final report

Project code:	A.MPT.0013
Prepared by:	Anita Sikes and Ron Tume
	CSIRO
Date submitted:	May 2010
Date published:	May 2011

PUBLISHED BY
Meat & Livestock Australia Limited
Locked Bag 991
NORTH SYDNEY NSW 2059

Potential of High Pressure Processing to improve tenderness and cook yields using low value meat cuts

Meat & Livestock Australia acknowledges the matching funds provided by the Australian Government and contributions from the Australian Meat Processor Corporation to support the research and development detailed in this publication.

EXECUTIVE SUMMARY

The Australian meat industry operates in a demanding and sophisticated market place. The consumer is becoming more knowledgeable, discerning and health-conscious and enjoys access to a greater range of food choices. One factor important to the consumer is eating quality, and it is important for the Australian red meat industry to continue to invest in this area to maintain a profitable and sustainable business. The adoption of new technologies is one pathway to achieve this which will enable the industry to remain at the cutting edge and remain competitive against alternative muscle food options (e.g. chicken, seafood).

One such technology is that of high pressure application to foods where its main commercial application has been for shelf life and improved food safety by inactivating food spoilage and pathogenic organisms on a variety of foods including cooked, packaged meats. In recent years there has been a large growth in the numbers and sizes of high pressure processing (HPP) equipment, particularly in Europe and the US. In addition to minimising microbial populations on foods, high pressure has been extensively studied for its effects on the properties of foods, for example, enzyme inhibition and textural changes. In the case of meat, much of the background understanding of effects on muscle proteins and texture we have today was initiated and formulated at CSIRO, Cannon Hill during the 1970 and the early 80s. With the extensive development of commercial high pressure equipment in the food industry in the last 10 years, and appreciation of the potential of such capability in the meat area our approach in this project was to confirm the tenderisation effect using a combination of high pressure and heat on low-value, post-rigor meat cuts. The critical part of this was to optimise the conditions and evaluate the potential for the commercialisation of the technology for modern high pressure processing equipment. We also sought to develop a process for a ready-to-eat product, having consistent eating quality and maximum yield. In this work we have also attempted to understand the mechanism by which pressure combined with heat leads to meat structures that are more susceptible to shear forces and therefore are more tender.

This Final Report summarises the important issues relating to the use of combined high pressure and heat (as a new technology) for improving the value of lower value meat cuts by tenderisation and/or improved yield.

For the work planned in this project, it was important to establish rigorous procedures using a "model muscle". The beef neck muscle (*M. sternomandibularis*) was chosen for this purpose so that meaningful textural objective measurement data could be obtained, with the ability to demonstrate an effect of the process on those components that contribute to texture – the myofibrillar proteins and collagen.

The initial approach was to process small pieces of beef neck muscle (50-80 g) at high pressures of between 200 to 800 MPa at 60℃ for 20 min. Application of pressures of 200 and 400 MPa for 20 min produced significant reductions in toughness when cooked (80℃ for 60 min) as assessed by Warner-Bratzler shear force. In addition,

weight losses during cooking were reduced compared with non-pressure treated.

Evidence for a reduction of pressure holding time at 200 MPa and 60°C suggested that 2-5 min provides satisfactory tenderisation of the beef neck muscle, provided that the interior of the meat attains a temperature between 50 and 60°C.

This process of specified high pressure (200 MPa) combined with heat (60℃) for 20 min was applied to commercially valuable muscles, each having a different inherent tenderness, broadly based on connective tissue content – topside (SM), eye round (ST) and strip loin (LD). The tenderising effects of pressure-heat found on the tough beef neck muscle was reproduced on the commercial muscles, resulting in a 40-65% reduction in Warner-Bratzler shear force value of the cooked samples. Ageing for 7 days did not further improve the tenderness of the treated samples. It was also found that pressure-heat treatment did not result in any large increase in lipid oxidation (TBARS).

Having established at a gross level the benefits of HPP on meat texture (200 MPa, 60℃, 20 min), the research direction was designed to understand what structural components were being modified, and how, by the pressure-heat treatments.

The approach we took, using beef neck muscle, was to investigate the effect of pressure-heat (200 MPa, 60°C, 20 min) compared with controls of heat treatment alone (60°C for 20 min) and raw muscle. Again we found pressure-heat treatment to have a marked tenderising effect on the meat when subsequently cooked at 80°C for 1 hour with Warner-Bratzler peak force being reduced from about 10 kg to less than 5 kg. The main tenderising effect resulted from a very large reduction in initial yield values suggesting that myofibrillar toughness was reduced by such treatment. To further elucidate the treatment effect we subjected each of the treated and control muscles to tissue separation into the major components, sarcoplasmic, myofibrillar and connective tissue fractions. Each fraction was analysed on the basis of its protein content and protein distribution by SDS-PAGE. Meat microstructure was also investigated by image analysis using light microscopy and the relationship between microstructure and textural properties was assessed.

Microscopy of whole muscle homogenates showed that there were differences in the appearance of the myofibres. Overall, myofibres from the pressure-heat treated samples were very short and appeared more swollen than any of the others, suggesting there had been greater proteolysis. Also, the broken ends of the fibres were very straight and perpendicular to the fibre length whereas for controls, ends were jagged with myofibrils protruding.

Application of high pressures (200 MPa) to muscle tissue, irrespective of temperature used, results in damage of lysosomal membranes with the release of a variety of enzymes, including the proteolytic cathepsins. At low temperatures (those normally found in chilled meat) the activities of the enzymes will be relatively very low and therefore proteolysis will not be very evident. However, application of high pressure at temperatures at 50 to 60°C will not only result in destruction of the lysosomes, but the released cathepsins will attack protein structures at about their optimal rate. Therefore it could be expected that disruption of some of the myofibrillar components would be achieved.

Not only does high pressure-heat treatment lead to greater proteolysis but we have shown that there is a strengthening of the myofibrillar structure making the structure more brittle. On the basis of this we suggested that the mechanism for this improvement in tenderness is the formation of a strengthened myofibrillar structure that, when sheared by mastication, allows the crack to pass through the meat rather than dissipate into a more visco-elastic structure. In this way, a more brittle fracture is achieved and the meat is perceived as more tender. The pre-requisite is that adequate enzymic activity has occurred.

The capability of the defined pressure-heat process was determined using a semi-commercial 35L HPP unit to improve the tenderness of single-serve steaks and larger meat cut portions. We demonstrated a significant improvement in tenderness with single serve steaks (100-200g, ~20mm thick) cut from the topside. However, pressure-heat treatment of larger meat portions (400-600g) did not result in tenderness improvement. It appears that the process with the defined conditions of pressure-heat is unsuitable for larger meat portions, probably due to the poor heat transfer from the compression fluid into the meat during the time the meat is under pressure.

With the success of the pressure-heat process on steak-sized portions, we investigated a one-step process to produce a ready-to-eat (reheat) product. Steaks (~25mm thick) were cut from lower-value meats and subjected to high pressures (200 MPa) for 20 min at temperatures ranging from 60 to 76℃. Steaks were not further cooked. Satisfactory “cooked” appearance was achieved with pressure at temperatures of 72 and 76℃. The internal appearan ce of these samples suggested a medium-rare degree of doneness. Application of pressure to samples whilst applying heat resulted in a very large reduction in treatment weight losses compared with heat application alone. This was evident at all temperatures studied but at the higher temperatures the differences were greatest. At 72 and 76°C with heat alone, the weight losses were 27.0 and 32.9% compared with just 7.0 and 9.5% for pressure-heat treated steaks respectively. The ability to retain over 90% of the original weight in a ready-to-heat product should be of great interest to the industry. Not only did the specified pressure-heat treatment result in greatly improved yields but there were also significant improvements in product tenderness. At 72 and 76°C with heat alone, the peak shear force values (using modified procedure for small samples) were 1.05 and 1.29 kgF compared with 0.75 and 0.64 kgF for pressure-heat treated steaks respectively. Therefore the one-step pressure-heat process described for steaks has great potential for value adding to lower cost meat products through generation of tender and succulent products through higher moisture retention.

The research work reported in this final report outlines a process capable of adding value through advanced processing, and therefore the opportunity to increase the profitability of the red meat industry. This value-added product meets consumer demands of consistent eating quality and convenience. The one-step process for a ready-to-eat product has distinct commercial advantages in terms of consistent quality and improved yield.

1 KEY QUESTIONS RELATING TO THE PROCESS

These main questions and answers summarise the important issues relating to the high pressure processes we have applied in an attempt to use this technology to improve the commercial value of lower cost meats by improving tenderness and/or reducing cooking losses. For further information, please refer to the key results areas in each of the individual Milestone Reports listed in the Appendix.

1.1 Why was beef neck muscle used?

Beef neck muscle (*M. sternomandibularis*) was used for much of the early experimental work and was chosen because of its shape and relative high degree of toughness due to a high content of connective tissue. The muscle is long and narrow with parallel muscle fibres from which many uniform portions can be obtained.

Key Results (refer Milestone Report No 2 – Appendix 2.2)

1.2 What are the optimum conditions for small muscle pieces?

We investigated treatment of small pieces of beef neck muscle (about 50 to 80 g) at high pressures of between 200 and 800 MPa at 60°C for 20 min. Compared with the non-pressurised (0.1 MPa), heated controls, treatment resulted in significant changes in tenderness when the product was later cooked. Following cooking, samples treated at 200 and 400 MPa showed the greatest improvement in texture. Using a Warner-Bratzler device, peak shear force was reduced by 40 to 50 % by pressure-heat treatment, resulting in a very tender meat product having values of about 4 kgF. Texture profile analysis also supported an improvement in hardness, gumminess and chewiness. In addition, the optimum conditions for texture improvement also resulted in lower treatment losses and lower cooking losses. For small meat pieces we concluded that optimal conditions to achieve tenderness were 200 MPa at 60°C for 20 min.

We were also able to demonstrate a significant improvement in tenderness (20-40% reduction in Warner-Bratzler shear force values) with pressure-heat treatment (200 MPa, 60°C, 16 min) of single serve steaks (100-200g , ~20mm thick) when pressure treated in the 35L semi-commercial unit.

There is also evidence that the duration of the treatment may be able to be reduced without affecting the beneficial effects required. Although the number of individual muscle samples was limited in these trials, the findings do indicate that under the conditions of pre-heat at 45°C in combination with pressure (400 MPa) and temperature (60°C) used, 2 to 5 minutes provides satisfactory tenderising of the beef neck muscles.

Key Results (refer Milestone Report No 3 – Appendix 2.3)

1.3 Optimising protocol for large meat pieces

The procedure successfully used for improving tenderness on small meat portions was ineffective when applied to larger meat portions. Meat portions (at 5°C) of approximately cubic shape, and ranging from 400 to 600 g, when subjected to 200 MPa pressure in a 35 L semi-commercial unit set at an initial temperature of 60°C showed no improvement in tenderness when subsequently cooked. Unlike the small meat portions, less than 25 mm in maximum thickness, the thicker portions did not reach the predicted minimum temperature required to achieve the effect. Even by warming large meat portions to an initial starting temperature of 45°C, the process was not satisfactory even though it can be calculated that with pressure, the temperature would have increased to about 53°C as a result of adiabatic heating. From this work it suggests that pressure and heat must be applied simultaneously.

Because of the large improvement in tenderness that is achievable by pressure-heat treatment it is recommended that alternative technologies be investigated to see if meat temperature can be efficiently raised to the desired temperature of about 60°C. If this can be achieved, it is recommended that a cooking step follow on immediately and then chilling to rapidly bring the meat temperature down close to 0°C.

Key Results (refer Milestone Report No 6 – Appendix 2.6)

1.4 Is cooking required post treatment in order to achieve improved tenderness?

To achieve improved tenderness it is necessary to cook the meat following pressure-heat treatment when using the defined optimum conditions. For food safety reasons the pressure-heat treated meat and the heated meat (60°C) has not been taste tested without cooking and our statement is based on shear force measurements alone.

Key Results (refer Milestone Report No 6 – Appendix 2.6)

1.5 Does heat (at 60°C) have to be applied during pressure treatment or can it be applied at a later time?

Unless the temperature of the meat is near 60°C during pressure treatment there is no improvement in tenderness when the meat is cooked. We found that when meat was subjected to pressure at low temperatures (5°C) and then immediately warmed to 60°C for 20 min, the meat did not show any improvement in tenderness when it was subsequently cooked. Had this been successful the process would have been of great value for larger meat portions.

Key Results (refer Milestone Report No 7 – Appendix 2.7)

1.6 Under what conditions will the optimised pressure-heat process not be effective for improving tenderness of meat?

The following Table shows those meat conditions where the optimised pressure-heat process is unlikely to produce a tender product.

Condition of meat	Meat is effectively tenderised	Possible reason
Pre-rigor meat	?	Pre-rigor meat is tenderised at low temperatures. May be less effective with heat
High pH meat (>5.9)	No	pH is too high for optimal proteolysis
Cooked meat	No	Proteolytic enzymes destroyed by cooking
Aged meat	No	Proteolyic enzymes inactivated by chilled storage

1.7 Is the optimised procedure effective on meats containing low and high contents of connective tissue?

For development of the procedure we chose a beef neck muscle which is well known for its inherent toughness because of its high content of connective tissue. Having established that very significant improvements in tenderness could be achieved in such a tough muscle, we then needed to determine that this pressure-heat protocol was suitable for a range of commercial beef cuts having varying degrees of tenderness as a result of different connective tissue contents. We were able to demonstrate that the procedure was effective in improving tenderness, not only in tough, but also in meat cuts that were initially very tender.

Key Results (refer Milestone Report No 5 – Appendix 2.5)

1.8 What is the evidence that connective tissue was affected by pressure-heat treatment?

Changes in the thermal properties of connective tissue measured by differential scanning calorimetry (DSC) provide the most convincing information that pressure-heat has impacted on its structure and properties. Although there was little change in transition melting temperature, DSC has shown that there was a large increase in heat input required (enthalpy) to melt the structure suggesting that there had been strengthening. Raw control and heated samples (60°C) exhibited lower enthalpies. This was further supported by the evidence obtained from hydrothermal isometric tension (HIT) measurements where the tension generated by connective tissue with heat was significantly greater in the pressure-heat samples compared with the raw or heat-only treated samples. In addition, the heat solubility of collagen was reduced following pressure-heat treatment.

These observations support the contention that high pressure increases the thermal stability of collagen through strengthening of hydrogen bonds and that pressure effects oppose the heat-induced changes.

Key Results (refer Milestone Report No 2, 6 – Appendix 2.2, 2.6)

1.9 Does pressure-heat treatment result in lipid oxidation and development of rancid aromas and flavours in the meat?

At the pressures and temperatures we have used and recommending for the process we have not found any increase in lipid oxidation products (TBARS) or off aromas even after 6 days of chilled storage. Other work, including our own, has found that some conditions of high pressure (usually greater than 400 MPa) can lead to promotion of lipid oxidation and aroma/flavour problems. However, we are recommending a maximum pressure of only 200 MPa which appears to do less damage to the membrane structures and therefore minimises the chances of lipid oxidation than the higher pressures used in other work.

Key Results (refer Milestone Report No 5 – Appendix 2.5)

1.10 Is the pressure-heat improvement in tenderness comparable with that achieved by ageing?

Chilled storage of SM, ST or LD muscles for 7 days did not significantly improve the tenderness of the meat whereas a pressure-heat treatment on day 1 gave an immediate and large improvement in tenderness.

Key Results (refer Milestone Report No 5 – Appendix 2.5)

1.11 Does pressure treatment at low temperatures affect the normal ageing process?

Pressure treatment applied at 5°C did not affect the normal ageing process. The pressure-treated meat showed the same improvement in tenderness at 2 weeks as was found in control meat stored at chiller temperatures for the same time. This shows that pressure (200 MPa) does not inactivate those enzyme systems (calpains) that contribute to tenderness improvement in aged meat.

Key Results (refer Milestone Report No 7 – Appendix 2.7)

1.12 Does the pressure-heat treatment affect the appearance of the meat prior to cooking?

The pressure-heat treatment does alter the colour of the meat giving it a slightly cooked appearance, particularly on the external surfaces. However, this colour change is less pronounced than in the heated control (60°C, 20 min). This means that the product could not be sold as a tender, raw meat in retail outlets. However, as it is necessary to cook the meat following this treatment, this should not be an issue for the food service industry, particularly as the final cooked product appears normal colour.

Key Results (refer Milestone Report No 5 – Appendix 2.5)

1.13 Is our working hypothesis still applicable?

We believe our working hypothesis is still largely applicable but clearly there are some areas that may need further explanation.

- Why is it necessary to have pressure and heat applied at the same time?
    - This suggests that when under pressure, the susceptible structural linkages within the myofibre are more accessible to the released proteolytic enzymes. When heat was applied at 60°C following

pressure treatment, although the enzymes may have been released from the fragmented lysosomes, and become activated by the higher temperature, they could not bind as readily to the substrates and therefore only limited proteolysis occurred.

- It is known that lysosomal structures are damaged by pressure at low temperatures and release various proteolytic and other enzymes. Therefore, why is it that a slow proteolysis doesn't occur at chiller temperatures over an extended period of time (e.g. 14 days of ageing) thus producing a tender product when ultimately cooked?
    - As explained above, the enzyme might have limited access to the substrate unless the system is under pressure, and
    - The proteolytic activity at ageing temperatures (0-1°C) will be low and may not result in significant substrate breakdown.

1.14 Is it possible to have a single step process that can produce a ready-to-eat or ready-to-heat product with improved tenderness and minimal cook loss?

One of the disadvantages of the optimised pressure-heat process is that the meat must be cooked in order to achieve tender meat. By using the same pressure and time conditions, but at a slightly higher temperature where the meat is effectively cooked, we have obtained significant improvements in tenderness as well as major improvements in product yield. By treating steak-size portions of tough meat at 200 MPa for 20 min at temperatures of 72 or 76°C we have produced tender meat that has a cook loss of less than 8 % compared with 25 to 30 % for meat heated to those temperatures or 40 % when cooked to 80°C. As there is minimal cook loss with these products the meat exhibits an attractive juicy steak having a 'medium to rare' cooked appearance.

The microbial status of this ready-to-heat product has not been assessed. However, since the surface of the steak was held at a minimum of 72 or 76°C for 20 min, it is unlikely that its consumption would pose a health risk. Food safety issues need to be substantiated in future work.

Key Results (refer Milestone Report No 7 – Appendix 2.7)

1.15 What is the unit cost of the optimised pressure-heat process?

The costs associated with processing were based on 2 suitable models of HPP equipment. Model parameters were developed for calculating costs based on equipment costs, manning, running costs and production practices. It was estimated that the process as outlined (application of pressure whilst applying heat, followed by cooking of the product) would add 0.12 to 0.14 AUD per kg to production costs.

Key Results (refer Milestone Report No 3 – Appendix 2.3)

1.16 Overview of effective treatments

The following Table provides a summary of the effectiveness of the various procedures trialled in this study.

Protocol for meat to 25 mm thickness	**Post treatment (Ageing or heating)**	**Cooking at 80°C for 60 min**	**Procedure resulted in improved tenderness/yield**
200 MPa, 60°C	-	Yes	**Yes**/No
200 MPa, 60°C	-	No cooking	No/No
200 MPa, 5°C	14 days, 4°C	Yes	No/No
600 MPa, 5°C	14 days, 4°C	Yes	No/No
200 MPa, 5°C	60°C, 20 min	Yes	No/No
600 MPa, 5°C	60°C, 20 min	Yes	No/No
Single-step process			
200 MPa, 72°C	-	No	**Yes/Yes**
200 MPa, 76°C	-	No	**Yes/Yes**