MEAT AGEING IMPROVEMENT IN WATERHOLDING: A BIOPHYSICAL PROCESS?

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***Abstract*— Water-holding capacity (WHC) of beef increases with ageing. This study tests the hypothesis that the improvement in WHC with ageing is due to the breakdown in meat structure and the creation of "sponge effect", which disrupts the moisture loss channels and physically entraps the free water in meat. Bovine *M. semimembranosus* from 8 animals were divided into 4 parts to represent 4 different ageing times (48 hours, 1, 3 and 6 weeks), vacuum packed and stored at -1.5°C. At the designated ageing time, the samples were removed from the -1.5°C chiller and frozen in -30°C freezer. The samples were cut from frozen into different sizes for various analyses including total moisture content; thaw loss; expressible water; meat spreadability; compression test; and protein extractability and SDS-PAGE of the extracted proteins. The WHC of the meat improved with post-mortem ageing as evidenced by the decline in drip loss ($p<0.01$) and expressible water ($p<0.01$) with ageing time. Meat protein extractability ($P < 0.05$), spreadability ($p< 0.01$) and compressibility increased with aging. Evidence of structural changes were seen in SDS-PAGE further confirming what was observed using physical methods. Spreadability was higher in samples with inherently higher pH relative to lower pH. The higher pH samples also had higher WHC. The outcome of this study confirms the "sponge effect" hypothesis and could be used in developing meat ingredients with tailored functionality in terms of waterbinding.**

***Index Terms*—Beef, ageing, waterholding capacity,"sponge effect"**

I. INTRODUCTION

Previous storage studies in our laboratory on lamb (Farouk, Tavendale, Lane, Pulford and Waller, 2007) and beef and venison (Farouk, Wiklund, Stuart and Dobbie, 2009) indicate that meat waterholding capacity improves with long term chilled storage. Previous studies on pork also reported improvement in waterholding capacity after several days of storage (Moeseke and Smet, 1999; Kristensen and Purslow, 2001). We hypothesised that the improvement in waterholding with ageing was due to the disruption of the channels through which water is lost as a result of muscle/meat structural breakdown and the formation of a "sponge effect" that traps the water (Farouk et al., 2009).

The aim of this study is to test this hypothesis by following changes in muscle structural proteins and relating those changes to the waterholding capacity of meat.

II. MATERIALS AND METHODS

A. Sample preparation

Eight young bulls (age 2-3 years) were slaughtered according to standard procedure at a New Zealand beef plant. All carcasses at this plant are hot-boned within 1 h post-mortem. The beef samples (*M. semimembranosus*) were collected at boning and transported chilled to AgResearch MIRINZ, stored at 15°C for 48 hrs. Samples were cut into four and the 4 sub-samples from each animal were weighed and then randomly assigned to one of four ageing times (48 hours, 1, 3 and 6 weeks) and at the designated ageing time, the meat samples were removed from the –1.5°C chiller and placed in a –30°C freezer. The meats were kept in the freezer for at least 4-5 days before further sample preparation to ensure the meat had completely frozen. The samples were cut while they were still frozen into various sizes for analyses.

B. pH and protein extractability

pH of the samples was measured after the storage period by inserting a calibrated pH probe (Mettler Toledo MP 125 pH meter with an Inlab 427 probe) directly into the meat. Duplicate readings were taken for analysis of each sample. Protein extractability was measured as described in Farouk and Swan (1997).

C. Total moisture

The meat was cut into 1.5 x 1.5 x 4 cm along the muscle fibre while it was still frozen and weighed (W1). To ensure there was minimal structural change due to ice crystal melting, the meat was ensured frozen when taken for drying. The meat was left to freeze dry for 3 days before being weighed (W2). The total water was calculated as Total water (%) = (W1- W2) x 100/W1).

D. Thaw loss

The Honikel bag method (Honikel, 1998) was used to gravimetrically measure thaw loss. Samples approximately 50g were cut from frozen, weighed, suspended in netting suspended over a plastic dish and then stored at 4°C for 72 h, removed from the netting, dabbed dry with a paper towel, and then weighed. Thaw drip loss was calculated as the difference in the weight of the samples before and after storage expressed as a percentage of the original weight of the samples before storage.

E. Expressible water and meat spreadability

For free water and spreadability measurements, samples of approximately 0.5 x 0.5 x 1.5 cm long were cut along the muscle fibre avoiding connective tissues and fat then accurately weighed to give approximately 0.5 g. Each sample was put on a layer of filter paper LabServ Code: LBS0002.090 (90 mm) and pressed between two glass discs parallel to the muscle fibre direction for 1 minute, the squashed meat was photographed using a digital camera, the digital images were analysed using Image Pro Plus to determine the inner and outer areas of the squashed meat sample. Values were corrected to 0.5 g of meat sample. The area of the outer circle represented the expressible water while the inner circle represented meat spreadability.

F. Cook loss and shear force measurements

Samples were cooked in a waterbath at 95°C to an internal temperature of 75°C (measured by thermocouples) and then immediately placed in ice-water slurry. The weight of the meat was recorded before and after cooking. After cooking the meat samples were blotted dry and re-weighed. The cook loss was calculated as weight lost expressed as a percentage of the original sample weight. Once cooled, 10mm x 10 mm cross section samples (n=10 from each sample) were cut out from the cooked meat samples and sheared with the MIRINZ Tenderometer. The results were expressed as shear force (kgF).

G. SDS-PAGE and Scanning electron microscopy

SDS-PAGE gels were run on the Bio-Rad Criterion Cell system. 100 µg protein was loaded per well. 5% Criterion precast Tris-HCl gels (Bio-Rad) was employed. The electrophoresis was conducted at constant current of 5mA/gel for 20hrs. MOPOS running buffer (Bio-Rad) was used. Gels were stained in Colloid Coomassie Blue G250 for 48hrs and scanned using the GS700 Calibrated Densitometer Scanner (Bio-Rad). Analysis of protein bands on the gel was using Quantity One software (Bio-Rad). The results were shown in peak density (ODu).

For scanning electron microscopy (SEM), meat samples were fixed in 2% gluteraldehyde in phosphate buffer (pH 7.2.), dehydrated in a series of ethanol, mounted onto an aluminium holder, dried under vacuum, coated with gold/palladium and examined using a Jeol JSM 7000F Field Emission Gun SEM operated at 5kV at a working distance of 15mm.

H. Statistical analyses

The experimental design was a randomised block where the blocks were the 8 animals. Two of the animals were high pH. The data were analysed using the ANOVA directive of GenStat (11th Edition), Version 10.2.0.175, Service Pack 1.

III. RESULTS AND DISCUSSION

The waterholding capacity of bovine *M. semimembranosus* improved (drip loss and expressible water decreased) significantly ($P < 0.01$) with ageing at –1.5°C for 6 weeks (Table 1). This improvement in waterholding capacity occurred without any significant changes ($P > 0.05$) in the pH or the moisture content of the meat (Table 1). Meat is composed of about 75% water; the bulk of the water is held either within the myofibrils, between the myofibrils, between the myofibrils and the cell membrane (sarcolemma), between muscle cells or between muscle bundles (Offer and Trinick, 1983). Bertram, Purslow and Andersen (2002) using NMR demonstrated that drip loss is an ongoing process involving the transfer of water from myofibrils to the extracellular space, which is affected by the structural features of a muscle tissue. Offer and Trinick (1983) hypothesised that the changes in the waterholding capacity of meat are due to changes in myofibrils as a result of the expansion or shrinkage in their filament lattice. Previous storage studies in our laboratory on lamb (Farouk et al., 2007) and beef and venison (Farouk et al., 2009) at conditions similar to that of the present study indicate that meat waterholding capacity improves with long term chilled storage. Studies on pork also reported improvement in waterholding capacity after several days of storage (Joo, Kauffman, Kim and Park, 1999; Moeseke and Smet, 1999), which was attributed to the lost of water early post-mortem in the form of drip or evaporation resulting in less water being lost at a later time post-mortem (the "leaking out" hypothesis). Kristensen and Purslow (2001) discounted the "leaking out" hypothesis and attributed the improvement in waterholding capacity to the degradation of cytoskeletal proteins during ageing, which reduces the rigor-induced lateral shrinkage of myofibrils, associated with the formation of drip and also enable the inflow of previously expelled water, thereby improving waterholding capacity. This hypothesis was supported by Zhang, Lonergan, Gardner and Huff-Lonergan. (2006) when they associated the degradation of integrin and desmin with reduced drip loss in fresh pork.

Table 1. *M. semimembranosus* waterholding and structural changes with chill (-1.5°C) storage time						
Attributes	Storage time (weeks)					
	0	1	3	6	SED	*P*-value
pH	5.7	5.6	5.6	5.6	0.04	NS
Total moisture (%)	75.2	75.5	74.9	75.0	0.16	NS
Thaw loss (%)	11.7	11.4	10.8	7.9	1.16	0.01
Expressible water (%)	65.4	62.6	62.9	56.1	2.55	0.01
Spreadability (%)	34.6	37.4	37.2	43.9	2.55	0.01
Protein extractability (%)	7.4	7.1	9.6	10.3	1.30	0.05
Compressibility (KgF)	5.9	5.5	5.3	5.3	0.35	NS

SED = Standard error of difference between means; *P*-value = Statistical significance; NS = Not significant

The results of the present study support the role of muscle protein structural breakdown in improving the waterholding capacity of meat. The results show that meat structure progressively broke down with ageing as evidenced by the significant ($P < 0.01$) and numerical increases in meat spreadability and compressibility respectively and the higher protein extractability with ageing time post-mortem (Table 1). These changes were accompanied by the breakdown of large molecular weight structural proteins such as titin, nebulin and a protein with a molecular weight in the range of 300-400KD, and the appearance and changes in the intensities of lower molecular weight proteins (Figure 1). These changes in structural proteins occurred faster and were more intense and extensive in higher pH meats compared to lower pH ones (Figures 1).

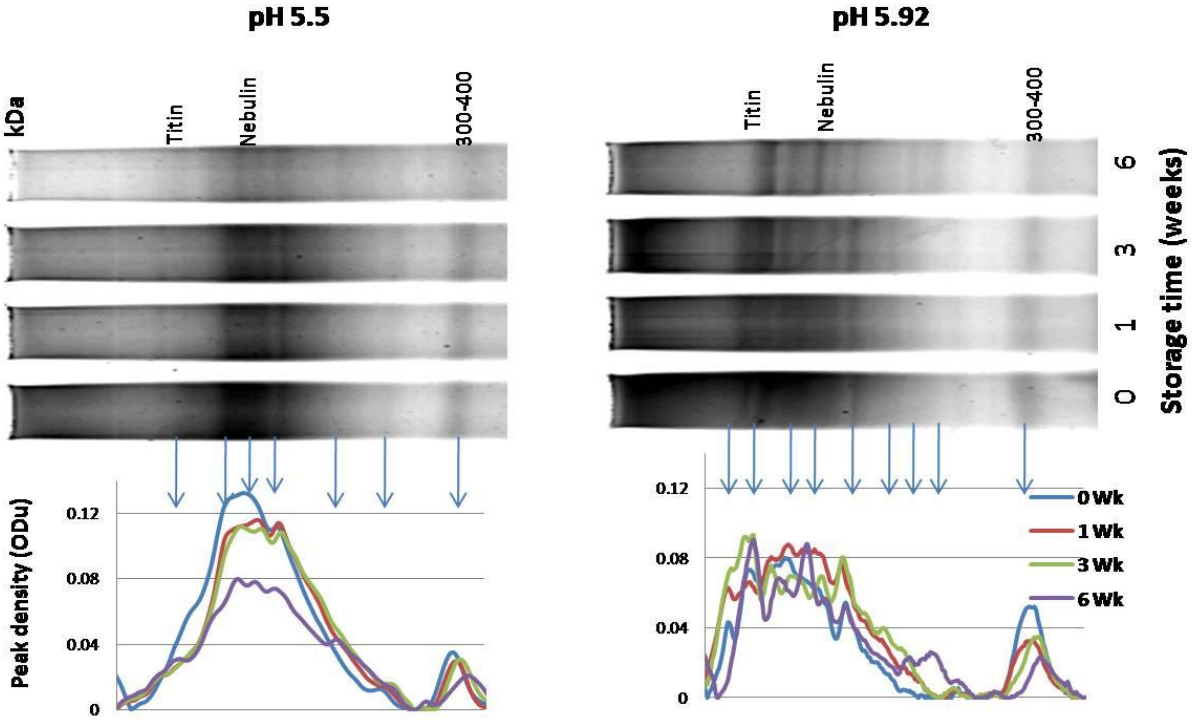

Figure 1. Changes in meat structural proteins with chill (-1.5 °C)storage time and pH

The results of the present study only partially supported earlier hypotheses that explain the structural basis for the improvement in waterholding capacity in terms of the reduction in rigor-induced lateral shrinkage of myofibrils. Reduction in the lateral shrinkage of myofibrils could not account for all the improvement in waterholding capacity observed in meat with ageing, nor provide structural reasons for the higher waterholding capacity of high pH meats compared to low pH or the lack of improvement in total waterholding capacity (purge + drip/thaw-drip + cook losses) with ageing. Reduced lateral shrinkage may be relevant in waterholding capacity changes during rigor development, but even then, Schafer, Rosenvold, Purslow, Andersen, and Henckel (2002) demonstrated that the degradation of structural proteins had little to do with it. We previously hypothesised that the improvement in waterholding with ageing is due to the disruption of the channels through which water is lost due to muscle structural breakdown and the formation of a "sponge effect" that traps the water and prevents it from getting lost (Farouk et al., 2009).

The results of the present study support our previous hypothesis in these ways: (1) waterholding capacity improved with ageing; (2) the improvement was accompanied by muscle structural breakdown demonstrated physically and chemically; (3) when data were analysed separately for pH, structural changes were higher and occurred much earlier in

high pH meats compared to lower pH meats thereby providing a physical explanation for the higher waterholding associated with high pH meats; (4) the structural basis for the "sponge effect" can be visualised at a gross level as seen in figure 2; and (5) the increase in protein extractability with ageing could lead to increased viscosity of meat water due to cold-induced partial gelation of the soluble proteins. This will reduce water mobility on its own and when combined with the "sponge effect" could significantly reduce water lost and consequently improve waterholding capacity.

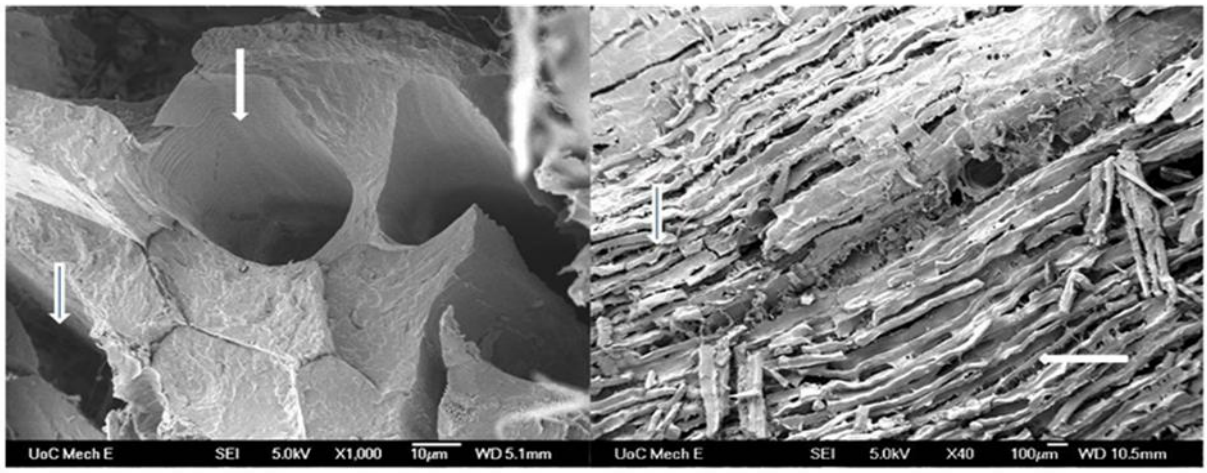

Figure 2. SEM micrographs of transverse (A) and longitudinal (B) fractured bovine *M. semimembranosus* showing intact cnannels through which drip could be lost (A)and aged muscle with less defined structure (B). White arrows point to channels and disrupted muscle structure.

IV. CONCLUSION

The waterholding capacity of bovine *M. semimembranosus* improved with ageing for 6 weeks. The improvement was related to meat structural changes observed physically and chemically. The data from the present study supports the hypothesis that meat structural breakdown results in the disruption of the channels through which water from meat is lost in the form of exudates and cookouts by forming "sponge like effect", which entraps water and prevents it from being lost. The extend of this structural breakdown explains the improvement in waterholding capacity with ageing and the higher water holding of high pH meat compared to low pH meats.

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