FIET Project update

The natural proteolytic enzymes in meat: their role in tenderising and their survival under storage and processing conditions

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The project team, left to right, Mike Boland, Seah Xin Hui and Lovedeep Kaur

Importance of tenderness in meat

Tenderness of meat is one of the most important characteristics that affects consumer preference, repeat purchase and ultimately the value of any meat cut. Cuts that are recognised as tender attract higher prices and can be used by the consumer in more different ways than tough cuts, the latter being usually restricted to recipes with long cooking times. We have estimated that, if the value of 50% of the tougher cuts in the New Zealand meat industry could be improved by processing to make them as tender as prime cuts, it could be worth $23 M per annum. There is a range of processes reported in the literature that can be used to tenderise meat (Table 1), but none of these on its own has been commercially successful to date. The FIET meat tenderisation project has an approach of adapting and combining different approaches with a view to maximum value addition through tenderisation, with minimum costs.

Table 1. Some different methods of meat tenderisation

Method	Principle	Advantages and disadvantages
Sous vide cooking – moderate temperature	Cooking at above 60°C for up to several hours denatures collagen	Produces tender meat. Takes a long time and requires special packaging.
Sous vide cooking- low temperature	Cooking between 50 and 60°C for several hours denatures collagen and allows endogenous enzyme activity	Produces tender meat. Takes a long time and requires special packaging. Food safety risks
High pressure treatment	Denatures myofibrillar and connective tissue proteins to tenderise meat	OK at modest pressures, higher pressure cause toughness. Equipment is expensive.
Pulsed electric field treatment	Electrical pulses make holes in membranes, may release endogenous enzymes from organelles, may release calcium ions to activate calpains	Very rapid processing. Equipment is expensive and full industrial scale does not yet exist.
Dry aging	Traditional method, allows natural enzymes to do their work.	Produces tender meat, but takes several weeks. Can result in off flavours (lipid oxidation / rancidity)
Added enzyme treatment	Has been shown to work well – hydrolyses proteins in the meat	Can over tenderise, causing sliminess; higher temperature sous vide can inactivate the enzyme
Shockwave treatment	Shockwaves break down muscle structures causing tenderness	Very rapid processing. Expensive equipment. Industrial scale does not yet exist

Table 2. Activities of cathepsins B and H (%) in 4 days post-mortem hot boned beef brisket stored at 4˚C (control) and subsequent storage at either 4˚C or -20˚C for 14 days

		Storage at 4 ˚C		Storage at -20 ˚C	
	4 days post-mortem meat stored at 4˚C (Control)	+ 7 days post-mortem meat	+ 14 days post-mortem meat	+ 7 days post-mortem meat	+ 14 days post-mortem meat
pH	5.67 ± 0.04	5.65 ± 0.06	5.51 ± 0.02	5.60 ± 0.12	5.55 ± 0.13
Cathepsin B (%)	100 a	137 ± 33	172 ± 16	132 ± 33	130 ± 31
Cathepsin H (%)	100 a	219 ± 78	105 ± 13	98 ± 67	97 ± 35

All values are mean ± standard error of mean for three replicates.

Reasons for toughness in meat

Toughness in meat is partly a result of processes that occur post-mortem. Because there is a cessation of ATP (energy) production in the muscle, the pH falls from neutral to about 5.5, leading to denaturation and loss of water binding capacity and the actin and myosin forming a rigid insoluble and inextensible protein complex. This causes rigor mortis. Another source of toughness is connective tissue – this is the collagen-based structure that gives the muscle structure and joins it to the bones. As an animal ages, the collagen forms crosslinks that make it stronger. This also makes the meat tougher.

Natural meat tenderisation by endogenous proteolytic enzymes

One method of tenderising meat is by using the natural proteolytic enzymes already in the meat to break down some of the myofibrillar proteins and connective tissue. This is used in the process of hanging, or dry aging, where the carcass is left to hang at chiller temperatures for a time of up to several weeks. This process is effective, but incurs a substantial cost for chilled storage and can result in off flavours due to lipid oxidation.

Another option for meat tenderisation is sous vide cooking at a temperature where at least some of the proteolytic enzymes are still active. Sous vide temperatures also tend to convert some of the collagen to gelatine by melting its triple helix structure, diminishing its contribution to toughness. This has been of particular interest for the FIET project, as it represents a method of rapidly achieving similar tenderisation as would be obtained from dry aging, without the potential for lipid oxidation. For this to work in the New Zealand industry, it would be necessary for at least some of the proteolytic enzymes to survive under both storage and cooking conditions.

Natural proteolytic enzymes in meat

The natural proteolytic enzymes in meat can tenderise the meat after rigor mortis by breaking down (hydrolysing) the structural proteins, both connective tissue and myofibrillar proteins. There are three kinds of proteolytic enzymes that are commonly discussed in this context:

- Calpains: The calpains are sulfhydryl proteases that depend on calcium ions for activity. There are two types of calpain, m-calpain and μcalpain, that require calcium in millimolar and micromolar concentrations, respectively. The calpains are considered by some researchers to be the major contributor to meat tenderness. Meat also contains calpastatin, a natural inhibitor of the calpains, and high levels of this inhibitor in the meat have been correlated with a lack of tenderness. However, the calpains are not heat-stable and would not be expected to persist during, for example, sous vide cooking.
- Cathepsins: The cathepsins are another class of proteolytic enzymes in meat. They occur in the lysosomes and are released when cell structure breaks down postmortem. Among the family of cathepsins, cysteine cathepsin B, H and L and aspartic cathepsin D are the most abundant in muscles. The cathepsins, other than cathepsin D, are more heat resistant and are potentially active at sous vide temperatures.
- Caspases are proteolytic enzymes that form part of the apoptosis (programmed cell death) process. There is not much evidence that they have a role in meat tenderising, but it has been suggested that they may break down calpastatin, enabling the calpains to be more active.
- Proteasomes have also been raised as a possible source of protein breakdown, but they have not been widely researched or discussed.

Effects of storage on the cathepsins

We explored the activity of cathepsins B, H and L using hot-boned briskets, obtained directly after slaughter and butchering from the ANZCO Manawatu meat processing plant at Bulls. Samples were taken immediately upon return to the laboratory and stored either at 4°C or frozen at -20°C. After various times, the samples were extracted and assayed for the different enzymes, using a range of assays. The results for cathepsins B and H are shown in Table 2.

It is apparent that these cathepsins survive well under freezing and may even increase under refrigerated conditions. The latter effect may be due to activation of proenzymes, but may also reflect better extraction possible from aged tissue (due to tissue breakdown during aging). It should be noted that there was considerable animal-to animal variation (up to 50%) in the amounts of enzyme measured (data not shown).

Survival under sous vide conditions

We then tested the survival of the calpains and cathepsins B, H and L under different sous vide temperatures, for varying lengths of time. The calpains were found to lose almost all activity in less than an hour at temperatures more than 50°C and a similar result was seen for cathepsin H. Cathepsins B and L were active at the lower sous vide temperatures as shown in Figures 1 and 2, with substantial activity remaining after 24 hours at 50°C. This demonstrates that cathepsins B and L show significant activity under lower temperature sous vide cooking conditions and could play an important role in tenderisation of meat at these temperatures.

Progress towards an industrial process for sous vide tenderisation

The use of temperatures between 50°C and 60°C is below what is generally accepted as safe for cooking meat – there is potential for survival of food-borne pathogens. The FSANZ guidelines state clearly "If storing sous vide red meat or poultry for longer than 2 days, do not cook at temperatures lower than 60°C". This is problematic if we are to make use of the cathepsin activity. Fortunately, there appears to be a way through: cooking for a period at low sous vide temperatures to allow the tenderisation to occur, followed by a further sous vide cook at higher temperature to kill off any pathogens. We are now researching this and expect to be able to present the results in a future FIET column.

Acknowledgements

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Further reading

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New Zealand Ministry for Primary Industries Template Food Control Plans: https://www.mpi.govt.nz/food-safety/food-act-2014/food-control-plans/template-food-control-plans/ accessed April 2020.

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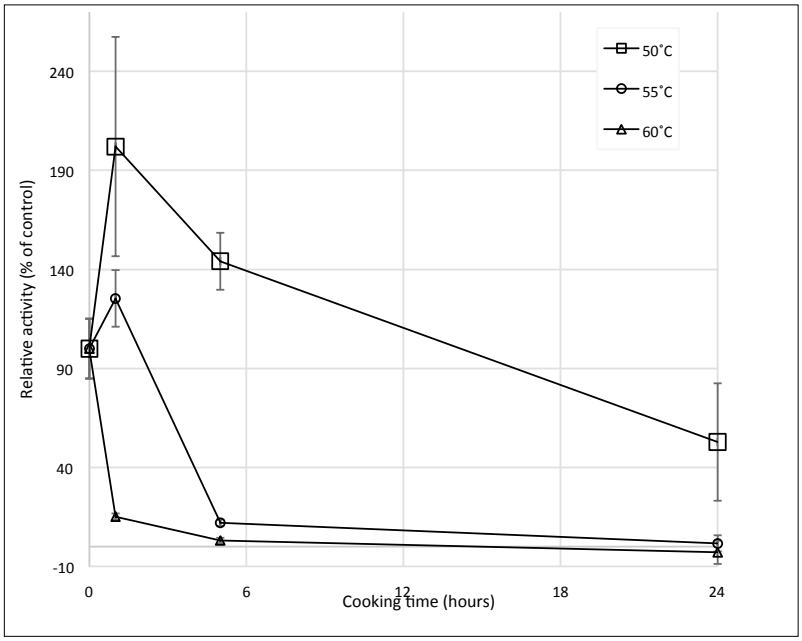

Figure 1. Relative activity of cathepsin B in hot boned beef brisket sous vide cooked at various temperatures and times.

Each data point represents the mean value of samples from three animals (error bars indicate SE).

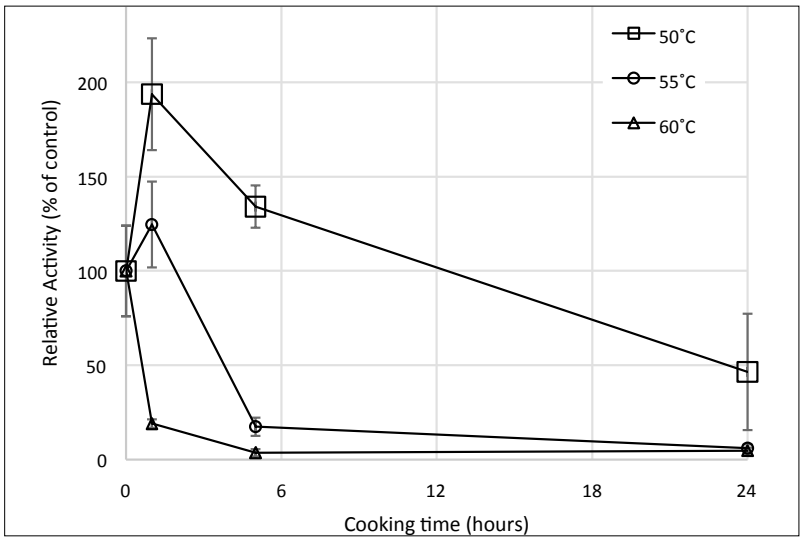

Figure 2. Relative activity of cathepsin L in hot boned brisket sous vide cooked at various temperatures and times.

Each data point represents the mean value of samples from three animals (error bars indicate SE).

Food Industry Enabling Technologies (FIET) is funded by the Ministry for Business, Innovation and Employment and its purpose is to support new process developments that have the potential to add significant value to our national economy. The programme has six partners, Massey University (the host), Riddet Institute, University of Auckland, University of Otago, Plant and Food and AgResearch. Funding is $18m over six years (2015-2021) and targets pre-commercialisation activities. If you are interested in more information, then please contact either Ross Holland (R.Holland1@massey.ac.nz) or Professor Richard Archer, Chief Technologist, (R.H.Archer@massey.ac.nz).