changes (as they would occur naturally) during frozen storage (lipid oxidation, free amino acids, increased solute concentration) and muscle condition at time of freezing (muscle rigor temperature, presence or absence of gases, and chemical leanness) on protein functionality frozen beef.

### Materials and methods

Heifers were captive bolt stunned and processed, with no electrical immobilization or stimulation, at a commercial abattoir. The *semitendinosus* muscle from the two hindquaters of each carcass was removed approximately 45 min after slaughter. Each muscle was weighed and individually sealed in a vacuum bag (Tuf-Flex Barrier Packaging, Trigon Plastics Ltd., Hamilton, N.Z.) without vacuum. For each animal, one muscle was submerged in a water bath at 10°C and the other was submerged at 35°C. After 24 h, muscles were ground through a kidney and 3-mm plate and samples were taken for protein solubility determinations (24-h time). The remaining mince from each muscle was divided into 64 treatment combinations corresponding to two levels each of vacuum (0 vs 99.9% vacuum); rigor temperature (10 vs 35°C); solute concentration (0 vs 0.2 mixed salts: 53% KCl; 27% $Ca(H_2PO_4)_2$; 13% NaCl; 7% $NaH_2PO_4$ ); oxidised fat [0 vs 1% added oxidised back fat (6.8 meq peroxide/kg fat free amino acids (0 vs 0.3% mixture of 50% glutamine, 25% carnosine, 25% phenylalanine) and chemical leanness (0 vs 15% added back fat). The weight of ground meat was adjusted for chemical leanness to give a total of 50 g sample. The levels and combinations of added substances were selected based on studies on chemical changes in meat during storage. The added substances were blended thoroughly into the ground meat using a small blender. The samples were then kept at -20°C for one month, after which they were thawed for 14 h at 4°C and protein solubility was measured as described in Farouk and Swan (1997).

The design was a complete $2^6$ factorial in blocks of 16 (total of 64 treatment combinations). Comparisons were made based on the significance of interactions in the ANOVA results.

### Results and discussion

Removal of gases (99.9% vacuum) improved total protein solubility (TPS) and myofibrillar protein solubility (MPS) but reduced sarcoplasmic protein solubility (SPS) (Table 1). The negative effect of vacuum on SPS could be due to purging of the sarcoplasmic proteins in meat juices and exposing them to surface denaturation. Vacuum improved TPS and MPS only in samples that had no added salts or free amino acids (Tables 3c and 3d). The adverse effect of vacuum on SPS was favoured by the combination of added oxidised lipids and low level of free amino acids (Table 3b). These data indicate that vacuum may help improve TPS and MPS only in short-term frozen storage, but with long-term storage, increased ionic strength due to solute concentration and/or free amino acids may neutralise any effect vacuum may have on protein solubilities. The data also indicate that gases are likely to have the most deleterious effect on protein solubilites compared to other factors during frozen storage of beef.

Increased ionic strength (added salts) alone did not affect protein solubility (Table 1). However, in meat stored frozen under vacuum, increased ionic strength had a negative effect on TPS and MPS; while in samples that were stored frozen without vacuum, increased ionic strength tended to improve TPS and MPS (Tables 2c & 3f).

Lipid oxidation (added oxidised lipids) alone at the level introduced into the samples did not affect protein solubility (Table 1). But in the absence of vacuum and when salts were not added, lipid oxidation tended to reduce MPS (Table 3e).

Free amino acids on their own tended to improve TPS (Table 1) but had no effect on SPS and MPS. The effect of free amino acids on TPS was favoured by the combination of low-low or high-high levels of oxidised lipids and vacuum (Table 2f). Free amino acids tended to increase MPS when interacting with high levels of oxidised lipids and low ionic strength (Table 3c).

Reducing chemical leanness (addition of fat) improved TPS and SPS but did not affect MPS. The effect of reduced chemical leanness on TPS was more pronounced in samples that went into rigor at 10°C than at 35°C (Table 2 b). The increased fat content might have "diluted" other potentially deleterious effects or protected the proteins from chilling injury or both.

Fresh (24-h) Samples that went into rigor at 35°C had lower ($P < 0.01$) protein solubility (TPS, SPS & MPS) than samples that entered rigor at 10°C (results not shown). However, after one month frozen storage and the various treatment conditions, rigor temperature alone did not affect protein solubility (Table 1).

The present study attempted to introduce some of the major changes that would occur naturally in meat during frozen storage. The data indicate that within the parameters of this study, some of the chemical changes that have been reported to cause protein denaturation (Matsumoto, 1979; Shenouda, 1980) do not on their own cause protein denaturation during frozen storage; rather, it is the interaction of these factors that is responsible for protein changes. In general, high rigor temperature, storage of meat in a gaseous enviroment, lipid oxidation and increased solute concentration tended to reduce protein solubility; whereas a reduced chemical leanness and an increased free amino acid content tended to increase protein solubility.

**References**

Farouk, M. M. & Swan, J. E. (1997) Effect of rigor temperature on the functional properties of beef. *Meat Science (submitted)*

Matsumoto, J. J. (1979) Denaturation of fish proteins during frozen storage. In *Proteins at Low Temperatures,* ed. O. Fennema, pp. 205-224, Avances in Chemistry Series 180. American Chemical Society, Washington, D.C.

Shenouda, S. Y. K. (1980) Theories of protein denaturation during frozen storage of fish flesh. *Advances in Food Research* **26**: 275-311.

Table 1. Main effects of treatments on protein solubility in frozen beef.

| Treatment* | | Protein solubility (%) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Vacuum | Mixed salts | Oxidised lipid | Free amino acids | Chemical leanness | Rigor temperature |
| Total proteins | - | 19.4 | 19.7 | 19.6 | 19.4 | 19.3 | 19.9 |
| | + | 19.9 | 19.6 | 19.7 | 19.9 | 20.0 | 19.4 |
| LSD (5%) | | 0.6 | 0.6 | 0.6 | 0.6 | 0.6 | 2.4 |
| Sarcoplasmic proteins | - | 8.1 | 7.7 | 7.5 | 7.6 | 7.4 | 8.2 |
| | + | 7.4 | 7.8 | 8.0 | 7.8 | 8.1 | 7.5 |
| LSD (5%) | | 0.6 | 0.6 | 0.6 | 0.6 | 0.6 | 1.6 |
| Myofibrillar proteins | - | 11.3 | 12.0 | 12.1 | 11.8 | 11.9 | 11.7 |
| | + | 12.6 | 11.8 | 11.8 | 12.1 | 12.0 | 12.2 |
| LSD (5%) | | 0.6 | 0.6 | 0.6 | 0.6 | 0.6 | 4.1 |

* - = 10°C rigor temperature, 95 CL (no added fat) and 0% for all other factors;
+ = full level of the factor (35°C rigor temperature; 80 CL; and 99.9% vacuum; 0.2% mixed salts; 1% oxidised lipids; 0.3% free amino acids).
LSD = Least significant difference for comparison between values in table.

Table 2. Effect of two and three-way interaction of treatments on percent total protein solubility

2a — V

| MS \ | - | + |
|---|---|---|
| - | 18.9 | 20.6 |
| + | 19.9 | 19.4 |

LSD = 0.8

2b — CL

| RT \ | - | + |
|---|---|---|
| - | 19.0 | 20.9 |
| + | 19.6 | 19.2 |

LSD = 0.8

2c — FAA

| OL \ | - | + |
|---|---|---|
| - | -0.1 | 0.6 |
| + | 0.4 | -1.2 |

LSD = 1.1

2d — OL

| MS \ | - | + |
|---|---|---|
| - | 0.1 | 1.4 |
| + | 0.8 | -0.2 |

LSD = 1.1

2e — OL

| FAA \ | - | + |
|---|---|---|
| - | 1.2 | -0.2 |
| + | -0.1 | 1.3 |

LSD = 1.1

2f — V

| OL \ | - | + |
|---|---|---|
| - | 1.1 | 0.1 |
| + | -0.2 | 1.1 |

LSD = 1.1

2a, b = 2-way interactions; 2c,d = response to added salts and free amino acids respectively in a 3-way interaction (salts x oxidised lipids x amino acids); 2e, f = response to vacuum and free amino acids respectively in a 3-way interaction (vacuum x oxidised lipids x amino acids)
MS = mixed salts; V = vacuum; RT = Rigor temperature; CL = Chemical leanness, FAA = free amino acids; OL = oxidised lipids

Table 3. Effect of two- and three-way interaction of treatments on percent sarcoplasmic and myofibrillar protein solubility

3a — V

| MS \ | - | + |
|---|---|---|
| - | 10.8 | 13.3 |
| + | 11.8 | 11.9 |

LSD = 0.8

3b — OL

| FAA \ | - | + |
|---|---|---|
| - | 0.3 | -1.7 |
| + | -0.8 | -0.5 |

LSD = 1.2

3c — OL

| MS \ | - | + |
|---|---|---|
| - | -0.5 | 1.2 |
| + | 0.5 | 0.1 |

LSD = 1.1

3d — OL

| MS \ | - | + |
|---|---|---|
| - | 1.3 | 3.7 |
| + | 0.3 | -0.1 |

LSD = 1.2

3e — V

| FAA \ | - | + |
|---|---|---|
| - | -1.5 | 0.9 |
| + | -0.2 | -0.7 |

LSD = 1.2

3f — V

| OL \ | - | + |
|---|---|---|
| - | 0.4 | -0.6 |
| + | 1.6 | -2.2 |

LSD = 1.1

3a = 2-way interaction effect on myofibrillar protein solubility; 3b = response to vacuum on sarcoplasmic proteins solubility in a 3-way interaction (vacuum x oxidised lipids x amino acids); 3c = response to amino acids on myofibrillar protein solubility in a 3-way interaction (salts x oxidised lipids x free amino acids); 3d, e, f = response to vacuum, oxidised lipids and salts respectively on myofibrillar protein solubility in a 3-way interaction (vacuum x oxidised lipids x amino acids); - & + are as described in table 1; MS, V, OL & FAA are as described in Table 2.