

# final report

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## **New products using pre-rigor beef**

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## Abstract

Two experiments were conducted at E.C Throsby abattoir at Singleton. The purpose of these experiments was to determine whether the quality of fresh processed meat products (sausages) could be improved using a combination of hot-boning, pre-rigor salting and rapid chilling using solid carbon dioxide.

The amount of salt to be added was studied in experiment 1, where salt concentrations of 0, 0.9 1.3 and 2% were added to pre rigor meat. Sausages produced using these salt concentrations were tested for thawing loss, shelf life (lab colour values) for 14 days, cooking loss and shear force. The tests identified that a salt content of 1.3% was sufficient to obtain beneficial effects of hot boning combined with pre-rigor salting and rapid chilling.

Experiment 2 was used to identify the differences between the control (1.3% salt post rigor), and two pre rigor salt treatments (1.3% pre rigor handmade and 1.3% pre rigor commercial preparation). It was again observed that pre-rigor salting effectively inhibited pH decline and improved water-holding capacity and colour attributes of the sausages.

Regarding the cooked colour of the sausages a number of issues were identified. As a result of the elevated pH, the sausages may appear undercooked when sausages produced using post rigor meat appear already fully cooked. After extended display storage (21 days) premature browning was observed for sausages cooked to a low endpoint temperature (65°C), whereas “pinking” was observed for sausages heated to a high endpoint temperature (95°C). Only the phenomenon of premature browning may represent some food safety risks. The other effects are only relevant in as far they affect consumer acceptance of the product.

Results from earlier studies cited in the literature review and results from the present experiment show that pre-rigor salting and solid carbon dioxide chilling results in a raw material with low bacterial counts. It is unclear whether the elevated pH of the sausages produced using this technique would favour the growth of spoilage bacteria during display storage. Further study of this issue is recommended.

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# Hot Boning Literature Review

## Introduction

There are a number of definitions and terminologies of hot boning in the literature which include:

-“Hot-boned meat is meat that has been removed from the carcass before the chilling process. The expression hot-boned meat may include pre-rigor meat as well as meat that has entered the onset of rigor” (Claus & Sorheim, 2006).

-“Hot processing designates the manufacture of meat products from hot boned meat. The term hot and pre-rigor processing are used interchangeably” (van Laack, 1989).

-“Hot-boning (HB) involves the removal of muscle or cuts up to about 90 min post-mortem, before the onset of rigor mortis ( $\text{pH} > 6.5$ ) (Keenan et al., 2010).

Within the context of this project the definition of Keenan et al. (2010) is most appropriate.

Hot-boning followed by immediate preparation is an age old practice to prevent bacterial spoilage in situations where chilling facilities are not available and is still practiced extensively in many developing countries. In modern slaughter operations, hot-boning is used because it has a number of economical advantages over cold-boning. These include lower labour requirements, a reduction in chiller space and energy inputs and increased product turnover (Keenan et al., 2010). However, a disadvantage of using hot-boned meat for the production of fresh meat is a toughening effect because the muscles are free to shorten during the onset of rigor once removed from skeletal restraint (van Laack, 1989). For this reason, hot-boning is mostly used for manufactured products such as hamburgers and sausages.

In this respect, the hot-boned meat can be manufactured pre-rigor or post-rigor. Advantages of using pre-rigor meat for products like beef patties include a superior water-holding capacity, resulting in improved juiciness and tenderness and a decrease in cooking loss (Keenan et al., 2010; van Laack, 1989; Sorheim et al., 2006; Berry et al., 1999). An additional advantage noted in some studies is an improvement in colour and colour stability (see Keenan et al., 2010 for a review).

## **Post mortem muscle metabolism**

To benefit from and understand the possible advantages of using pre-rigor meat for manufactured products some knowledge about post mortem muscle metabolism is useful. During the conversion of muscle to meat the pH in the muscles declines from about 7.2 to 5.4-5.8 and muscles enter rigor at a pH of about 6. The rate and extent of pH decline affect a number of quality characteristics. Since the ultimate pH of meat is relatively close to the iso-electric point of muscle proteins, a general decrease in water-holding capacity occurs. In addition, as a result of a rapid pH-decline at a relatively high carcass temperature, an additional decrease in water holding capacity can occur as a result of protein denaturation (van Laack, 1989). In tandem with the reduction in water holding capacity, the colour of the meat changes due to a change in light reflectance and absorption. As a result, high pH meat is relatively dark in colour, whereas normal ultimate pH meat is relatively light in colour. Other factors which are less pertinent to quality aspects of manufacturing beef is that the rate and extent of pH- and temperature decline affect the tenderness of meat through an effect on the contraction status of the meat at rigor and the tenderizing response during aging of the meat (Hwang and Thompson, 2001).

Therefore, to benefit from the properties of pre-rigor meat, it has to be processed in the pre-rigor state (early after slaughter), otherwise the superior functionality will be lost (Sadler and Swan, 1997b).

## **Preservation of the Functionality of Hot Boned Muscle**

Pre-rigor meat has a higher water-holding capacity and better fat-emulsifying properties than post-rigor meat, which makes it more suitable for meat products such as such as sausages and patties (Sadler and Swan, 1997a). As explained above, part of this effect is due to an elevated pH. In addition, pre-rigor meat has a greater emulsifying capacity because the salt-soluble proteins are easier to extract before actin and myosin combine to form actomyosin during rigor development (Pearson & Gillett, 1996). Addition of salt (NaCl) can maintain the high binding properties of pre-rigor meat by extraction of salt-

soluble proteins and limiting the extent of pH decline. However, grinding of meat accelerates the rate of pH decline (Sorheim et al., 2006). Thus, the window of opportunity to benefit from the properties of pre-rigor meat is dependent on the rate of pH-decline in the muscles and at what pH salt is added to extract protein and arrest further pH decline. In order to obtain the full effect of salting of pre-rigor beef, salt should be added at a pH above 5.9-6.0 (Sorheim et al., 2006).

Recommendations regarding the concentration of salt to be used to effectively inhibit pH decline vary from 1.3% to 2% (Astruc et al., 2008; Boles and Swan, 1996; Claus and Sorheim, 2006).

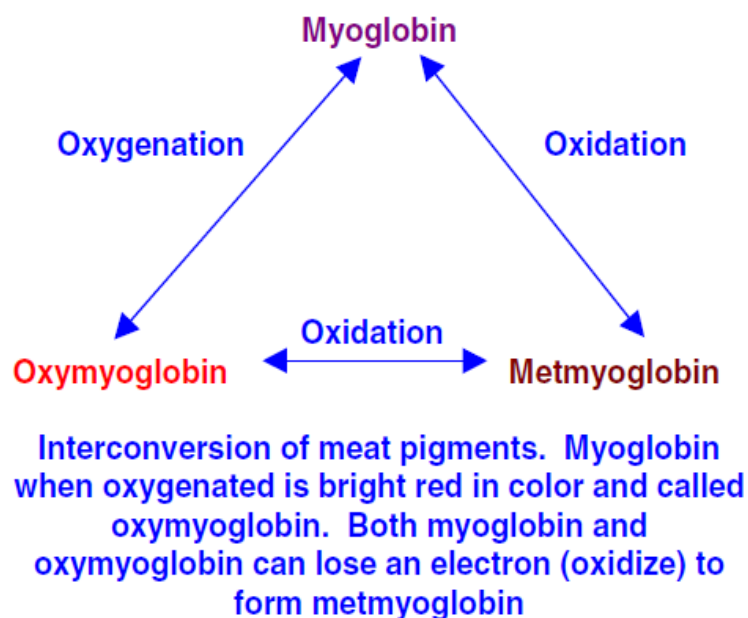
### **Food safety considerations**

Hot boning and/or processing differ from cold processing in various aspects, many of which may have an impact on the microbiological conditions of the end product (Van Laack 1989). Many conditions during hot processing would seem to favour the growth of micro-organisms including high temperatures, high pH, and high humidity". In addition, exposure of whole carcasses to contamination would be less than that of the individual primal, subprimals or beef trimmings (Quilo et al., 2009). However, results from studies on hot-boned ground beef are contradictory. A number of studies found no adverse effect of processing of hot-boned beef on bacterial counts (Emswiler and Kotula, 1979; Kotula et al, 1987; Jacobs and Sebranek, 1980; Contreras et al., 1981; McMillan et al., 1981). Other studies observed higher colony counts when processing hot-boned meat (Lin et al., 1979; Bentley et al., 1987; Legarreta et al., 1987; Newsome et al., 1987). As suggested by van Laack (1989), these differences may be due to chilling procedures like the addition of dry ice. Carbon dioxide in solid form (dry ice) at -78.9°C may reduce growth of spoilage and pathogenic bacteria in meat as a consequence of both rapid temperature decline and bacteriostatic properties of the gas (Sorheim et al., 2006). Results from a study by Abu-Bakar et al., (1988) revealed that the microbial quality of beef patties prepared after hot-boning and CO<sub>2</sub> chilling were superior or equal to controls during 21 days of storage at 0°C.

## Meat quality aspects

### Colour and Colour Stability

Myoglobin is the principal protein responsible for meat color, although other heme proteins such as hemoglobin and cytochrome C may also play a role (Mancini & Hunt, 2005). The purple-red myoglobin turns to cherry-red oxymyoglobin by oxygenation (binding of molecular oxygen). This is called the 'blooming' of meat. The other form of myoglobin, resulting from the oxidation of the iron in myoglobin, is metmyoglobin, which is greyish-brown in colour (Figure 1)



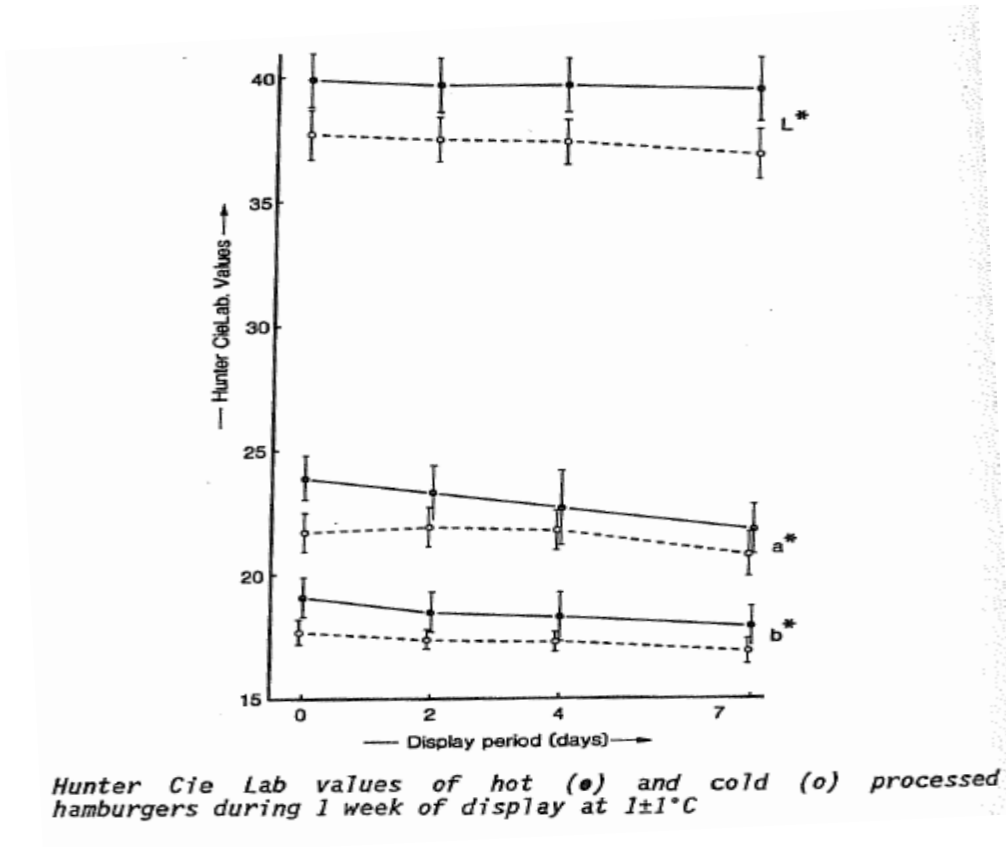
**Figure 1.1** Interconversion of meat pigments (Boles & Pegg (2005))

Autoxidation of myoglobin and oxymyoglobin (browning) has been investigated in detail, and the rate found to be influenced by several factors, including the oxygen partial pressure, exposure to light, product pH, salt concentration and temperature, and the presence or absence of certain lipid oxidation products (Mikkelsen et al., 1999).

Although it could be expected that pre-rigor salting would result in a darker colour of product as a result of an elevated pH, the opposite has been observed in a number of studies. An increased redness and lightness of was observed for beef patties produced



in this fashion (van Laack, 1989; Boles et al., 1998; Sorheim et al., 2006). A possible explanation for the increase in lightness is a more even distribution of fat in hot processed products (van Laack 1989). The reason for an increase in redness is unclear.



**Figure 1.2:** Lab colour values during storage of pre- and post-rigor salted hamburgers (van Laack, 1989).

### Cooked Meat Colour

The change in meat colour from red to greyish brown is due to denaturation of myoglobin. This denaturation is a function of pH and temperature (Terra et al., 2009). As pH increases a higher final endpoint temperature is required to denature similar amounts of myoglobin. As a result, high pH meat may appear undercooked after a cooking treatment at which normal pH meat looks cooked. Premature browning is an opposite phenomenon. Premature browning is defined by an internal cooked color of meat that is brown at temperatures when it should still appear red in color and is related to the oxidative state of meat before cooking (Grobbel et al., 2008). The oxidative state of

meat is affected amongst other factors by storage time and packaging method. Not surprisingly, premature browning is associated with modified atmosphere packaging with high concentrations of oxygen. Since appearance cooked appearance is not a good indicator for doneness, the internal temperature of ground beef patties should be determined using a thermometer or thermocouple, rather than by visual color appraisal (Brewer and Novakofski, 1999).

Regarding the effect of pH on denaturation of myoglobin, it is not surprising the problem of “hard-to-cook” hamburgers is associated with high pH beef (Berry, 1998; Berry and Bigner-George, 1999). Another colour problem that is associated with high pH meat is the formation of a pink hemochrome. As a result, products that initially appear fully cooked may gradually undergo color reversion, becoming pinker over time (Mancini and Hunt, 2005). Other reasons for an undercooked appearance may be contamination with nitrite or carbon monoxide, both of which react with myoglobin to form a relatively stable red compound (Boles and Pegg, 2005)

Results on the cooked appearance of pre-rigor salted and carbon dioxide chilled beef are limited. However, Berry et al. (1999) observed that the degree of doneness of patties produced in this fashion was significantly less than the cold-boned controls after grilling. In contrast, Sorheim et al. (2006) did not observe an effect on degree of doneness. The most likely explanation for this difference is a difference in internal temperature. In the first study patties were cooked to an internal temperature of 71°C, whereas the temperature was 80°C in the latter study.

## **Water-holding capacity**

A consistent observation when using pre-rigor salted beef is an increase in cook yield (van Laack, 1989; Farouk and Swan, 1997; Berry et al., 1999; Sorheim et al., 2006; Claus and Sorheim, 2006). Differences in cooking loss in the order of 12-15% are not uncommon and thus have a significant impact on yield of cooked product. This improvement in water holding capacity was also noted for the raw product by Farouk and Swan (1997). Thaw loss was 0% for pre-rigor salted beef and 2-3% for non-salted

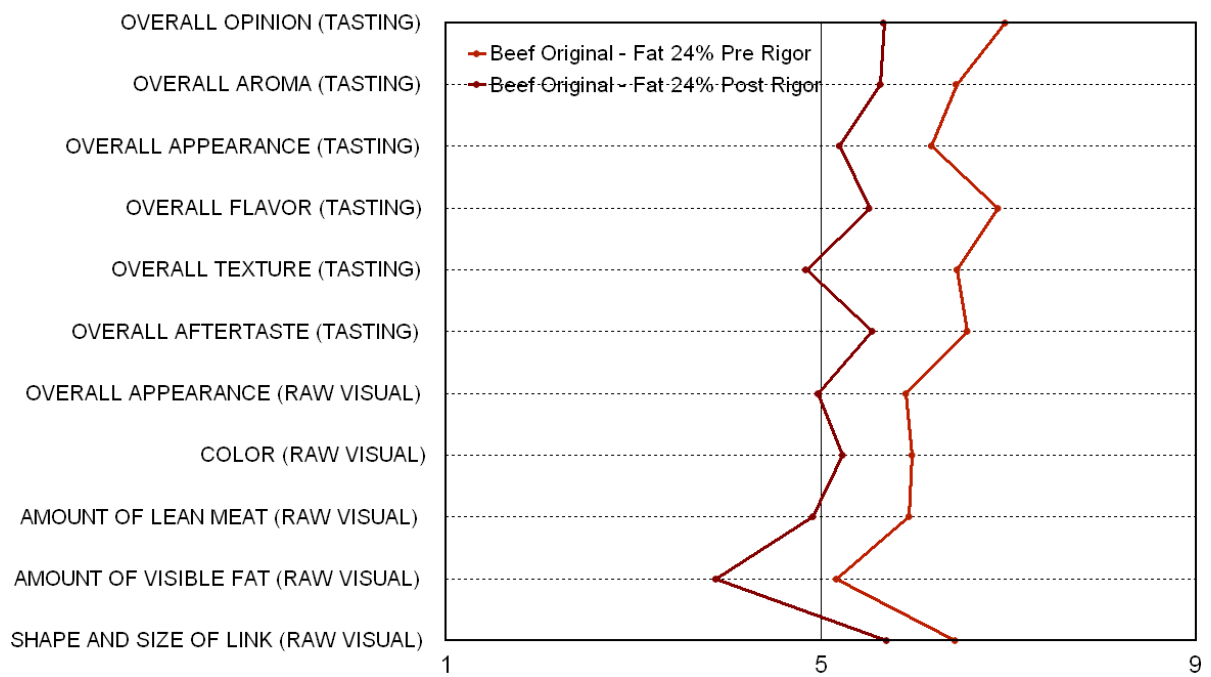
controls. Similarly, Sorheim et al. (2006) observed no drip loss from beef patties produced in this fashion during 1 day of chilled storage.

## Sensory quality

The sensory quality of meat and meat products has been considered an important factor since the beginning of the food industrialization process due to its influence on the overall quality of the product. Quality, in terms of sensory properties, is related to the adequate levels of sensory attributes considering the appearance, aroma, flavour, and texture (Deliza and Gloria 2009).

There are a number of studies in which the sensory qualities of pre-rigor salted beef products have been evaluated. Regarding juiciness, either no effect (Berry, 1996; Berry and Leddy, 1988; Berry and Stiffler), or an improvement (Cross et al., 1979) was observed. Similarly, beef flavour was either not affected (Abu-Bakar et al., 1988; Berry and Leddy, 1988; Cross et al., 1979) or improved (Berry et al., 1999; Williams et al., 1994).

The most relevant sensory analysis results regarding the current project were generated by Sensometrics for Beak and Johnston. This study involved 173 untrained consumers and compared the quality of sausages produced from pre- and post-rigor salted beef. The results of this study are summarized in Figure 1.3. The results clearly show an improvement in most, if not all, sensory quality aspects as a result of pre-rigor salting of the raw material.



**Figure 1.3** Consumer evaluation of beef sausages produced from pre- and post-rigor salted beef.

# Experiment 1.

## Using Pre-Rigor Meat to Produce Manufacturing Beef

### Introduction

Hot-boning and pre-rigor processing of meat offers potential benefits in reducing the costs of whole carcass chilling, and using the superior water holding capacity characteristics of pre-rigor meat to improve both sensory quality and yield of ground meat products. However, reliable application of hot-boned meat for the production of ground meat products depends on stringent process control during the slaughter process to maintain pre-rigor characteristics until further processing. Furthermore, the first step in further processing (grinding and salting) should preserve the beneficial characteristics of pre-rigor meat.

Beak & Johnston (B&J) intend to use hot-boned beef trim for the production of beef sausages. To this end, B&J have planned a series of experiments at EC Throsby (Singleton, NSW) to address pertinent issues. These include:

- Verifying the rate of pH decline at EC Throsby
- Assessing the functional properties of pre-rigor salted beef as affected by salt concentration and chilling conditions
- Assessing the functional, sensory and microbiological characteristics of pre-rigor processed beef sausages during chilled storage.

The described experiment was aimed at determining the optimal salt concentration to maintain the functional characteristics of pre-rigor meat and quality aspects of sausages produced using this material.

### Materials and methods

#### Sample collection and processing

16 kilograms of trim from each of 6 carcasses was collected on the processing chain at EC Throsby. The trim was ground with the aid of a mincer to approximately twenty five millimetres in diameter. Once ground, the trim was mixed with dry ice (solid carbon dioxide pellets) to achieve rapid chilling and salt was sequentially added to reach final concentrations of 0%, 0.9%, 1.3% and 2%. Four kg batches were removed from the mixer before each of the salt addition steps.

After 5 days of chilled storage, the ground trim for each treatment (n=4) was mixed and processed into sausages at the B&J facilities in Sydney. Sausage formulation included addition of salt to a final concentration of 1.3%. Therefore, the final salt concentration in the sausages was 1.3%, with the exception of the sausages produced with trim that was treated with 2% salt pre-rigor. For each of the treatments and carcasses, a series of sausages were packaged (3-4 on a polystyrene tray covered with cling wrap), frozen at -20°C and transported to the Meat Science Department at the University of New England for further analysis.

## pH

The initial pH measurements (about 1 hour post slaughter) were taken on sub-samples (ca. 100g) of the different batches (6 carcasses x 4 treatments) which were homogenized to a fine mince using a blender. Samples were stored at about 1°C, and additional pH measurements were performed after 1 and 4 days of storage. A TPS pH meter equipped with a glass electrode was used to perform the pH measurements. The reported values reflect the average of three measurements at different locations in the samples.

pH measurements were also performed on two sausages (three locations within each sausage) out of each batch.

## Thawing loss

Thawing loss was determined by weighing the sausages (3-4 sausages/polystyrene tray) before and after thawing for about 48 hours at 1°C. Thaw loss was determined by weighing the sausages and packaging material before thawing, and the sausages and dried packaging material, individually, after thawing. Thawing loss is expressed as the percentage weight loss of the sausages during the thawing period.

## Colour

Colour of the sausages was determined after 1, 3, 5 and 7 days of display storage (polystyrene trays covered with cling wrap) at 1°C using a Minolta CR300. Colour is expressed as L<sup>\*</sup>-, a<sup>\*</sup>, and b<sup>\*</sup>-values, representing lightness, redness, and yellowness, respectively. Reported values represent triplicate measurements on each of three locations of each sausage.

## Cooking loss

Cooking loss was determined after thawing the sausages (n=6 /treatment), and after 7 days of display storage (n=6 /treatment). During the initial cooking trial, the weighed sausages were suspended, in a plastic bag, in a waterbath of 70°C during 30 min., chilled in a bath of running tap water and stored overnight at 1°C, after which the weight of the cooked sausages was recorded. Cooking loss was expressed as a percentage of the weight of the samples before cooking.

After the initial cooking treatment it was noted that some of the sausages appeared undercooked (redness in the centre of the sausages). Therefore, during the second cooking trial, temperature probes were placed in the centre of the sausages to record the temperature. The total cooking time during the second cooking trial was 53 min.

## Shear force

Shear force of the cooked sausages was determined using the Warner Bratzler shear force method using 6 samples per sausage (n=6 /treatment). Means for each sausage, after removal of outlier values, were used for statistical analysis.

## Microbial Testing

The microbial testing was conducted by Silliker Inc., who examined the Total Plate Count and E.coli present in the material. For experiment one, Silliker examined the different salt treatment groups (0%, 0.9%, 1.3% & 2% salt) of the raw material. In addition to this, Silliker measured the fat in the 0% salt treatment. Measurements were taken at production (P) +2, P+5 and P+10 for all the raw material samples. Silliker measured the different treatment groups manufactured sausage microbial content. This was conducted across all the different salt treatment groups mentioned above. Silliker also examined the microbial content found between frozen and fresh manufactured sausages. Measurements were conducted on production (P) +3, P+7 and P+14.

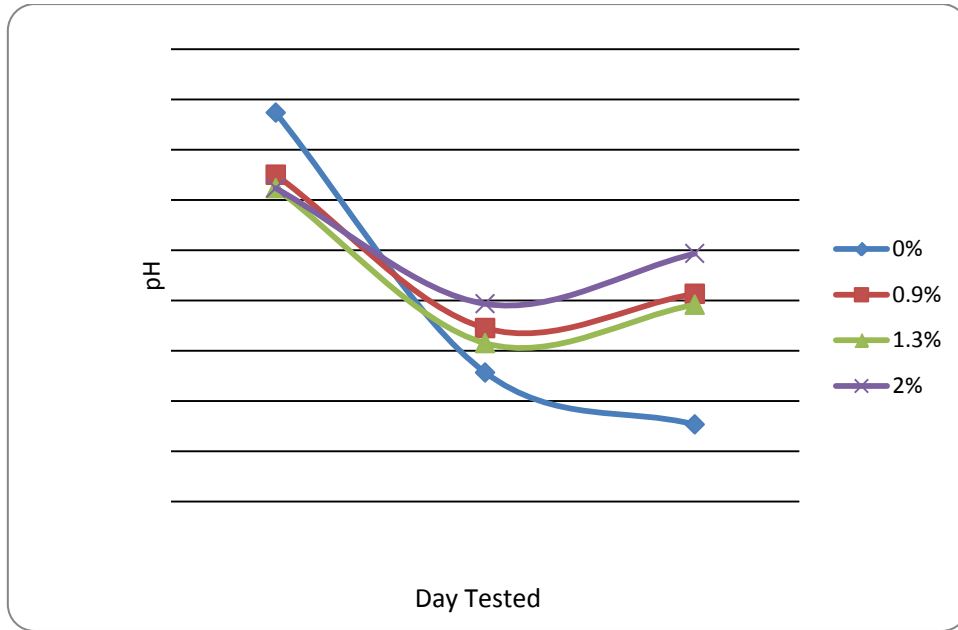
## Results and discussion

### pH

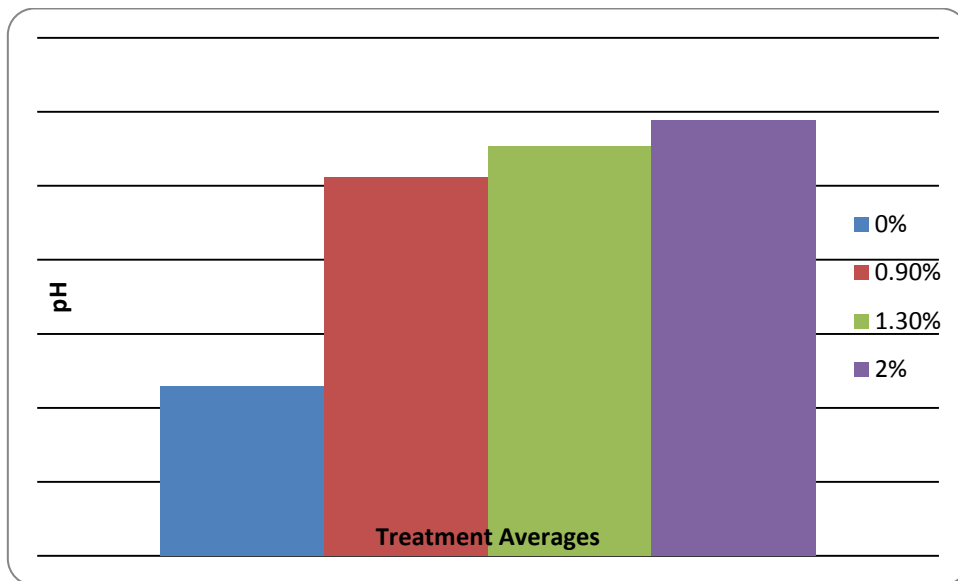
Results of the pH measurements at the different times post-processing are shown in figure 1 and appendix 1. From these results, it is clear that the different salt treatments initially accelerated the rate of pH decline, but limited the extent of pH-decline. These results are consistent with reports in the scientific literature on the subject.

From the results of the pH measurements in the ground product (figure 1) and the sausages (figure 2), it is clear that the salt treatments persistently limited the extent of pH-decline.





**Figure 2.1:** pH values of beef mince at different times post-processing as affected by addition of different salt concentrations.

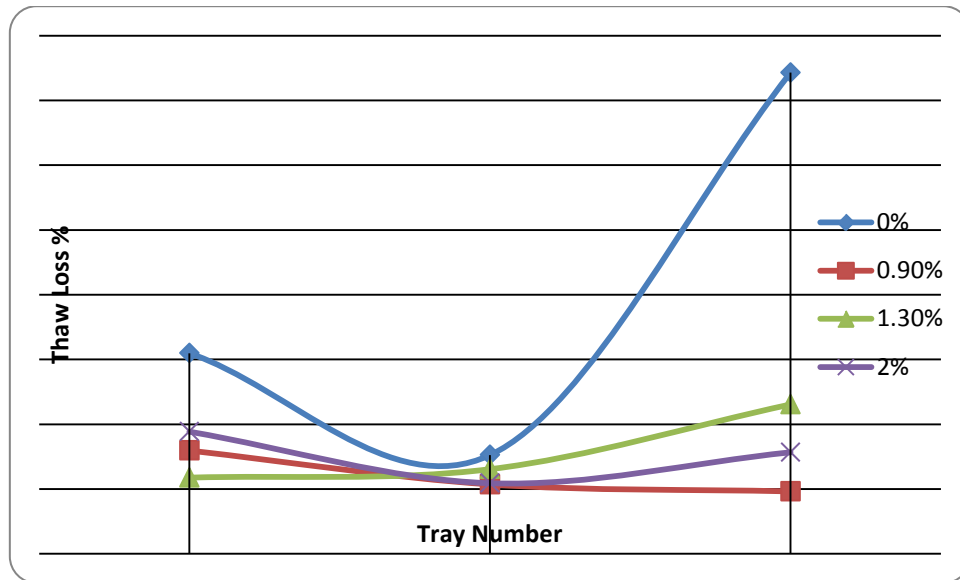


**Figure 2.2:** pH of the sausages as affected by pre-rigor addition of different concentrations of salt.

### Thaw loss

Thaw loss was determined by weighing the combined weight of the frozen sausages and packaging material (n=3 per treatment) prior to defrosting, and the weight of the

individual sausages and the dried packaging material after defrosting. Since only three trays of sausages per treatment were available, the data regarding thawing loss are limited (figure 3, appendix 2). However, the results indicate that pre-rigor salt treatment may limit the amount of thaw loss. This result is consistent with the known effects of pH on the water holding capacity of meat products.



**Figure 2.3:** Percentage of thaw loss across salt treatments.

### Colour

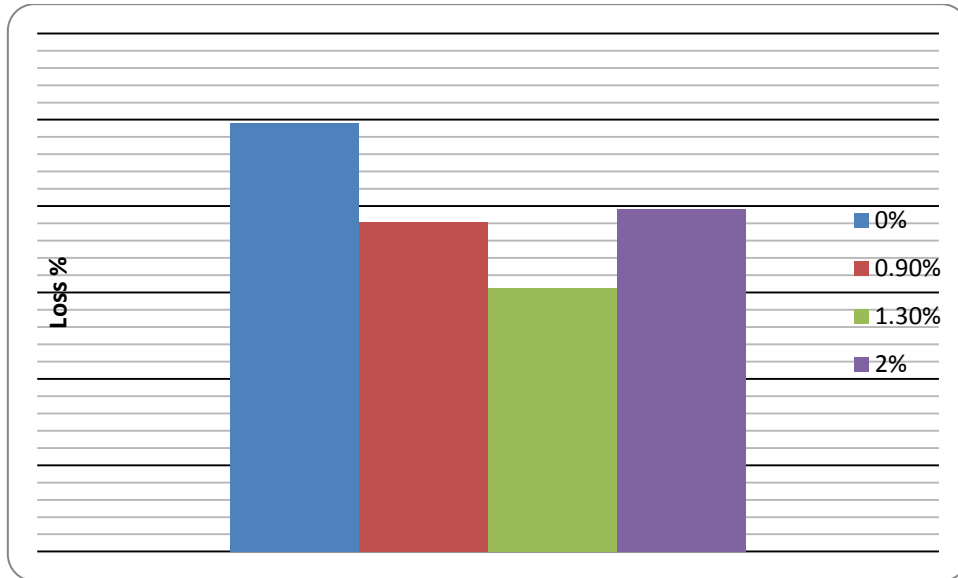
Colour measurements for samples were taken at 2 day intervals (d 1, 3, 5, and 7). This was measured as  $L^*$ ,  $a^*$ , and  $b^*$  values. It is worth noting that  $L^*$  value measures the colour range from black (0) to white (100),  $a^*$ -value measures the colour spectrum from green (negative values) to magenta (positive values), and the  $b^*$ -value represents the blue (negative) to yellow (positive) spectrum.

The results of the colour measurements can be found in appendix 3. The most noticeable effect of the pre-rigor salt treatment is that it increased the  $a^*$ -value (redness) of the sausages and that this effect persisted throughout the storage period.

### Cooking Loss

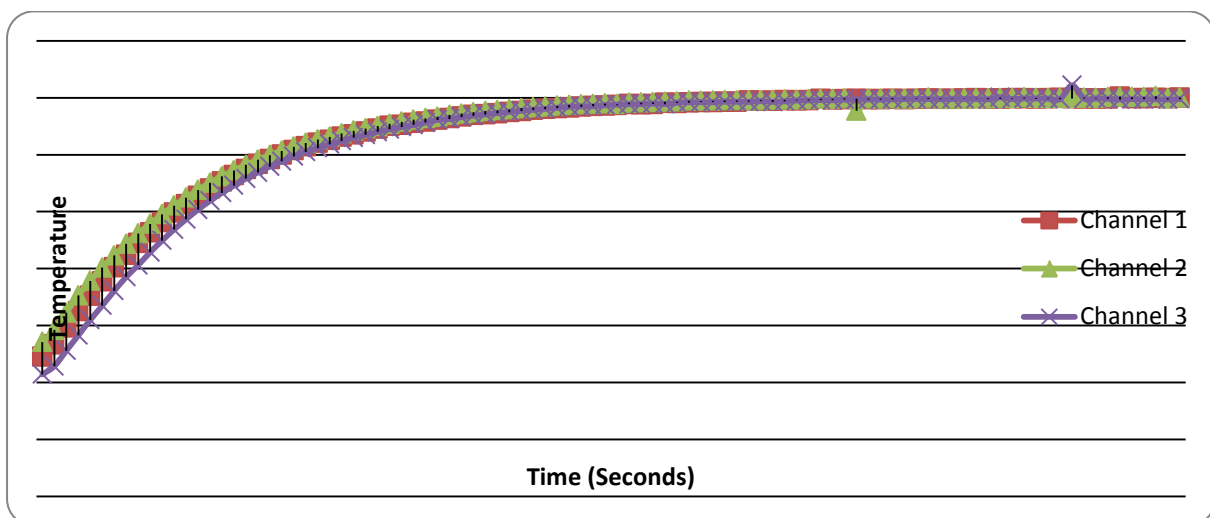
The initial cooking trial involved cooking the sausages for 30 minutes at 70°C. The effect of this cooking method on cooking loss is shown in figure 4. These results show that pre-

rigor salt treatment decreased cooking loss by about 20%. This result is consistent with the known effects of pH on the water holding capacity of meat.



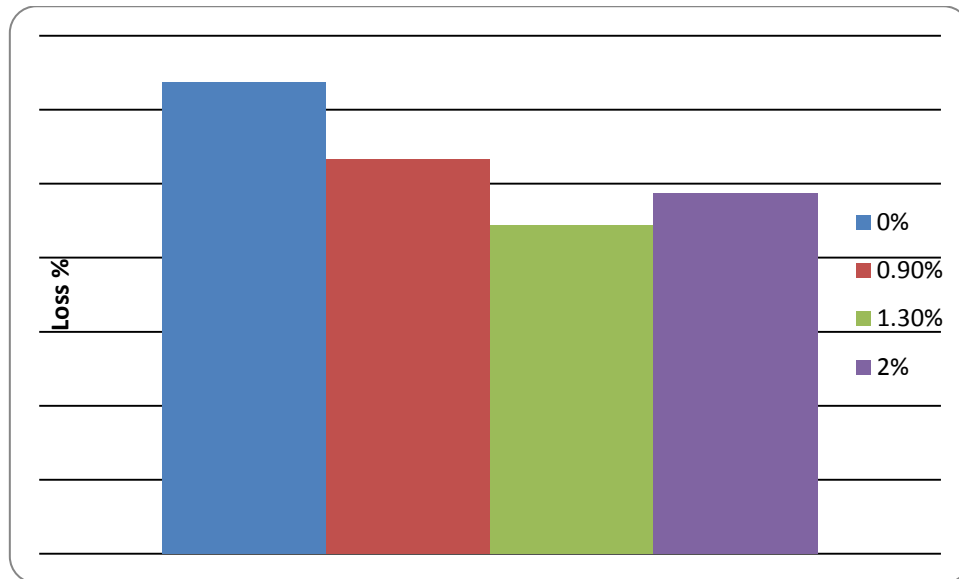
**Figure 2.4:** Cooking loss percentage (trial 1)

After the first cooking trial it was noted that the interior of the sausages was still reddish/pink in colour. This raised the question whether the cooking treatment was sufficient to thoroughly heat the interior part of the sausages. To address this question, temperature probes were inserted in the sausages during the second cooking trial (sausages cooked after 7 days of display storage). The temperature recordings show that the centre of the sausages reached the final cooking temperature within 30 minutes (Figure 5).



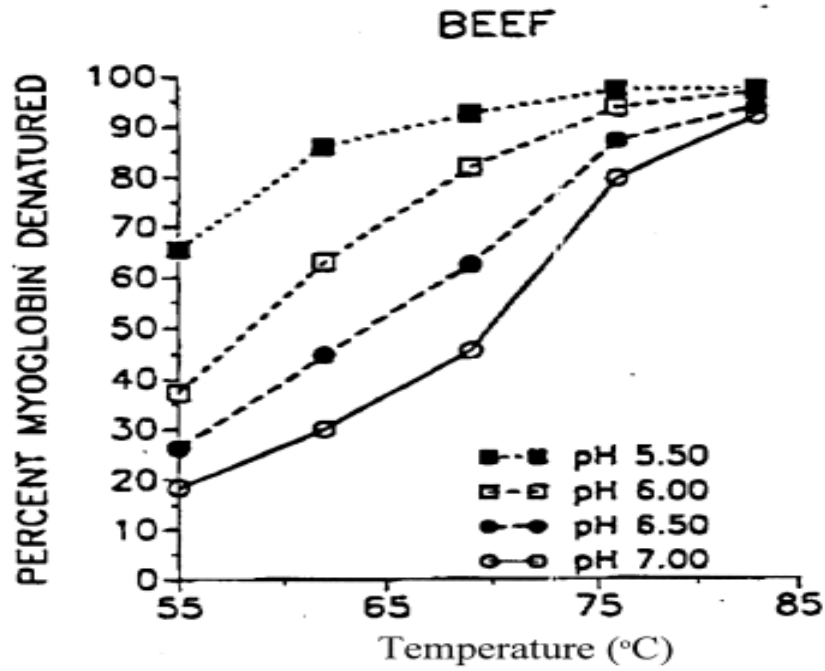
**Figure 2.5:** Temperature in the centre of the sausages during cooking.

In the second cooking trial, the sausages were heated for a period of 53 minutes. Consequently, the cooking loss increased as compared to trial 1 (Figure 6). However, similar to the results of the first cooking trial, pre-rigor salting resulted in a decrease in cooking loss.



**Figure 2.6:** Cooking loss percentage(trial 2).

After the second cooking trial careful attention was paid to evaluation of the meat colour in the centre of the sausages, and it was noted that the control samples appeared fully cooked, whereas the salt-treated samples were still reddish/pink in the centre. The explanation for this is that denaturation (browning) of (oxy)myoglobin (reddish/pink) is both pH and temperature dependent (Figure 7). Therefore, meat (products) with a relatively high pH require a greater heat load to appear fully cooked than meat(products) with a relatively low pH.



**Figure 2.7:** Denaturation of beef myoglobin as affected by pH and temperature.

### Microbial Testing

The results for the microbial counts for the raw material can be observed below.

**Table 2.1:** Average Total Plate Count and E.coli counts of the raw material for the salt treatment groups.

	P+2		P+5		P+10	
	TPC (CFU/g)	E.Coli (CFU/g)	TPC (CFU/g)	E.Coli (CFU/g)	TPC (CFU/g)	E.Coli (CFU/g)
Raw Beef Fat 0% Salt Sample	975	<10	3,580	<10	1,030	<10
Raw Beef 0% Salt Sample	240	<10	80	<10	25	<10
Raw Beef 0.9% Salt Sample	200	<10	275	<10	570	<10
Raw Beef 1.3% Salt Sample	255	<10	130	<10	390	<10
Raw Beef 2% Salt Sample	330	<10	230	<10	375	<10

Pre-rigor salting and carbon dioxide chilling resulted in a raw material with low bacterial counts during chilled storage.

**Table 2.2:** Fresh Manufactured sausages Total Plate Count and E. coli count.

	P+3		P+7		P+14	
	TPC (CFU/g)	E.Coli (CFU/g)	TPC (CFU/g)	E.Coli (CFU/g)	TPC (CFU/g)	E.Coli (CFU/g)
<b>Beef Sausage (0% Salt RM) Fresh</b>	6,900	<10	2,200	<10	7,900	<10
<b>Beef Sausage (0.9% Salt RM) Fresh</b>	44,000	<10	1,300	<10	150,000	<10
<b>Beef Sausage (1.3% Salt RM) Fresh</b>	3,700	<10	2,100	<10	1,200	<10
<b>Beef Sausage (2% Salt RM) Fresh</b>	7,300	<10	1,800	<10	730	<10

Sausages that were produced using raw material that initially salted with 0.9% salt displayed relatively high bacterial counts. Since the raw material exhibited low bacterial counts this must be due to contamination during sausage production. Results presented in table 2.2 suggest that further growth occurred during display storage. However, results presented in table 2.3 suggest that no further growth occurred during display storage. A count of 150,000 CFU/g (Table 2.2) classed as marginal (action required) under the Australian and New Zealand Food Standards (Meat Standards).

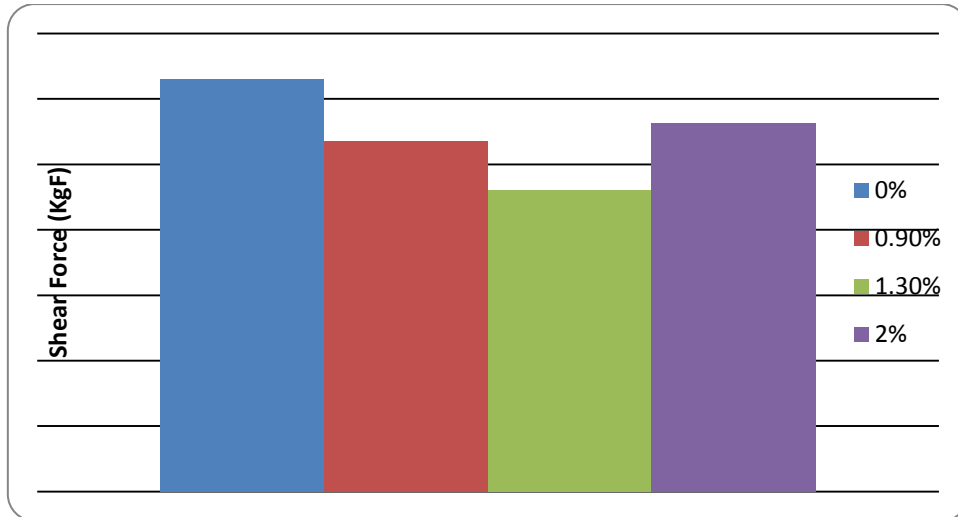
**Table 2.3:** Frozen Manufactured sausage Total Plate Count and E.coli count.

	P+2		P+5		P+10	
	TPC (CFU/g)	E.Coli (CFU/g)	TPC (CFU/g)	E.Coli (CFU/g)	TPC (CFU/g)	E.Coli (CFU/g)
<b>Beef Sausage (0% Salt RM) Frozen*</b>	7,700	<10	2,400	<10	5,000	<10
<b>Beef Sausage (0.9% Salt RM) Frozen*</b>	51,000	<10	8,300	<10	42,000	<10
<b>Beef Sausage (1.3% Salt RM) Frozen*</b>	6,500	<10	1,100	<10	790	<10
<b>Beef Sausage (2% Salt RM) Frozen*</b>	5,500	<10	2,400	<10	4,500	<10

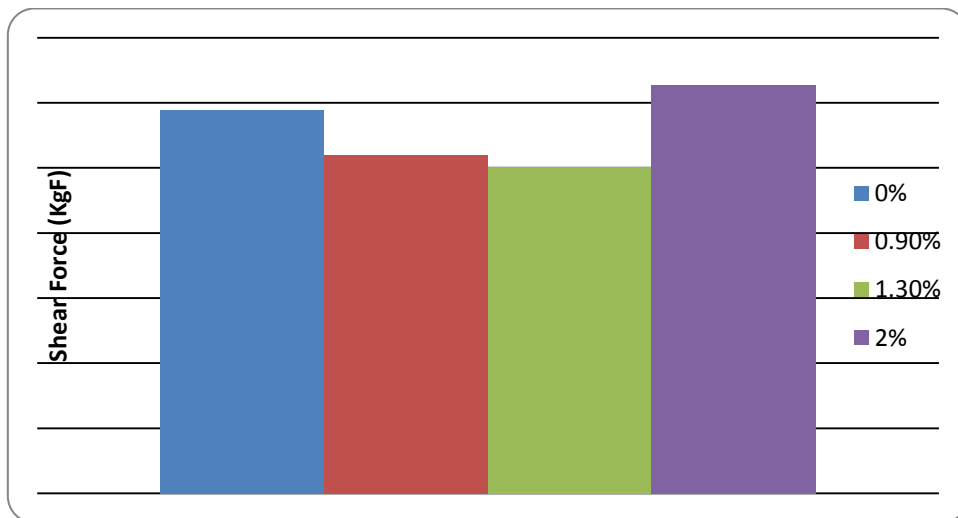
None of the samples performed unsatisfactorily regarding E. coli counts. An E.coli higher than 100 CFU/g is classed as marginal (action required).

### Shear Force

The shear force results are depicted in figures 8 and 9. Statistical analysis of the results showed that the treatments did not have a significant effect on the shear force (data not shown).



**Figure 2.8:** Shear force of the sausages as affected by the treatments (trial 1).



**Figure 2.9:** Shear force of the sausages as affected by the treatments (trial 2).

## Conclusions

- Pre-rigor salt treatment effectively arrested the pH decline in beef trim, and this effect persisted throughout storage and sausage production.
- The elevated pH of pre-rigor salted beef resulted in improved waterholding capacity of the sausages as indicated by reduced thaw and cooking losses.

- The use of pre-rigor salted beef for sausage production resulted in an increased redness ( $a^*$ -value) which persisted throughout display storage for 7 days.
- Shear force was not significantly affected by the treatments.
- Pre-rigor salting may affect the appearance of the cooked product. This observation may require further attention in following trials.



# Experiment 2

## Refinement of the Usage of Pre-Rigor Manufactured Salted Beef

### Material and Methods

#### Carcase and Processing

One hundred and thirty kilograms of 80CL lean beef carcass trim was hot boned at E.C Throsby abattoirs at Singleton (23.2.2010). The 130 kilograms were obtained from six batches (corresponding to six carcasses) of approximately 22 kilograms. The different batches of trim were passed through a mincer to grind the trim to approximately 25 millimetres in diameter. After mincing, a sample of about 400 g was removed for pH measurements. The remainder of the batch was transferred to a mixer, chilled rapidly using carbon dioxide pellets, and salted to a final concentration of 1.3%. After this stage, an additional sample of about 400 g was removed for pH determinations. Of the final batch, about 3 kilograms was removed for a control sample (1.3% salt added post rigor). The control sample and about 3 kg of pre-rigor salted trim were used to produce hand-made sausages at the laboratories of Beak and Johnsons. The remainder of the salted beef trim was used to manufacture commercial sausages. Sausages were frozen at -20°C and transported for further analysis to UNE.

#### pH

pH measurements on the control and salted trim were conducted using a TPS pH meter and probe. The pH measurements reflect an average of measurements taken at three separate sites on each sample. pH decline measurements were conducted over a ten day period of chilled storage (~3°C). The measurements were taken on days 0, 1, 3, 5, 7 and 10 post mortem. This allowed for a detailed examination of the rate of pH decline over the period of 10 days.

#### Thawing Loss

Frozen sausages were weighed individually, placed in styrofoam trays covered with clingwrap and allowed to thaw over a period of approximately 48 hours at 3°C. After thawing, the sausages were patted dry with kitchen paper and weighed again. Thaw loss was calculated as a percentage of the initial weight of the sausages.

### Lab Colour

Ten sausages per treatment group were packaged in styrofoam containers and covered with cling wrap. Colour was measured using a Minolta CR 300. The Lab colour measurements were conducted after 0, 3, 6, 9, 12, 15, 18 and 21 days of display storage.

### Sausage pH

Two sausages per treatment group were used for pH measurement. The pH measurements were taken with a TPS pH meter and a TPS pH probe. Measurements were taken from three locations in the sausages, and the average was recorded for further analysis. The pH of the sausages was measured after 0 and 21 days of display storage.

### Cooking Loss

Sausages (n = 13 per treatment) were weighed, placed in plastic bags and suspended in a 100°C water bath. The internal temperature in the sausages was measured by inserting a temperature probe into the centre of one of the sausage per cooking batch. Sausages (n = 3/treatment) were cooked to an internal temperature of 65, 75, 85 or 95°C. After cooking, the bagged sausages were chilled in ice water, after which fluids were drained from the bags and sausages chilled overnight at 3°C. Sausages were patted dry using paper towels and weighed. Cooking loss was determined after 0 (n=13/treatment) and 21 (n=10/treatment) days of display storage.

## **Cooked Colour**

After determination of the cooking loss, each sausage was halved, and a Lab colour measurements were taken with a Minolta CR 300 on both sides of the halved sausages. Colour data was recorded, and a photo was taken using a Fujifilm S5800 digital camera. Cooked colour measurements were taken on sausages after 0 and 21 days of display storage.

## **Shear Force**

Shear force measurements were conducted using the Warner Braztler method. From each sausage (n = 3 per treatment/cooking temperature), 6 samples were prepared for shear force measurements. The average shear force for each sausage was recorded and used for further analysis.

## **Microbial Testing**

During experiment 2, Silliker examined the Total Plate Count and E.coli content found in the raw material. This measurement was only taken once on production +5.

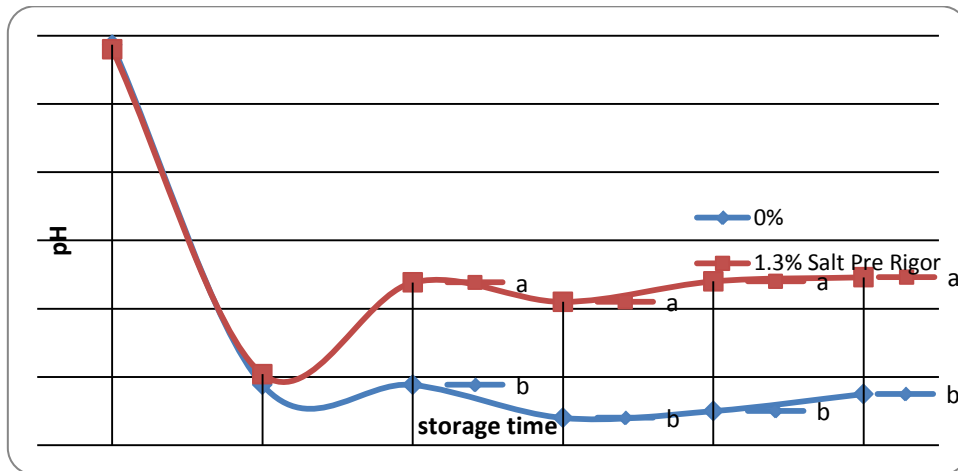
## **Statistical Analysis**

Statistical analysis was performed using either Student's T-test or analysis of variance (ANOVA). ANOVA's were conducted for the cooking loss, cooked colour and for shear force with each factor (1.3% post rigor, 1.3% pre rigor handmade and 1.3% pre rigor commercial) being treated as fixed effects. Student's T-test was conducted for the pH, thawing loss and sausage pH.

## **Results and Discussion**

## pH

The results of the pH measurements of the raw material are given in figure 1.

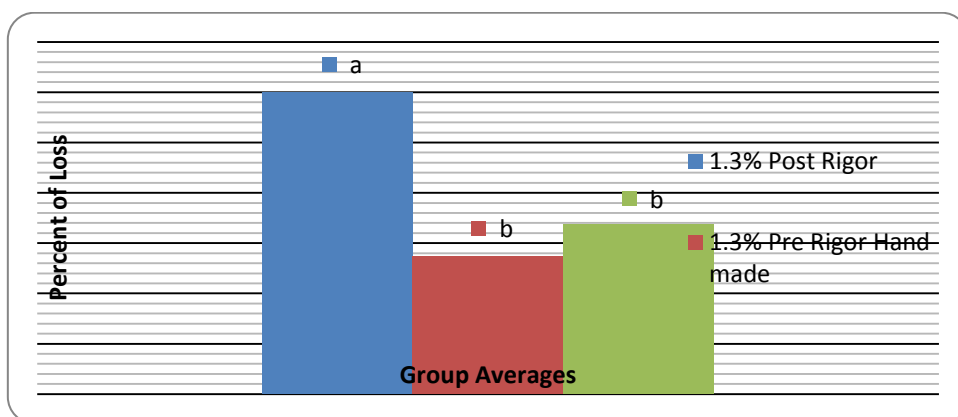


**Figure 3.1:** pH of pre-rigor salted (1.3% salt) and unsalted beef as affected by storage time. <sup>ab</sup> Means, not containing a common superscript, differ significantly, ( $p < 0.05$ ).

From the results presented in figure 1 it is clear that pre-rigor salting limited the extent of pH decline over the 10 day trial period. The difference in ultimate pH is about 0.17 units with a standard deviation of 0.06 units, and this difference was stable over the storage period of the raw material. It is unclear why the pH values of both treatments were similar at 1 day after slaughter.

## Thawing Loss

The results of the thaw loss determinations are depicted in figure 2. Although thaw losses were low in general, pre-rigor salting resulted in a reduced thawing loss.



**Figure 3.2:** Thaw loss comparison across all groups. <sup>ab</sup> Averages not containing a common letter in the superscript are significantly different ( $p < 0.05$ ).

### Lab colour

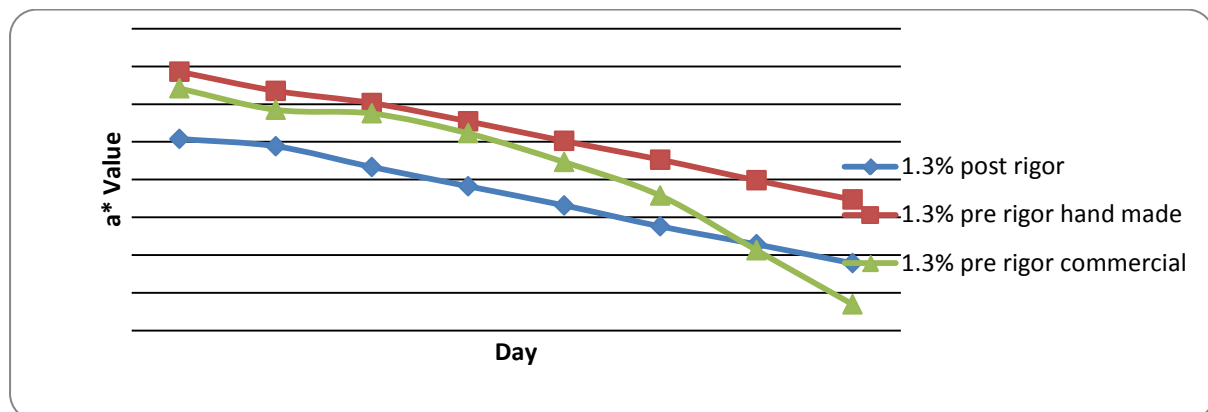
An assessment of colour was conducted during a sausage shelf life test (n=10 per treatment). For the purpose of this experiment, only the evolution of a\*-values (redness) are reported (Table 1; Figure 3).

**Table 3.1:** The means and standard errors of the a\* values for sausage colour

Day	1.3% post	1.3% pre hand made	1.3% pre Commercial
Day 0	20.16 ± 0.42 <sub>g</sub>	23.72 ± 0.25 <sub>a</sub>	22.83 ± 0.34 <sub>b</sub>
Day 3	19.77 ± 0.25 <sub>g</sub>	22.70 ± 0.19 <sub>bc</sub>	21.07 ± 0.36 <sub>de</sub>
Day 6	18.67 ± 0.18 <sub>h</sub>	22.06 ± 0.18 <sub>cd</sub>	21.50 ± 0.36 <sub>de</sub>
Day 9	17.65 ± 0.20 <sub>ij</sub>	21.09 ± 0.19 <sub>ef</sub>	20.45 ± 0.32 <sub>fg</sub>
Day 12	16.63 ± 0.14 <sub>k</sub>	20.04 ± 0.19 <sub>g</sub>	18.93 ± 0.31 <sub>h</sub>
Day 15	15.52 ± 0.18 <sub>i</sub>	19.05 ± 0.17 <sub>h</sub>	17.14 ± 0.22 <sub>jk</sub>
Day 18	14.56 ± 0.18 <sub>m</sub>	17.96 ± 0.23 <sub>i</sub>	14.25 ± 0.22 <sub>m</sub>
Day 21	13.56 ± 0.14 <sub>n</sub>	16.94 ± 0.16 <sub>k</sub>	11.38 ± 0.20 <sub>o</sub>

abcdefghijklmno Means not containing a common letter in the superscript are significantly different  $P < 0.05$ .

Pre-rigor salting resulted in an increased redness which persisted throughout display storage for 12 days for both hand-made and commercially produced sausages. After 12 days of storage, a relatively sharp decline in a-values (browning) was observed for the commercially produced sausages (figure 3). At present, the reason for the difference in colour development between the hand-made and commercially produced sausages is unclear.



**Figure 3.3:** a\* value decline over 21 days of display storage.

## Sausage pH

The pH of the sausages was determined to assess whether the differences in pH in the raw material would be persistent (Table 2). throughout sausage production and display storage

**Table 3.2:** Means and standard errors of the sausage pH after 0 and 21 days of display storage.

	1.3% post	1.3% pre handmade	1.3% pre commercial
Day 0	5.68 ± 0.04 <sup>c</sup>	5.94 ± 0.01 <sup>b</sup>	6.12 ± 0.01 <sup>a</sup>
Day 21	5.67 ± 0.01 <sup>c</sup>	5.91 ± 0.02 <sup>b</sup>	5.96 ± 0.01 <sup>b</sup>

<sup>abc</sup> Means not containing a common letter in the superscript are significantly different  $P < 0.05$ .

From these data it can be concluded that the initial difference in pH of the raw material is persistent throughout production and display storage of the sausages.

## Cooking Loss

**Table 3.3:** Cooking loss, as affected by production method and endpoint temperature (Means and standard errors).

Temperature	1.3% Post Rigor	1.3% pre Rigor Hand Made	1.3% Pre Rigor Commercial
65°C	13.43 ± 2.5 <sup>cd</sup>	2.53 ± 0.5 <sup>e</sup>	2.68 ± 0.6 <sup>e</sup>
75°C	16.19 ± 1.3 <sup>c</sup>	2.49 ± 0.4 <sup>e</sup>	3.78 ± 0.5 <sup>e</sup>
85°C	20.33 ± 0.8 <sup>b</sup>	3.30 ± 0.4 <sup>e</sup>	4.61 ± 0.4 <sup>e</sup>
95°C	26.34 ± 1.2 <sup>a</sup>	10.67 ± 0.7 <sup>d</sup>	10.71 ± 1.0 <sup>d</sup>

<sup>a,b,c,d,e</sup> Means, not containing a common letter in the superscript, are significantly different ( $P < 0.05$ ).

From the results presented in table 3, it is evident that pre-rigor salting had a large effect on cooking loss, which persisted regardless of the endpoint temperature of the cooking procedure. Obviously, this observation can be explained by an increase in water holding capacity of the meat due to an elevated pH. Analysis of the sensory evaluation results is needed to establish whether this effect translates in to an improvement in sensory characteristics.

The results above relate to sausages which were cooked immediately after thawing. A similar test was conducted after 21 days of display storage (Table 4). Results from this analysis show that the observed differences in cooking loss persisted throughout display storage. However, cooking loss in general decreased with storage time. A number of hypothesis can be formulated to explain this observation. However, further research is needed to test the validity of these hypotheses.

**Table 3.4:** Cooking loss, after 21 days of display storage, as affected by production method and endpoint temperature (Means and standard errors)

Temperature	1.3% post rigor	1.3% pre hand made	1.3% pre commercial
65°C	4.19 ± 0.64 <sup>d</sup>	3.65 ± 0.65 <sup>d</sup>	3.91 ± 0.40 <sup>d</sup>
75°C	8.91 ± 2.18 <sup>b</sup>	4.04 ± 0.17 <sup>d</sup>	4.51 ± 0.25 <sup>d</sup>
85°C	7.90 ± 0.01 <sup>b</sup>	5.47 ± 0.22 <sup>cd</sup>	5.76 ± 0.24 <sup>cd</sup>
95°C	19.37 ± 0.73 <sup>a</sup>	7.11 ± 0.47 <sup>bc</sup>	9.09 ± 0.49 <sup>b</sup>

<sup>abcd</sup> Means, not containing a common letter in the superscript, are significantly different ( $P < 0.05$ ).

### Cooked Colour

Because of the known effect of pH on the colour of cooked meat, the effect of heat treatment on cooked appearance was assessed by measuring the redness ( $a^*$ -value) in the centre of the cooked sausages (Table 5).

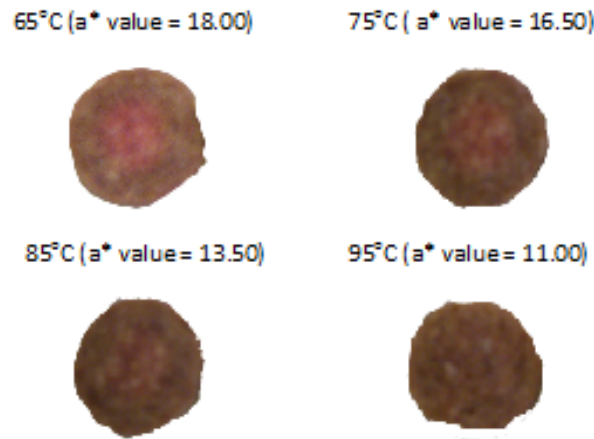
**Table 3.5:** Redness ( $a^*$ -value) in the centre of the sausages, as affected by production method and endpoint temperature (Means and standard errors)

Temperature	1.3% Post Rigor	1.3% pre Rigor Hand Made	1.3% Pre Rigor Commercial
65°C	8.47 ± 0.1 <sup>ef</sup>	8.99 ± 0.4 <sup>ef</sup>	17.65 ± 0.8 <sup>a</sup>
75°C	8.46 ± 0.2 <sup>f</sup>	8.54 ± 0.2 <sup>ef</sup>	16.42 ± 0.5 <sup>a</sup>
85°C	8.74 ± 0.2 <sup>ef</sup>	9.73 ± 0.1 <sup>de</sup>	13.59 ± 0.5 <sup>b</sup>
95°C	9.30 ± 0.4 <sup>def</sup>	10.52 ± 0.5 <sup>cd</sup>	11.27 ± 0.6 <sup>c</sup>

<sup>a,b,c,d,e,f</sup> Means, not containing a common letter in the superscript, are significantly different ( $P < 0.05$ ).

The expected effect of pH on cooked appearance was only evident in the commercially prepared sausages, which required a more intensive heat treatment to appear fully cooked. It is unclear why this effect was not observed in the hand-made sausages.

The effect of endpoint temperature on the redness (a\*-value), and therefore, cooked appearance of the commercially produced sausages is shown in figure 4.



**Figure 3.4:** Internal colour of commercially produced sausages using pre-rigor salted beef as affected by endpoint temperature.

Cooked meat colour was also assessed after 21 days of display storage (Table 6). After this storage period all of the sausages appeared fully cooked, even after heating to an internal temperature of 65°C. The explanation for this observation is that during the storage period, most of the oxymyoglobin (red) present in the sausages had already been oxidized to metmyoglobin (brown).

**Table 3.6:** Redness (a\*-value) in the centre of the sausages after 21 days of display storage, as affected by production method and endpoint temperature (Means and standard errors)

Temperature	1.3% post rigor	1.3% pre hand made	1.3% pre commercial
65°C	8.45 ± 0.28 <sup>def</sup>	8.08 ± 0.16 <sup>ef</sup>	9.80 ± 0.32 <sup>bcd</sup>
75°C	7.75 ± 0.10 <sup>f</sup>	8.89 ± 0.08 <sup>cdef</sup>	9.34 ± 0.22 <sup>cde</sup>
85°C	8.21 ± 0.28 <sup>ef</sup>	9.44 ± 0.18 <sup>cde</sup>	10.25 ± 0.32 <sup>bc</sup>
95°C	9.36 ± 0.38 <sup>cde</sup>	10.97 ± 0.35 <sup>ab</sup>	12.32 ± 0.33 <sup>a</sup>

abcdef Means, not containing a common letter in the superscript, are significantly different ( $P < 0.05$ ).



## Shear Force

Shear force analysis did not reveal any major differences in this objective measure of tenderness between the treatment groups (Table 7).

**Table 3.7:** Shear force, as affected by production method and endpoint temperature (Means and standard errors).

Temperature	1.3% Post Rigor	1.3% pre Rigor Hand Made	1.3% Pre Rigor Commercial
65°C	1.03 ± 0.1 <sup>ab</sup>	1.05 ± 0.1 <sup>ab</sup>	1.03 ± 0.0 <sup>ab</sup>
75°C	1.07 ± 0.1 <sup>ab</sup>	0.93 ± 0.0 <sup>b</sup>	1.06 ± 0.1 <sup>ab</sup>
85°C	1.19 ± 0.1 <sup>a</sup>	0.92 ± 0.0 <sup>b</sup>	1.05 ± 0.1 <sup>ab</sup>
95°C	1.21 ± 0.1 <sup>a</sup>	1.00 ± 0.1 <sup>ab</sup>	1.04 ± 0.1 <sup>ab</sup>

<sup>a,b</sup> Means, not containing a common letter in the superscript, are significantly different ( $P < 0.05$ ).

## Microbial Testing

Microbial testing was limited for this experiment (Table 3.8).

**Table 3.8:** Raw Meat Total Plate Count and E.coli count.

	P+5	
	TPC (CFU/g)	E.Coli (CFU/g)
Raw Material with 1.3% salt	680	<10
Raw Material with 0% salt	5550	<10

Pre-rigor salting resulted in a product with numerically lower plate counts than non-salted raw material.

## Conclusions

- The addition of 1.3% salt to pre rigor beef limited the extent of pH decline and this effect was persistent throughout production and storage of the sausages produced using this meat.
- The use of pre-rigor salted beef improved the water-holding capacity of sausages as evidenced by a decrease in thawing and cooking loss.
- The use of pre-rigor salted beef increased the redness (a\*-value) of sausages, and this effect persisted throughout 21 days of display storage.

- The use of high pH beef to produce sausages may result in a product that needs a more intensive heat treatment to appear fully cooked.

## **General Discussion**

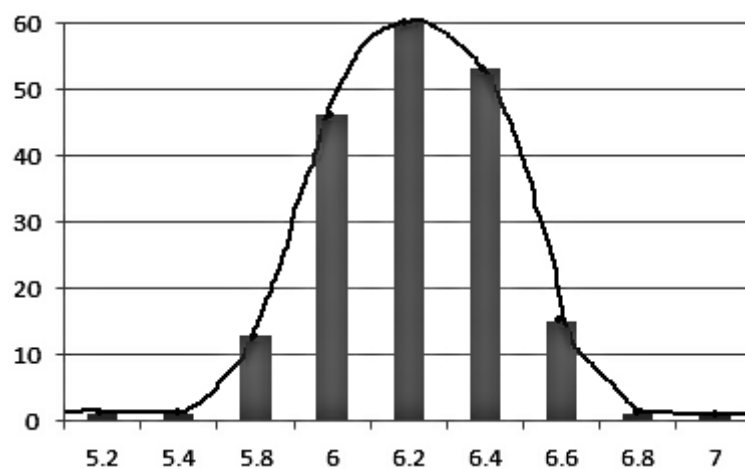
### **Salt Level**

The level of salt selected was of great importance for these experiments. The literature suggests levels of salt from 1.3% to 2% salt to be sufficient to limit the pH decline. The results from the present experiment suggest that beneficial effects of salting pre-rigor muscle can be obtained with salt concentrations as low as 0.9% (experiment 1). However, since the target salt concentration in the sausages was 1.3% this concentration was used for experiment 2. Nevertheless, it should be noted that a lower salt concentration can be used if one would like to produce low-salt sausages.

## pH

Maintaining an elevated pH in the raw material is essential to benefit from the improved manufacturing characteristics of pre-rigor salted meat. The findings in this project indicate that pre-rigor salting effectively limits the extent of pH decline, and that no further drop in pH occurs during storage of the raw material or the sausages produced thereof.

As mentioned in the literature review, the pH of the raw material should be pH 6 or higher to benefit from the process of hot-boning and salting. In the present project pH data were only collected from a limited number of carcasses. To obtain a better understanding of the variation in pH decline at E.C. Throsby we used a dataset that was collected by Edwina Toohey (DPI) at E.C. Throsby abattoirs on the loin and topside of a larger number of carcasses at about 1 hour after slaughter. As can be seen in figure 4.1, more than 90% of the pH values are above 6 at about 1 hour after slaughter. Thus, the conditions at E.C. Throsby appear suitable for this processing technique. However, it is evident that when this processing technique is adopted a pH monitoring program would be useful to ensure that intended benefits of this processing technique are maintained.



**Figure 4.1:** pH distribution of beef loins and topsides at about 1 hour after slaughter.

## Thaw loss

Although thawing loss is a minor issue regarding the quality criteria of the product under study, the results of the present experiments indicate that pre-rigor salting improved the water-holding capacity of the sausages. As indicated in the literature review, this effect can be largely explained by an elevated pH.

## Lab Colour

### Raw Sausage Colour

The results from the experiments in this project confirmed the results from studies cited in the literature review. This processing technique results in an increased redness of the product. However, the rate of decrease in redness during display storage appears similar. Thus, if visual acceptability of the product is determined by the redness of the product, this production technique has the potential to extend the shelf life of the product.

Having said this, it is also evident that the method used to produce the sausages may have a large influence on the colour stability. This was evident from the results in experiment 2 where sausages were either hand-made or produced under commercial conditions. Whereas the handmade sausages displayed a constant decline in redness over the 21 day storage period, the redness of the commercial sausages declined sharply after the day 12. The reason for this is unclear. The difference between day 12 and day 21 can be observed below in Figure 2.

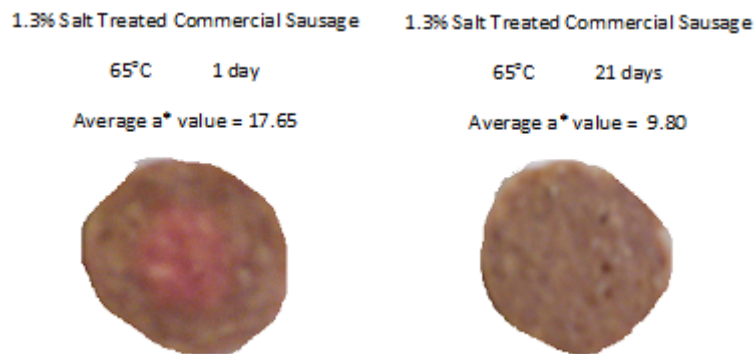


**Figure 4.2:** The visual difference between day 12 and day 21 for 1.3% salt treated commercial sausages.

### Cooked colour

The cooked colour measurements were conducted only as part of experiment 2. The colour was measured because a few anomalies were noted with the cooked sausages in experiment 1. In experiment 1, most of the sausages appeared uncooked even though they were cooked to a core temperature of 70°C.

The colour examination was conducted for Experiment 2 for both cooking trials. An image for the sausage ~~colours for~~ [colours for](#) the commercial sausages can be seen below.



**Figure 4.3:** Comparison between day 1 and day 21 cooked colour for the 1.3% commercial sausages.

During this project three different colour issues mentioned in the literature review were observed. Firstly, the elevated pH resulted in an undercooked appearance when sausages with a low pH already appeared fully cooked. Secondly, after extended storage and cooking, the commercially produced sausages looked fully cooked whereas the fresh product did not appear fully cooked (figure 4.3). This can probably be described as premature browning as a result of the oxidative status of the product after extended display storage. Thirdly, an increased redness was observed with an increase in cooking temperature in the commercially produced sausages, which was most notable after extended display storage. This can probably be described as pinking. It is unclear which reaction is responsible for this effect. Only the phenomenon of premature browning may represent some food safety risks. The other effects are only relevant in as far they affect consumer acceptance of the product.

### Cooking Loss

In both experiments, the well-documented effect of this processing technique on cooking loss was confirmed. Pre-rigor salting resulted in a large decrease in cooking loss. This effect has obvious consequences for yield of the cooked product. It may be speculated that this effect is largely responsible for the improvement in the texture of the sausages as assessed by consumers and the reduction in shear force as observed in this project.

## Microbial Analysis

Results on microbial analysis appear to confirm results from studies mentioned in the literature review that hot boning and chilling with solid carbon dioxide results in a product with very low initial bacterial counts. In addition, the bacterial counts remained very low during 10 days of chilled storage of the raw material.

A point of concern may be that, potentially, the elevated pH in the final product may favour the growth of spoilage bacteria. In experiment 2 high total plate counts were observed for one of the batches of sausages with an elevated pH (Table 2.2 and 2.3). Since the raw material used to produce these handmade sausages had low bacterial counts, contamination likely occurred during sausage manufacturing. Results in Table 2.2 suggest that further bacterial growth occurred during display storage whereas results in Table 2.3 suggest no further growth during display storage. To assess the risk of bacterial spoilage it may be considered to perform a study in which high and low pH sausages are inoculated with spoilage bacteria and their growth is monitored during display storage.

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## Appendix 1.

Day 0	Salt Treatment pH			
	0%	0.9%	1.3%	2%
Animal 1	6.54	6.29	6.24	6.19
Animal 2	6.38	6.29	6.19	6.24
Animal 3	6.5	6.28	6.39	6.34
Animal 4	6.43	6.33	6.23	6.28
Animal 5	6.28	6.16	6.16	6.17
Animal 6	6.11	6.15	6.13	6.12
Average	6.37	6.25	6.22	6.22
Std	0.16	0.08	0.09	0.08
CV	0.02	0.01	0.01	0.01

Day 1	Salt Treatment pH			
	0%	0.9%	1.3%	2%
Animal 1	6.03	5.96	5.95	6.04

<b>Animal 2</b>	5.91	5.86	5.9	5.95
<b>Animal 3</b>	5.74	6.11	5.86	5.97
<b>Animal 4</b>	5.7	5.96	5.95	6.07
<b>Animal 5</b>	6.03	5.93	5.92	5.92
<b>Animal 6</b>	5.73	5.85	5.91	6.01
<b>Average</b>	5.86	5.95	5.92	5.99
<b>Std</b>	0.15	0.09	0.03	0.06
<b>CV</b>	0.03	0.02	0.01	0.01

<b>Day 4</b>	<b>Salt Treatment pH</b>			
	0%	0.9%	1.3%	2%
<b>Animal 1</b>	6.00	6.01	6.04	6.19
<b>Animal 2</b>	5.78	5.87	6.00	6.02
<b>Animal 3</b>	5.63	6.22	5.88	6.03
<b>Animal 4</b>	5.68	6.08	6.08	6.25
<b>Animal 5</b>	5.7	6.00	6.03	6.01
<b>Animal 6</b>	5.73	5.90	5.92	6.06
<b>Average</b>	5.75	6.01	5.99	6.09
<b>Std</b>	0.13	0.13	0.08	0.10
<b>CV</b>	0.02	0.02	0.01	0.02

## Appendix 2.

### Sausage weight before thaw (g)

<b>Treatment</b>	<b>0%</b>	<b>0.90%</b>	<b>1.30%</b>	<b>2%</b>
<b>1</b>	401.05	376.32	406.26	361.61
<b>2</b>	435.62	404.82	433.09	484.34
<b>3</b>	353.78	454.36	406.64	382.66

### Sausage weight after thaw (g)

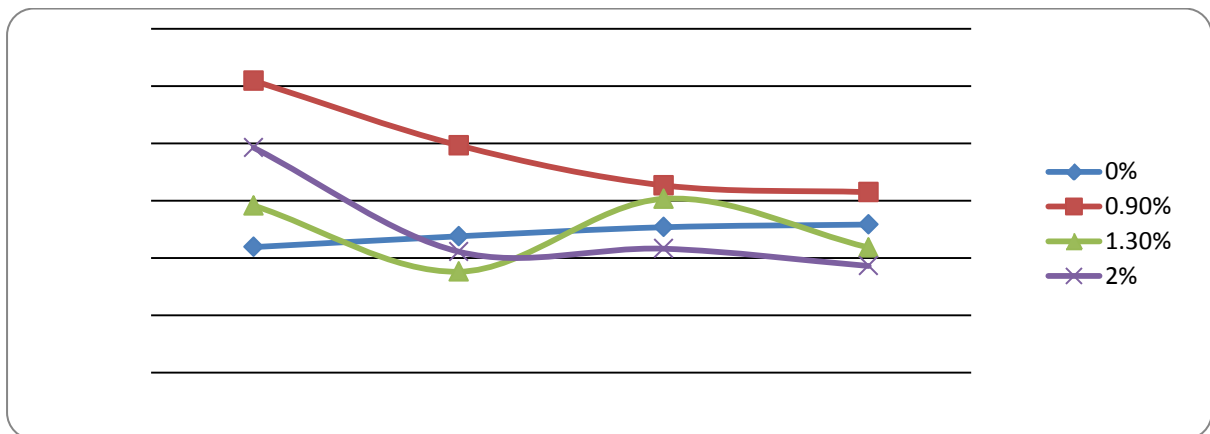
<b>Treatment</b>	<b>0%</b>	<b>0.90%</b>	<b>1.30%</b>	<b>2%</b>
<b>1</b>	394.84	373.32	403.87	358.2
<b>2</b>	432.3	402.65	430.26	481.7
<b>3</b>	340.64	452.17	401.95	379.66

### Thaw loss %

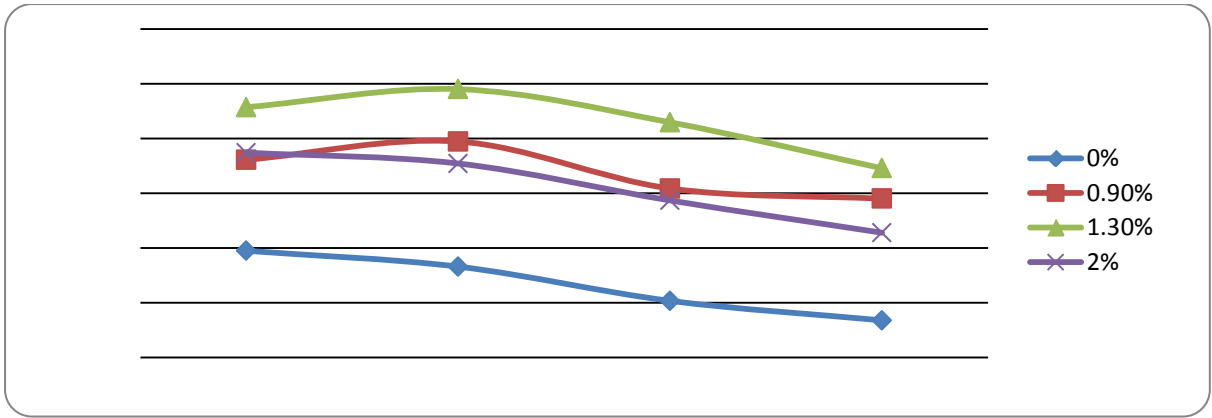
<b>Treatment</b>	<b>0%</b>	<b>0.90%</b>	<b>1.30%</b>	<b>2%</b>
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<b>1</b>	1.55	0.80	0.59	0.94
<b>2</b>	0.76	0.54	0.65	0.55
<b>3</b>	3.71	0.48	1.15	0.78

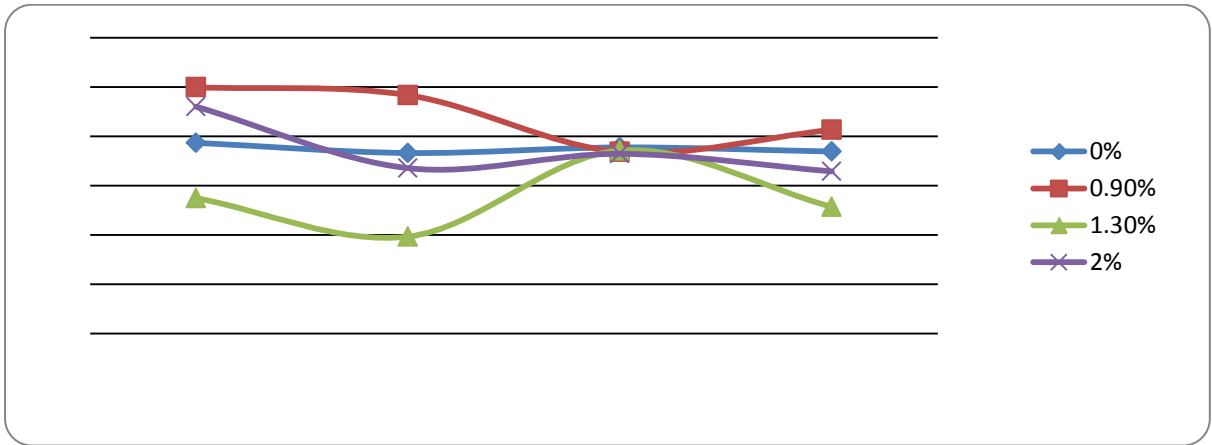
### Appendix 3.



L\* Values



a\* values



b\* values