Effect of Mechanical Work on the Meat Used for Making Reformed Meat Products

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Abstract

Tumbling, a process commonly used during production of reformed meat, applies mechanical work against the meat pieces to break down the meat structure, enhance brine absorption and extract solubilized myosin to the meat surface. The myosin acts as glue to bind meat pieces together when heated. The work done in the tumbler is currently unquantifiable and its relationships with total protein and myosin extraction and binding strength (Tensile Adhesive Strength, TAS) of two meat pieces are unknown.

Much of this project was allocated to developing and evaluating an instrument called the Impact and Friction Mechanical Robot (IFMR), which is able to repeatedly apply a desired impact and to vary the rate of repeated impacts and the time gap between each impact. The degree of sample compression could also be varied. The work done as a consequence of the hitting process can be calculated for each individual hit and summed to give the total work impacted on the meat.

Four groups of 20 mm^3 meat cubes were prepared for the hitting treatments. One group was used as the control while the other three were pre-soaked in 0.396, 0.713 or 1.146 mol/L of brine consisting of NaCl and salts of phosphate. The meat cubes were hit so maximum impact force was 10 N with an average 0.665 s between each hit for 0, 400, 800, 1200, 1600 or 2000 hits. The exudate on the hit meat surface was scraped off and examined for total protein and myosin.

The total protein extracted was not influenced by the work ($p=0.764$) applied on meat cubes pre-soaked in different concentrations of brine ($p=0.123$). Myosin extracted increased with total work done ($p=0.006$) on the meat and concentration of brine ($p<0.001$) used for soaking.

Two meat cubes were attached together at the hit surfaces, cooked at 70 °C with a 250 g weight applied, and tested for tensile adhesive strength (TAS). The TAS between meat pieces increased with increased total work ($p=0.0001$) done on the meat and increased brine concentration ($p<0.001$) for pre-soaking. The TAS also increased as myosin concentration increased ($p=0.001$). A good TAS of the meat pieces could be achieved by adequately solubilising the myosin using brine and applying sufficient total work to the meat pieces.

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List of Abbreviations

IFMR	Impact and Friction Mechanical Robot
TAS	Tensile Adhesive Strength
NaCl	Sodium chloride
STPP	Sodium tripolyphosphate
TSPP	Tetrasodium pyrophosphate
SHMP	Sodium hexametaphosphate
SDS PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
HH	Hitting head
VB	Visual Basic
DOS	Disk Operating System
GUI	Graphical User Image
BCD	Binary-Coded Decimal
AU	Arbitrary Unit

Nomenclature

S_R	Scanning rate
S_T	Total data to be scanned
n	Number of hits
H_G	average gap time between hits
H_T	average time per step
F_T	force target
D_T	distance target
S_N	initial step number
k	hit number
F_P	Peak force
F_T	Target force

CHAPTER 1

Introduction

The science and technology of processing has long been applied to meat commodities by humans without realizing it. Basic processing methods such as fermenting, salting, drying, smoking and cooking were used to prolong the shelf life and to modify the flavour. Later, more methods, which involved physical and chemical treatments on meat, such as by size reduction, mechanical mixing, curing, addition of spices, stuffing, and heating were introduced to produce a greater variety of meat product (Heinz & Hautzinger, 2007). These processing methods have been regularly modified and improved upon over the years to minimize the processing period and cost, and at the same time maximize the outcome and quality of the meat products.

Among the meat products produced, reformed meat has been well received by consumers. Significant developments occurred to reformed meat about four decades ago to fulfil the demand from consumers for steak meat. The concept of reformed meat is to fabricate a steak-like meat product by joining small pieces of meat, usually from lower-quality parts of carcasses (Varnam & Sutherland, 1995). The process involves reducing the size of meat pieces, applying mechanical treatment to the meat, and attaching the meat pieces by heating them in a mould. Salt and phosphate are added before or during the mechanical treatment to change the physiochemical properties of the meat proteins, such as by solubilizing the salt-soluble protein myosin.

The equipment commonly used today to apply mechanical treatment to the meat pieces is a tumbler or massager, which operate by conveying impact and friction forces to the meat. The work done on the meat modifies the meat structure and allows accumulation of solubilized myosin on the meat surface. Myosin acts as glue to stick the meat pieces together when heat is applied. The work produced from tumbling and massaging also enhances the curing process, distributing the flavour and colour more uniformly. The mechanical treatment also causes the meat texture to become more pliable and allows the moulding process to be done efficiently.

Many developments have ocurred that increase performance of the tumbler and massager, such as using different numbers of paddles, using different sizes of meat container, introducing a vacuum, and by varying the temperature during processing. Processing parameters have also been studied to optimize final product quality. This can be done, for example, by varying rotating speed and/or by adjusting processing time, and/or by introducing rest times between periods of tumbling. A wide diversity of processing methods has been applied with the broad aim of optimizing the properties of

meat pieces used in reformed products (Boles & Shand, 2002; Lin, Mittal, & Barbut, 1990; Pietrasik & Shand, 2004).

Until now no studies have accurately controlled the amount of force and measured the total work applied to meat during the tumbling or massaging process with a view to relating the changes in the physico-chemical properties of the meat to the amount of work that has been applied to the meat. The effect on the meat in terms of amount of protein extracted and subsequent binding strength between meat pieces after treatment with known forces or after application of a known amount of total work has not been reported. Therefore, this study aimed to contribute to information on these subjects. The main objectives of the study were:

1. To develop equipment that could apply pre-determined forces to a meat sample. The equipment envisaged needed to be able to record the force applied and the extent to which the meat was compressed so work done to the meat could be calculated. Variations in the number of the force applications and the speed and gap time between each application also needed to be set as required.

2. To determine a method of harvesting exudate from the treated meat surface for protein analysis, and then develop a standard protocol to combine two pieces of meat and measure binding strength after cooking.

3. To determine the effect of different amounts of work done on 20 mm meat cubes that had been soaked in different concentrations of brine on the amount of total protein and myosin extracted and on the binding strength of the attached meat cubes.

4. To evaluate the relationship between the total protein and myosin in the exudate and the binding strength of attached meat cubes.

CHAPTER 2

Review of Literature

2.1. Reformed and restructured meat products

Meat restructuring is a process of transforming smaller cuts of meat into a new meat product, generally known as restructured meat, through the process of reducing the meat size, blending and reforming (Smith, 1984). The term restructured meat is often used interchangeably with reformed meat, although they can be used to define two different categories of processed meat. Reformed meat products require intact meat pieces to be bound together while restructured meat products are extensively minced prior to restructuring (O'Kennedy, 2000). However, since both use partially disassembled meat which is then reassembled, they can be considered as the same product, technically (Pearson & Tauber, 1984). This review has used these definitions to distinguish between the two products.

In producing reformed meat products, the process of meat size reduction is usually carried out by flaking, chunking or sectioning (Secrist, 1987). The meat pieces have salt and phosphate added prior to or during the blending process to enhance the meat functional properties (Xiong, 1994). Tumblers and massagers are the established equipment used for meat blending. The tumbling or massaging process is not only used to mix the meat but, more importantly, to apply mechanical energy through physical treatment of the meat (Martin, 2001). The purpose of the physical treatment is to firstly loosen up the meat structure, which allows and enhances the penetration of salt and phosphate into the meat to solubilize the salt-soluble proteins. Then the solubilized meat proteins are brought to the surface by the tumbling or massaging process via a pumping action (Pearson & Gillett, 1996). The repetition of physical treatment on the meat pieces produce a gummy-like protein exudate on the meat surface (Kerry, Stack, & Buckley, 1999).

Another effect of the physical treatment on meat is to make the meat softer and more pliable so that it can be reformed using a mould or casing into a desired shape and size during the reforming process. The extracted protein exudates act as glue when the reformed meat pieces are heated (Pearson & Tauber, 1984), though some products are sold cold without going through the cooking process (Sheard, 2002).

2.2. Tumbling and Massaging

Both tumbling and massaging are crucial methods known to impart forces to the meat but they are sometimes differentiated according to the level of the physical treatment (Knipe, 2004a). Tumbling is a more vigorous process where the meat pieces are lifted up by a baffle and fall down from the top of the rotating drum due to the force of gravity (Barbut, 2002). The falling meat impacts other meat pieces, the rotating drum wall, or other moving baffles. This procedure conveys impact forces to the meat (Claus, Colby, & Flick, 1994). In contrast, massaging is a more delicate process where the paddles turn around horizontally and rub the meat slowly (Barbut, 2002). This action causes the meat pieces to rub against the wall and other meat pieces. As a result, friction forces are produced against the meat (Arboix, 2004). Nevertheless, both tumbling and massaging processes have the same purpose; to convey sufficient energy via physical treatment to the meat pieces to achieve the targeted degree of meat disintegration.

An early method of tumbling was reported as a simple rotating drum that massaged the meat gently. The principal of rotating the drum, like a cement-mixer was applied on a larger size drum so the meat pieces could drop at least one metre (Woolen, 1971). Improvements such as vacuum and temperature control were also incorporated into the tumbler treatment. Vacuum is applied firstly to prevent air bubbles accumulating between adjacent pieces of meat and thus weakening the tensile force between adjacent meat pieces after the meat is cooked; and secondly to enhance the protein extraction (Arboix, 2004). Vacuum also improves brine absorption into the meat by opening up the cell structure of the meat, which results in a more even flavour and colour throughout the product (Koch Equipment LLC, 2009). Temperature is usually controlled between –1 °C to 10 °C during the tumbling process, depending on the species and muscle type, to avoid microbial growth due to the increase of temperature and length of processing (Heinz & Hautzinger, 2007). Lower temperatures (–0.9 °C to 4.4 °C) during tumbling have been shown to improve binding strength of hams compared to 10 °C (Knipe, 2004a). This was related to the fact that the salt-soluble protein was extracted from lean meat more readily at 2.2–3.3 °C (Knipe, 2004a).

The tumbler and massager have not only been applied to small pieces of meat for reformed meat production, but also for a variety of meat sizes. The designs of both tumblers and massagers allow the equipment to cater for different portion sizes and types of meat ranging from cured pork bellies, bone-in products, boneless chunks of meat and

even to smaller meat pieces such as coarsely ground meat (Claus et al., 1994). Tumbling or massaging of larger pieces of meat such as hams, loins or roasts is carried out to make use of the physical treatment as a method of distributing the brine evenly, thus ensuring that the colour of the product is homogenous (Hullberg, Johansson, & Lundström, 2005). The texture of these bigger products such as hams can also be altered by the tumbling process but the height of the fall needs to be chosen carefully to avoid excessive damage due to the lower amount of meat inside the tumbler. Arboix (2004) suggested that tumblers be 40-50% full, while Pearson & Gillett (1996) recommended 50 to 60% full for achieving satisfactory results.

2.3. The mechanics of falling meat in the tumbler

No specific detailed explanation of the basic energy conversions during meat tumbling has been found from previous research. Only the simple concept of the impact force applied via tumbling, which results in kinetic energy being transferred to the meat has been reported (Addis & Schanus, 1979). As an alternative, concepts from physics (Wenham & Ovens, 2010; Wilson, 1977) and sport mechanics (Burkett, 2010) were used to describe what happens inside a tumbler.

There are three impacts acting on the meat pieces inside the tumbler during the process of meat tumbling; first there is the impact between the meat and the baffles, second, the impact between the meat and the rotating drum wall or floor, and third, the impact between falling meat and other meat pieces. In the tumbling process to produce reformed meat, the meat pieces are carried up to the upper part of the tumbler by the baffles (Karales, 2000). The meat at this point is going to fall off the baffle of the rotating tumbler and will possess a certain amount of gravitational potential energy. The meat then falls down due to gravity. The gravitational potential energy is converted into kinetic energy. The kinetic energy is at the maximum just prior to the impact on the floor which is equal to the original amount of potential energy before falling (Wilson, 1977). The mass of the meat and the velocity of its falling are important factors, as meat with a larger mass possesses greater kinetic energy (Burkett, 2010). The final velocity of meat falling inside the tumbler will be proportional to the square of the height it has fallen. However, if the tumbler is occupied with a vacuum system, the meat will fall at the same velocity regardless of its size but the bigger meat pieces will have more kinetic energy because of its larger mass.

When the falling meat reaches the rigid tumbler floor, an impact force is produced against the meat. The force compresses the meat and does work on the meat. From an energy conversion perspective, the meat falling on the tumbler floor transfers the kinetic energy to the floor. Because of the rigidity of the tumbler floor, the kinetic energy is dissipated back into the meat as elastic potential energy as a result of the meat compression (Wenham & Ovens, 2010). The elastic potential energy is then converted to heat and sound energy. The sound energy is dissipated through the air, while the heat energy increases the temperature of the floor and meat. This changes the properties of the meat, especially the protein structure breakdown (Wilson, 1977).

2.4. The effects of time, speed, and pattern of tumbling on product characteristics

2.4.1. Time of processing

Time is an important factor for the tumbling or massaging processes to allow sufficient work to be applied on the meat. The length of time can be varied from a short to a long duration, based on the nature of the product wanted. Too short a tumbling time can produce a low-quality product that disintegrates readily after cooking because of inadequate myofibrillar-protein extraction and solubilisation (Lin et al., 1990; Lindley, 1991). The quality of the ham, such as the cooked yield and texture, are not improved due to the short time of tumbling (30 minutes) when compared to the non-tumbled samples (Ockerman, Plimpton, Cahill, & Parrett, 1978).

Increasing processing time was reported to improve the quality of the meat products. For example, tumbling boneless chicken meat for 15, 30 and 45 minutes to produce chicken tikka resulted in significantly ($P<0.05$) higher values for cooking yield and moisture retention for product tumbled for the longer times (Bharti, Anita, Das, & Biswas, 2011). Another study, in which restructured buffalo meat was produced by tumbling for 0, 1, 2 or 3 hours, showed that tumbling for 3 hours produced a better salt-soluble extraction thus leading to better binding, cohesiveness and tenderness when compared to the tumbling times of 0, 1 and 2 hours (Sharma, Kumar, & Nanda, 2002).

Increased tumbling time has also been shown to reduce the cooking loss of goat ham and at the same time reduce the shear force because the muscle fibre has been

disrupted (Dzudie & Okubanjo, 1999). The colour of the reformed ham was also improved by prolonging the processing time. A longer time increased cell breakdown and with the help of solubilized myofibrillar protein, the brine and myoglobin were able to migrate more easily during the process (Gillett, Cassidy, & Simon, 1981). However, overworking the meat too long may produce an unacceptable rubbery texture in the reformed meat (Lin et al., 1990; Lindley, 1991). Therefore, it is important to ensure the tumbling or blending time is optimized to produce the best reformed meat products.

2.4.2. Speed of processing

Speed of tumbling is also one of the factors that could determine the quality of the reformed meat. A study by Ghavimi, Althen, & Rogers (1987) reported that there were no differences among tumbling speeds of 5, 10, 15, and 20 rpm during 12 hours of intermittent tumbling (10 min of tumbling per hour) for yield, Hunter colour scores, handling-ability, Allo-Kramer shear values, and voids between chunks of sectioned and formed beef meat. However, tumbling at 15 and 20 rpm did result in reformed meat with higher values of breaking strength measured by Instron Universal Testing Machine compared to meat tumbled at 5 or 10 rpm. This might be due to the fact that more extracted protein was produced on the meat surface with the higher tumbling speeds. In the production of hams, different tumbling speeds of 15 or 25 rpm, and cumulative revolutions of the tumbler (3000, 6000 or 9000), had no effect on shrinkage and water holding capacity. However, products processed at 25 rpm with 3,000 revolutions were harder, gummier, and chewier than products processed with 6000 and 9000 revolutions (Lin et al., 1990).

Drum rotation speed determines the intensity of tumbling and if it is carried out too slowly, even for longer periods of tumbling, may not result in the desired physico-chemical property changes in the required final product. However, at some rotational speed, the intensity could overwork the meat and result in an unacceptable broken-meat texture (Woolen, 1971). The choice of tumbling speed could be crucial to achieving the desired meat product quality. Even though increasing the speed can result in better protein extraction, it is important to consider the time of tumbling to prevent unacceptable quality characteristics of the reformed meat.

2.4.3. Continuous or intermittent tumbling

Certain tumbling and massaging processes are carried out continuously (Gillett et al., 1981), while others are intermittent (Bedinghaus, Ockerman, Parrett, & Plimpton, 1992). The methods have different effects on the resulting meat product. As an example, the cell structure of the meat tumbled intermittently (10 min per hour for 18 hours) resulted in more alterations especially in deep tissue such as significant nuclei disorganization ($p<0.05$) and significantly less clarity of the striation pattern ($p<0.01$) compared to meat tumbled continuously for 3 hours – the same effective actual tumbling time (Cassidy et al., 1978). The result is thought to be related to the degree of brine absorption, i.e. the NaCl and phosphates need time to diffuse into the meat, and as a consequence rest time during tumbling has been shown to be more effective than continuous tumbling because it is the total time that is important and not the actual tumbling time. A study by Ockerman & Organisciak (1978a) showed that a significant increase in brine infusion started after 1.5 to 2 hours (9-12 hours in real time) out of a total of 3 hours total tumbling time for intermittent tumbling (10 min per hour for 18 hours) compared to 3 hours continuous tumbling, probably due to the increased soaking time. It is understood that brine absorption improves with the loosening of muscle structure due to the tumbling process. However, the maximum absorption will occur without agitation because agitation (pumping action) tends to counteract the added uptake of the brine by the loosened structure of the muscle. The uptake reaches its maximum only when agitation ceases (Pearson & Gillett, 1996).

Tumbling for 18 hours intermittently (10 min per hour) resulted in improved ham quality and yield compared to continuously tumbling for 3 hours (Krause, Ockerman, Krol, Moerman, & Plimpton, 1978). In contrast, Boles & Shand (2002) found that processing and tenderness characteristics of roast beef showed no significant differences between continuous and intermittent tumbling. This might be due to lesser tumbling time, as the intermittent tumbling was only carried out for 4 hours with 10 minutes on and 20 minutes off, while the continuous tumbling was done for total of 80 minutes. One proposal for intermittent tumbling was to tumble the meat for 1-2 hours followed by rest for 18-24 hours, and then continue tumbling until an appropriate amount of protein exudate was obtained (Knipe, 2004a).

2.5. Effect of physical treatments on meat structure and brine absorption

The salt-soluble proteins used to bind meat pieces originate from the myofibrillar proteins. In meat structure, myofibrillar proteins are located within the muscle fibrils that are within muscle fibres, and the fibres are within a framework of connective tissue. The physical treatment is crucial to create paths for the salt and phosphate to penetrate the muscle. Figure 2.1 (a) shows the structure of skeletal muscle which is kept intact by connective tissues at three sites termed the epimysium, the perimysium, and the endomysium.

A study carried out by Nishimura, Hattori, & Takahashi (1994) showed that the epimysium is composed of outer and inner layers of thick sheets. The outer layer contains collagen fibres laying transversely to the muscle fibre axis, which is in contrast to the inner layer where the collagen fibres are parallel to the muscle-fibre axis. The thickness of the epimysium varies according to the muscle location within a carcass, as reported in a study with beef carried out by Field, Riley, & Chang (1969). The epimysium plays a role in meat toughness as the removal of this connective tissue has been shown to reduce the shear force for beef steaks from semitendinosus muscle(Janz, Aalhus, & Price, 2006). Another study reported that hams made from meat where all the removable connective tissue had been removed and then tumbled, significant improved ($p>0.05$) sliceability, yield and external appearance compared to hams made from meat where only the fat had been trimmed (Krause, Ockerman, et al., 1978).

Epimysium covers about 10 to 100 or more bundles of muscle fibres (Amitrano & Tortora, 2012). The targeted myofibrillar proteins are located within these muscle fibres. Each fascicle, or bundle, is covered by connective tissue called the perimysium. The perimysium is composed of several layers of thick sheets (100-200 μm thick) consisting of collagen fibres made up of tightly bundled fibrils and connected to sheaths of the endomysium (Nishimura et al., 1994). Two parts of the perimysium have been identified, the thicker primary perimysium, and a thinner secondary perimysium (Schmitt, Degas, Perot, Langlois, & Dumont, 1979).

Brooks & Savell (2004) report that the perimysium thickness differs between beef carcasses of similar age and between beef muscles within a carcass. The toughness of meat from various chicken skeletal muscles (Liu, Nishimura, & Takahashi, 1996), from different muscles of the same beef carcass (Li, Zhou, & Xu, 2007), and from different muscles of a roe deer (Zochowska-Kujawska, Lachowicz, Sobczak, & Gajowiecki, 2007), have been reported to be related to the thickness of the primary perimysium. Secondary

perimysium thickness has also been reported to contribute to the toughness of different muscles of pork (Nishimura, Fang, Wakamatsu, & Takahashi, 2009).

Inside the fascicle there is another type of connective tissue named endomysium, which surrounds individual muscle fibres. The endomysium is composed mostly of reticular fibres and tightly arranged collagen fibrils. It has been shown that removing endomysium from rabbit *longissimus dorsi* caused greater swelling in the muscle fibres when treated with salt (Knight, Elsey, & Hedges, 1989). This showed the possibility that more myofibrillar protein can be solubilized when there is no endomysial barrier covering the muscle fibres. Some studies have been carried out on the effect of physical treatments on connective tissue. Endomysium of porcine muscle was shown to have been clearly disrupted after massaging for 4 hours (Theno, Siegel, & Schmidt, 1978b). A similar result was observed on the connective tissue of pork biceps femoris muscle after massage treatment was applied for 20 minutes, followed by a resting time of 24 hours and then a second massage for 10 minutes (Rejt, Kubicka, & Pisula, 1978).

In more recent studies, the thickness of the connective tissue was observed and measured before and after the physical treatment. The changes in the connective tissue thickness were an indication of the level of damage that occurred to myofibrils, which led to loosening and separation of the fibres. For example, massaging pork ham muscles intermittently (30 minute massaging and 30 minute rest) for a total processing time of 2-6 hours resulted in significantly thicker perimysium and endomysium (Lachowicz, Sobczak, Gajowiecki, & Żych, 2003). This result agreed with results from massaging chicken and turkey breast muscle, where the perimysium and endomysium for both muscles were thicker than non-massaged samples (Zych, Lachowicz, Gajowiecki, Sobczak, Zochowska-Kujawska & Kotowicz, 2007). In beef *semimembranosus* muscle, increases in perimysium and endomysium thickness were only detected after massaging for more than 6 hours. However, no significant increase was observed when the same treatments were applied to *biceps femoris* muscle (Lachowicz, Gajowiecki, Zych, Zochowska, Sobczak & Kotowicz, 2003). In contrast, massaging decreased the endomysium thickness for *longissimus* (1-15%) and *quadriceps femoris* (1-29%) muscles of wild boar (Zochowska-Kujawska, Lachowicz, Sobczak, Gajowiecki, Kotowicz, Zych & Medrala, 2007). The variable results could be due to the different muscle types and different species involved. Nevertheless, physical treatments proved to be able to disrupt and change the physical attributes of connective tissue which could improve the penetration of salt and phosphate and at the same time reduce meat toughness.

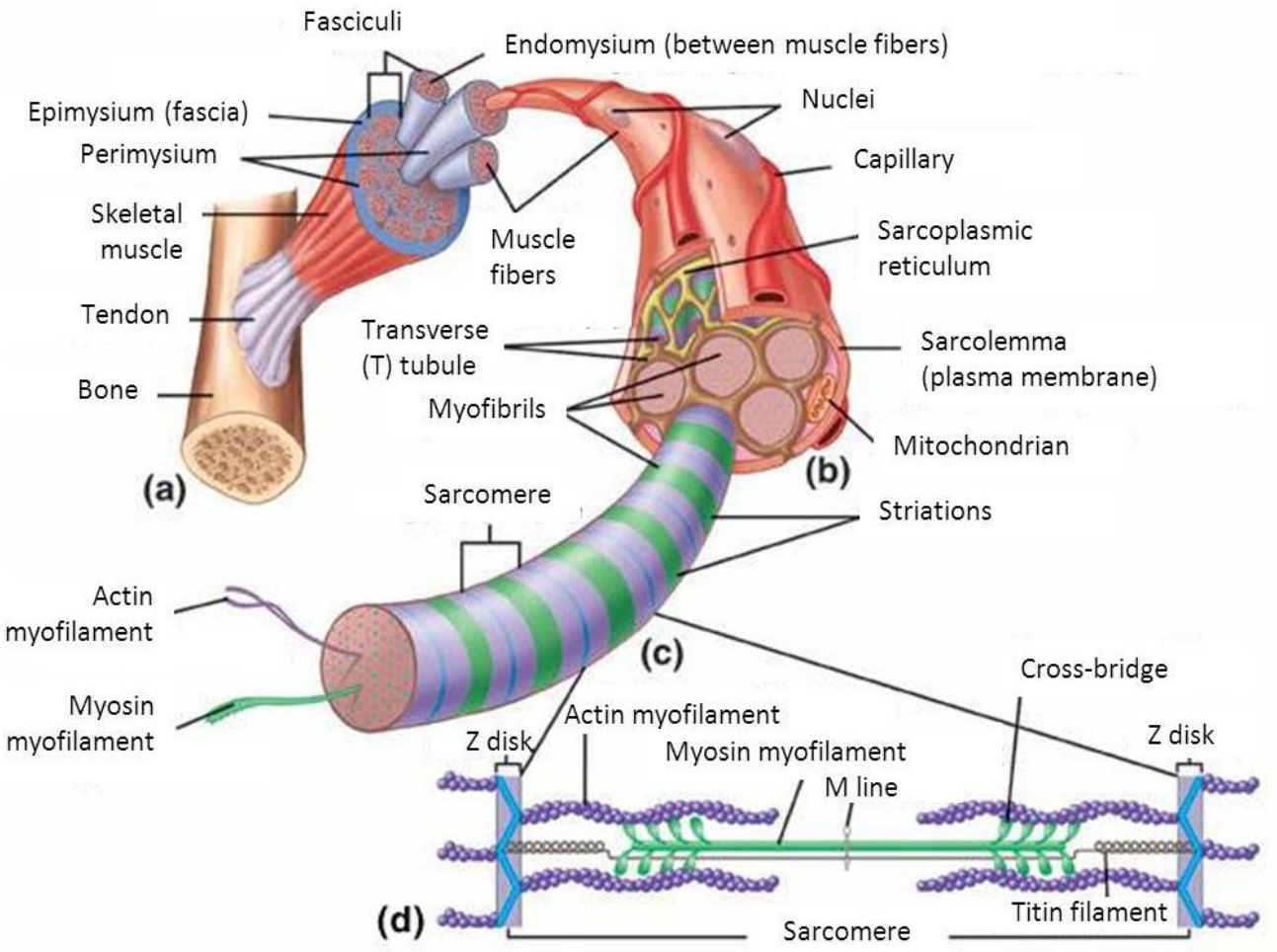

Figure 2.1. (a) Structure of the skeletal muscle, (b) The muscle fibre (myofibre) and its contents, (c) myofibrils and myofilaments of the skeletal muscle, (d) One sarcomere of myofibril, adapted from Silvernagel (2013).

Physical treatments will not only disintegrate the connective tissues but also the muscle fibres located within the endomysium. Muscle fibres, also known as myofibres (Figure 2.1 (b)), represent 75–90% of muscle volume and their diameter ranges from 10 to 100 μm. Several different classes are recognised according to their contractile and metabolic properties (Lefaucheur, 2010). Muscle fibres are covered by a cell membrane called the sarcolemma, which is associated with capillaries and nuclei. Under the sarcolemma, and covering the myofibrils, are the sarcoplasmic reticulum, and mitochondria. Muscle fibres are composed of many myofibrils - long, thin cylindrical rods, usually 1-2 μm in diameter, which run within and parallel to the long axis of the muscle fibre. Myofibrils are made up primarily of thick and thin myofilaments (Amitrano & Tortora, 2012).

Physical treatments applied to the muscle can alter the properties of the muscle fibres. The tumbling process has been reported to affect muscle fibres with the disruption of the sarcolemma leading to increased juiciness and tenderness of goat hams (Dzudie & Okubanjo, 1999). Tumbled porcine muscle has also showed significant disruption of the sarcolemma, more disorganized nuclei, and a decreased clarity of the muscle striation pattern compared to non-tumbled meat (Cassidy et al., 1978). Massaging porcine muscle for four hours created spaces between myofibrils and disrupted the sarcolemma, but when salt and phosphate are added, the myofibrils became more fragmented, the sarcolemma was completely destroyed and fibres were shredded and coated with solubilized protein. After 24 hours of massaging, the fibres of porcine muscle were excessively shredded and disrupted while the sarcolemma was completely destroyed. With the addition of salt and phosphate the separation of myofibrils was more pronounced, the fibres lost their integrity and firmness and were covered with larger amounts of solubilized protein (Theno et al., 1978b).

A study using another method of physical treatment showed that mechanical tenderization of pork muscle using a meat activator (Gunther Wensing, Stadtlohn, Germany) disrupted the sarcolemma, destroyed mitochondria and fractured myofibrils, which led to improved brine holding capacity and reduced pork toughness (Tyszkiewicz & Jakubiec-Puka, 1995). By damaging the muscle fibres through physical treatments, the myofibrillar proteins in the myofibrils were exposed to salt and phosphate, thereby facilitating solubilisation of the salt-soluble proteins.

Sodium chloride migrated significantly more into tumbled meat compared to non-tumbled meat over the same time. This effect was recorded initially after 3 hours of tumbling and significantly higher sodium chloride contents were recorded for every hour

up to 16 hours (Ockerman & Organisciak, 1978b). In another study, tumbling with added phosphate significantly enhanced distribution of salt and nitrite in porcine muscle compared to non-tumbled samples. The sample was injected with brine prior to tumbling and the distance the brine travelled was measured after tumbling for 10 min for every hour for 18 hours (Krause, Plimpton, Ockerman, & Cahill, 1978). Once the brine reached the targeted protein, solubilisation of the protein occurred, and physical treatment once again helped to extract these out to the meat surface.

2.6. Proteins for Binding Meat Pieces

One of the objectives of applying physical treatments to meat is to disintegrate the muscle structure so that salt and phosphate can reach the myofibrillar proteins. The main function of myofibrillar proteins within the living muscle is to control the process of muscle contraction (Marianski & Marianski, 2011; Warriss, 2000). Myofibrillar proteins consist of several types of protein which are soluble in high concentrations of salt. The myofibrillar proteins form the structure called myofibrils. Every fibril has a striated repeated pattern or sarcomere, which is the distance between two adjacent Z-Lines (Z-Disks) (Figure 2.1 (c) and (d)). The length of one sarcomere is approximately 2.3 μm (Elliot & Elliot, 2001) with an M-line in the middle of it. The fibril consists of thick and thin filaments. The thick filaments consist mainly of myosin and titin, while actin, troponin and tropomyosin are the main components of thin filaments. The thick filaments are located within the A-band while the thin filaments can be found within the I-band and some parts of the A-band.

Myosin (100 nm in length and 1.5-2 nm in diameter) is the main protein to act as the glue for making reformed meat products (Marianski & Marianski, 2011; Xiong, 2004). The myosin of skeletal muscle (myosin II) has been distinguished from the other myosin superfamily members based on its unique self-associated long tail to form myosin filaments (Craig & Woodhead, 2006). As a motor protein, myosin is able to convert chemical energy to mechanical energy through structural changes (Choi & Kim, 2009).

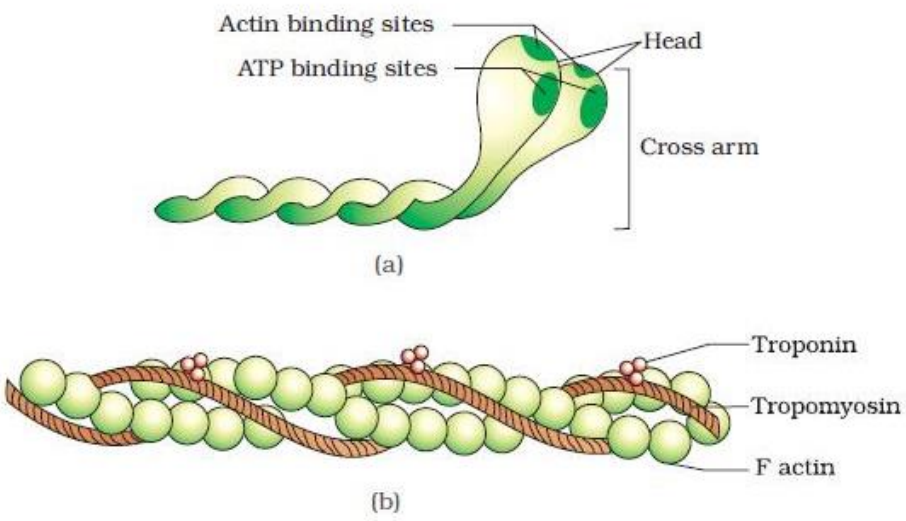

Figure 2.2. (a) Myosin, (b) Actin, Troponin and Tropomyosin (adapted from Kshitij Education India Private Limited (2012)).

One pair of heavy peptide chains and two pairs of light chains are the main structural features of the myosin molecule. The two heavy chains coil around each other and form a long rigid tail, also known as an alpha-helical coiled coil rod. At the N terminal of the heavy chain are laid two globular heads known as sub fragment 1 (S1), with the pairs of light chains attached to each head (Sobti, 2008). The light chains are divided into two, the essential light chain (ELC) and the regulatory light chain (RLC) (Gordon, Homsher, & Regnier, 2000). The S1 region functions as the actin-binding site and ATP-binding site (Jung, Komatsu, Ikebe, & Craig, 2008), which is crucial in the process of muscle contraction. Myosin molecules can be divided into heavy meromyosin (HMM) and light meromyosin (LMM) sections. From the N terminal of the heavy chain to a point on the tail where there is a hinge (a flexible joint) is the area of the HMM. The area between the hinge and the C terminal is known as LMM (Sobti, 2008). A hinge also exists at the neck of the myosin, and the part between the two hinges is known as sub fragment 2 (S2).

Besides myosin, other proteins such as actin can also influence the binding of the meat pieces (Marianski & Marianski, 2011). Figure 2.2 (b) shows the relationship between actin, troponin and tropomyosin within the thin myofilament. A single globular molecule of actin, also known as G-actin, binds to another G-actin molecule via polymerization to form F-actin (1.0 μm in length), a double helical chain (Elliot & Elliot, 2001; Pearson & Young, 1989). The process occurs under certain conditions which basically relate to concentrated salt solutions (Warriss, 2000). Gordon et al. (2000) have

explained in their review that the polymerization to form the thin filament backbone occurs spontaneously.

Tropomyosin molecules are polymerized along the actin filament head-to-tail such that one tropomyosin molecule spans every seven actin monomers (Zot & Potter, 1987). In the form of homodimer or heterodimer, tropomyosins are two alpha-helical chains arranged as a coiled coil which are ~42 nm in length (Gordon et al., 2000). The troponin protein complex is composed of three components; C, I and T. These different names are based on the activating and separating factor (Ohtsuki & Morimoto, 2008). The properties of these troponin components are: 1) troponin-C binds Ca^{2+}, 2) troponin-I inhibits the actomyosin ATPase, and 3) troponin-T attaches the troponin to tropomyosin and actin (Gordon et al., 2000).

Myofibrillar proteins extracted from the treated meat form the creamy and tacky exudate, visible on the meat surface after tumbling. In addition, other components such as the sarcoplasmic proteins, fat, water and broken muscle fibres can also be found inside the exudates (Siegel, Theno, & Schmidt, 1978).

The effectiveness of tumbling on protein extraction has been studied by Ghavimi et al. (1987). Tumbling for 12 hours with increasing rotation per minute for 5, 10, 15 and 20 rpm was reported to have an effect on amount of protein extraction. The extracted protein was scraped from the surface and measured by using the Bio-Rad protein assay technique. Tumbler rotation at 20 rpm showed significantly more total protein extraction compared to 5 rpm. Among all the compounds extracted, the myosin is the most important in binding the meat pieces together as comparative studies have shown that the binding strength of reformed beef semitendinosus muscle was superior for myosin compared to actomyosin (Macfarlane, Schmidt, & Turner, 1977) and against other compounds such as sarcoplasmic protein, non-protein control, muscle homogenate and fat-free washed homogenate (Siegel & Schmidt, 1979). The comparisons were made by applying the crude myosin or other tested materials in between two pieces of meat.

2.7. Factors Affecting Muscle Protein Solubility and Extractability

2.7.1. Salt and Type of Phosphate

Salt (sodium chloride) and salts of phosphate are among the most commonly used ingredients for curing in meat processing (Shahidi & Samaranayaka, 2004). Salt has three main functions in meat products 1) to enhance the physical processing effects, 2) to improve the sensory properties and 3) to act as a preservative (Matthews & Strong, 2005). Phosphate is used to control the colour and flavour deterioration rate by reducing myoglobin oxidation and lipid oxidation. Phosphate may also improve the functional properties (water holding capacity, fat emulsification capacity, juiciness and tenderness) of the meat products (Trout & Schmidt, 1987).

No food regulations prescribe an upper level of salt in meat products. Instead consumer considerations (unpalatable saltiness taste) dictate maximum addition in the end product (Sebranek, 2009). Commonly, the percentage of salt used in meat products ranges from 1-2% (sausages), 2-3% (hams), 1.2-1.8% (bacon) and 2-4% (jerkies) (Shahidi & Samaranayaka, 2004). However, health authorities would like to see a reduction in the above levels because salt has been implicated in raised blood pressure (Desmond, 2006). In contrast to salt, phosphate has been limited to 0.5% in meat products measured as phosphorus pentoxide values as regulated by European Parliament and Council Directive (2006) in Directive No. 95/2/EC. Usage of phosphate over the level permitted will result in an unpleasant astringent metallic flavour, and excessively rubbery texture but, more importantly, the excessive phosphorus could lead to reduced calcium absorption in the human body (Trout & Schmidt, 1987).

Different types of mineral salts have been studied with regard to their effect on protein extraction. Comparisons made under several conditions between different types of salts for their ability to extract protein from Red Hake fish muscle showed that lithium chloride (LiCl) extracted more protein than sodium chloride (NaCl) and potassium chloride (KCl) (Kelleher & Hultin, 1991). Munasinghe & Sakai (2004), on the other hand found that NaCl was a better protein extractant compared to KCl and LiCl for pork lean meat. This was measured by the higher amount of myosin, other individual proteins, and total protein extracted from the meat. However, NaCl is the most common salt being used in the industry because of its availability, cost and it is categorized as Generally Recognized as Safe (GRAS) (Sofos & Raharjo, 1995).

Phosphate used in meat processing is categorized by its chain length (link of PO_4 structural units by sharing the oxygen atoms) (Trout & Schmidt, 1987). According to Long, Gál, & Buňka (2011), two chains length structure of tetrasodium diphosphate (also known as tetrasodium pyrophosphate (TSPP)) was the most effective in solubilizing protein because of its ability to react with actomyosin directly. However, it has the weakness of poor solubility in water. Therefore, sodium tripolyphosphate (STPP) and sodium hexametaphosphate (SHMP), which have longer chains, are usually added with TSPP to enhance its solubility. The STPP is also used extensively because it can be hydrolysed into pyrophosphate in the meat, thus having the same effect as TSPP in solubilizing meat protein (Knipe, 2004b).

Both salt and phosphate are usually incorporated together in the brine for the manufacturing of meat products due to their synergistic effect on meat properties. For example, mixing of 1% salt with 0.5% of different phosphates individually (tetrasodium pyrophosphate (TSPP), sodium tripolyphosphate (STPP) and sodium hexametaphosphate (SHMP)) increased the water holding capacity and reduced shrinking after cooking of chicken breast compared to phosphate treatment only (Shults & Wierbicki, 1973). The concentration of salt also could be reduced to achieve a better quality product when used together with phosphate. It has been reported that significantly higher cooking yields and binding strengths were obtained for beef rolls produced with 2% salt and 0.5% STPP compared to 3% salt only (Moore, Theno, Anderson, & Schmidt, 1976).

The synergistic effect of salt and phosphate on meat has been explained by Offer & Trinick (1983) in detail. Two processes occur when salt and phosphate are added to meat as shown in Figure 2.3. In process 1, the chloride ions of salt produce an electrostatic repulsive force between the thin and thick filaments while phosphate dissociates the actomyosin into actin (thin filament) and myosin (thick filament). The thick filaments are then depolymerised into myosin monomers with the addition of high concentrations of salt (process 2).

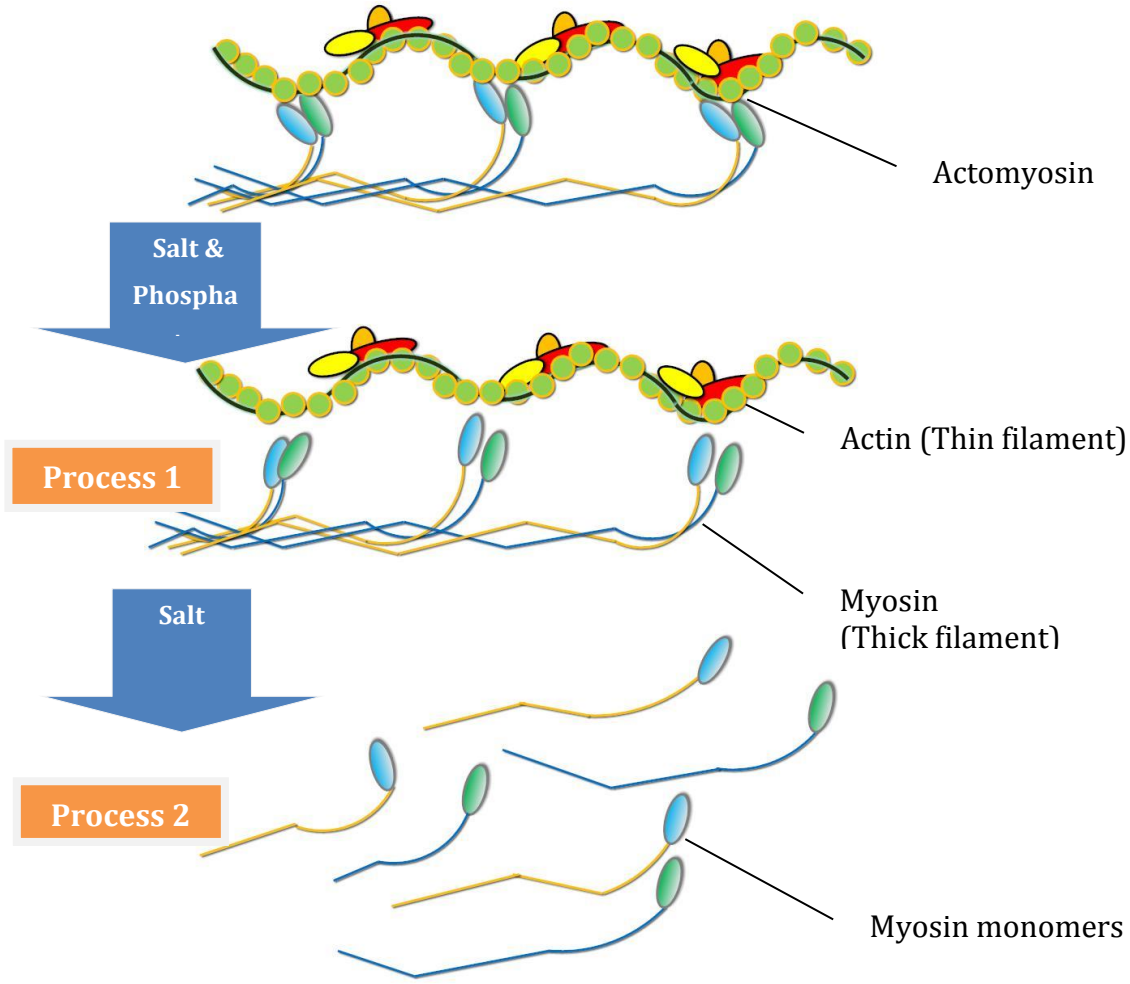

Figure 2.3. Dissociation of actomyosin into actin and myosin (process 1) and depolymerisation of thick filament into myosin monomers (process 2).

Mixing of any of the alkaline phosphates and salt would also increase the pH value of a meat or meat product. For example, 1% of salt and 0.5% of TSPP elevated the pH value of beef semimembranosus (pH=6.20) compared to treatment with TSPP independently (pH=5.90). In this study, TSPP produced the highest pH followed by STPP (pH=5.75), while SHMP showed no changes (pH=5.50) (Shults, Russell, & Wierbicki, 1972). The pH of pork patties was also significantly increased when treated with 2% salt and 0.5% phosphate (combination of 75% STPP and 25% SHMP) (pH=6.48) compared to treatment with salt (1%) only (pH=6.18) and the non-treated sample (pH=6.17) (Keeton, 1983).

The pH level affects the water holding capacity (proportional to swelling) of the meat and provides an indication of the solubility of the protein. For example a study carried out by Richardson & Jones (1987) on turkey breast meat showed a steep increase of protein solubility from pH 5.5 to about 6.0. Then, the rate of increase started to slow down from pH 6.5 to 7.5. This result was in agreement with the study by Vega-Warner, Merkel, & Smith (1999) that showed an increased solubility of salt-soluble protein of bovine *M. semimembranosus* and *M. vastus intermedius* in 0.6M (3.5%) NaCl and 0.05M (0.6%) sodium phosphate as the pH increased. The solubility rose quickly from pH 5.5 to 6.0 from~20% to ~80%, and as the pH was increased from pH 6.5 to 7.5 the solubility continued to increase, but more slowly, ~80% to near 100%. Therefore, a pH of from 6.0 to 7.5 is suggested to obtain the maximum extraction of the protein.

The concentration of salt is also an important factor for solubilizing the myosin as well as swelling the myofibrils. Offer & Trinick (1983) reported that shrinking of myofibrils occurred when applied with 0.1M (0.58%) of salt and only slight changes were observed when the salt concentration was increased to 0.2M (1.17%), 0.3M (1.75%), 0.4M (2.38%) and 0.5M (2.92%). However, at 0.6M (3.5%) of salt, the A-band, where the myosin and actin are located was extracted suggesting firstly, the salt concentration needed to reach 0.6M to get significant break-up of the thick filament in the absence of mechanical energy and secondly, that the protein in the thick filament had been solubilized. The solubilisation increased as the salt concentration increased until it reached a maximum at 1.0M (5.8%) of salt, at which concentration the myofibril diameter had expanded up to 2.8 times compared to the application of 0.1M salt (Knight & Parsons, 1988; Offer & Trinick, 1983). In the production of meat products, low concentrations of salt have been used (0.5-1.5%) for health and flavour purposes. According to Sheard (2002), water added as brine to the meat usually is in the form of ice and therefore the salt concentration is much higher than 0.5-1.5% thus allowing greater amounts of solubilisation to take place than might be the case if the salt concentration was 0.5-1.5%. Lower concentration of salt can still be applied for solubilisation of the myosin as proved by Offer & Trinick (1983) where at 0.4M (2.38%) of salt and addition of phosphate (sodium pyrophosphate) (10mM), the A-band of myofibril was extracted thus causing the swelling of myofibril. It is concluded that brine with a salt concentration of 0.6M is the most suitable to trigger the solubilisation of myosin, but with the presence of phosphate the salt concentration can be reduced to 0.4M.

2.7.2. Animal species and muscle type

In general, the flesh products from cattle, pigs and sheep are categorized as red meat. They are distinguished from the flesh of poultry (chicken, turkey, duck, pigeon, and guinea fowl), which is termed white meat (Bender, 1992). In early studies muscle fibres were grouped according to the colours of the meat. More recently, the classification of muscle fibres has evolved so that many other ways of classification have been used, including classification according to metabolic types, histochemical characteristics, functionality and morphology. Different muscle fibre types (β-red, α-red or α-white) or (Type I, Type IIA or Type IIB) have different myosin isoforms (Pette & Staron, 2000) that could exhibit different myosin properties.

Myosin from different fibre types has been shown to produce gels of different gel strength. For example, a study on the gelation properties of myosin types showed that myosin from white fibre types was a superior gel former compared to myosin from red fibre types when tested: 1) at all the temperatures above a minimum for gel induction, 2) in both 0.2 and 0.6M of NaCl, 3) for all protein concentrations used (≤ 20 mg/mL) and 4) at pH ≥ 5.8 (Fretheim, Samejima, & Egelandsdal, 1986). The study showed that it was possible to produce a diversity of reformed meat binding strength. The myofibrillar proteins from different muscle fibre types have also shown variation in their functional properties such as protein solubility, viscosity and gelation (Xiong, 1994). Table 2.1 shows the distribution of different muscle fibre types in different muscles from the same species and also different species. The table shows that the proportions of the various fibre types differ between muscles from the same species and also between species for the same muscle. Therefore, the binding properties of the reformed meat will be affected by different muscle types and different animal species.

Table 2.1. The distribution of muscle fibre types for different muscles and different species.

Muscle type	Species	Meat / Breed	Classification scheme	Muscle fibre distribution			References
				Red (%)	Intermediate (%)	White (%)	
Longissimus dorsi	Bovine	Beef	β-red, α-red, α-white	22.3	22.9	54.8	Kirchofer, Calkins, & Gwartney (2002)
	Ovine	Lamb (Suffolk ram X Suffolk-Rambouillet)	β-red, α-red, α-white	7.1	44.5	48.4	Solomon, Moody, Kemp, & Ely (1981)
		Lamb (Suffolk ram X Finnish Landrace-Southdown)	β-red, α-red, α-white	6.4	37.0	56.6	Solomon et al. (1981)
	Porcine	Pork (domestic pigs)	I, IIA, IIB	6.5	3.2	90.3	Ruusunen & Puolanne (2004)
		Pork (wild pigs)	I, IIA, IIB	13.0	17.3	69.7	Ruusunen & Puolanne (2004)
Longissimus thoracis	Bovine	Beef (Mishima-Japanese native steers)	I, IIA, IIB	37.1	17.0	46.0	Morita, Iwamoto, Fukumitsu, Gotoh, Nishimura, & Ono (2000)
	Ovine	Lamb (Segureña)	I, IIA, IIB	10	36	53.7	Peinado et al. (2004)
	Porcine	Pork	β-red, α-red, α-white	8.38	7.36	82.8	Eliáš, Hluchý, & Mlynek (2007)
Semi-membranosus	Bovine	Beef	β-red, α-red, α-white	21.1	28.5	50.4	Kirchofer et al. (2002)
	Ovine	Lamb (Suffolk ram X Suffolk-Rambouillet)	β-red, α-red, α-white	15.4	43.3	41.3	Solomon et al. (1981)
		Lamb (Suffolk ram X Finnish Landrace-Southdown)	β-red, α-red, α-white	13.1	37.5	47.3	Solomon et al. (1981)
	Porcine	Pork (domestic pigs)	I, IIA, IIB	6.6	3.6	89.8	Ruusunen & Puolanne (2004)
		Pork (wild pigs)	I, IIA, IIB	16.6	16.1	67.3	Ruusunen & Puolanne (2004)

2.8. The effects of fibre orientation and heating treatments on binding strength

2.8.1. Meat fibre orientation

The orientation of muscle fibres at the binding surface affects the binding properties of the meat. Purslow, Donnelly, & Savage (1987) tested the binding strength of meat pieces by applying crude myosin in between meat pieces and then cooked at 80 °C. They found that the muscle fibres were perpendicular to the adhesive junction (90°/90°) was stronger than when both meat pieces were parallel (0°/0°) to the junction, or when one meat piece had fibres perpendicular and the other had fibres parallel to the adhesive junction (90°/0°). Figure 2.4 shows the orientation of the meat pieces against the layer of the adhesive for a 90°/0° junction.

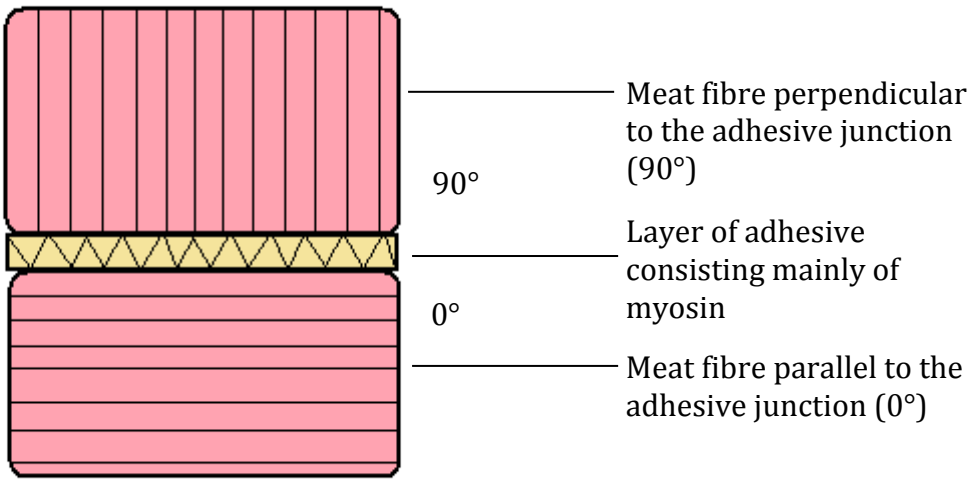

Figure 2.4. Two possible orientations of meat fibres during meat binding.

The 90°/90° junctions showed that when the two pieces of meat were pulled apart the fracture occurred within the myosin gel used to bind the meat pieces. While for the 90°/0° and 0°/0° junctions the fracture was within the muscle fibres of the meat piece with fibres parallel to the junction. It can be concluded that the strength of the meat with

fibres perpendicular to the adhesive layer is greater than the strength of the myosin adhesive layer, which is greater than the strength of the meat with fibres parallel to the adhesive layer (Donnelly & Purslow, 1987). However, studies by Farouk, Zhang, & Cummings (2005) showed that the fibre alignment for restructured beef steaks did not produce any significant difference in the binding strength, either when the fibres were aligned parallel, perpendicular or a mixture of both. Their result might be due to the use of the enzyme transglutaminase as the glue rather than the crude myosin used by Purslow et al. (1987). The binding junctions of the sample by Farouk et al. (2005) were numerous while only one binding junction was tested by Purslow et al. (1987). These orientation variables and the nature of the "glue" could play an important role in the binding strength of neighbouring meat pieces.

2.8.2. The effects of heat treatments on gel-network formation by myosin

The proteins in meat are important, especially myosin, because they act as glue for attaching meat pieces together in the production of reformed meat products. Heat treatment is often used to allow the myosin to coagulate and form a network, thus binding the neigbouring pieces of meat. Myosin forms a gel network in four sequential steps. These are: 1) the unfolding of the myosin heads (S1 region), 2) disulfide bonding and hydrophobic interactions between the myosin heads causing intermolecular interaction of S1 regions, 3) the unfolding of myosin tail or Light Meromyosin (LLM), and 4) interaction of the tails to form a gel network (Lanier, Yongsawatdigul, & Carvajal-Rondanelli, 2014). It appears that both the head and the tail of myosin play roles in forming the gel network with the myosin head role involving sulfhydryl groups, while the myosin tail consists of conformational changes of the helix-coil of the rod (Samejima, Ishioroshi, & Yasui, 1981).

The thermal denaturation of myosin was reported to have occurred at transition temperatures of 47, 54, 57 and 63 °C by Smyth, Smith, & O'Neill (1998). However, the S1 regions start to unfold when the temperature rose to 37°C (Burke, Zaager, & Bliss, 1987). This was consistent with the results of Smyth, Smith, Vega-Vargas, & O'Neill (1996), which showed that the unfolding of the S1 region of chicken breast muscle occurred at 36 °C and with aggregation when the temperature reached between 45 to 54 °C. Smyth et al., (1998) suggested that the forming of disulfide bonds occurred between 47 to 53 °C, which

contributed to gel network formation. For the myosin tail, King & Lehrer (1989) reported that the helix structure of the myosin tail unfolded in three transitions at 43, 47 and 53 °C. At 47 °C however, the unfolding process occurred at the S2 region. The formation of a myosin gel by heating has been demonstrated by Morita & Yasui (1991) who examined changes occurring to the hydrophobicity of the myosin tail during heating. At 35 to 40 °C, the hydrophobic amino acid residues of the myosin tail started to be exposed and reached a maximum exposure at 65 °C. When the meat was heated to temperatures higher than 65 °C, it resulted in a decrease in the hydrophobicity of the myosin tail. This could be due to the hydrophobic residues taking part in protein-protein interactions, where a network is formed to produce a gel for meat adhesion. The helix content of the myosin tail or the LMM began to decrease at 30 °C and attained a minimum at 70 °C. Therefore, it can be concluded that, to have maximum myosin gel formation, the reformed meat would be heated to between 65 to 70 °C. This would maximise the bonding between meat pieces. However, this may not always be the best temperature range as different meat samples from different species-containing different fibre types, salt and phosphate levels, and pH of the meat-could mean that different temperature ranges would be optimal either for myosin denaturation or aggregation (Lanier et al., 2014; Visessanguan, Ogawa, Nakai, & An, 2000).

2.9. Effects of mechanical work applied to meat and binding strength

Reformed meat product quality is partly determined by the binding strength between meat pieces, which should be such so that the product characteristics are similar to intact steak. Tumbling or massaging processes are crucial factors in producing a reformed meat product with a desired binding strength. For example, a study by Gillett et al. (1981) with ham massaged for 4, 8, 12, 16, 20 and 24 hours at 5 rpm resulted in adequate binding of ham slices (at 4 hours of massaging time), and binding strength increased further as the time of massaging increased up to 20 hours, but remained constant from 20 to 24 hours. The duration of massaging with the binding value in brackets was as follows; 4 hours (~260 gm/cm^2), 8 hours (~310 gm/cm^2), 12 hours (~395 gm/cm^2), 16 hours (~470 gm/cm^2), 20 hours (~510 gm/cm^2) and 24 hours (~525 gm/cm^2).

Increased binding strength can usually be achieved by increasing tumbling or massaging time. For example, mixing for 16 minutes and 24 minutes increased binding strength by 60% and 72%, respectively compared to 8 minutes (Booren, Jones, Mandigo, & Olson, 1981). A similar pattern was also observed when increasing mixing time from 5 to 10 or 20 minutes increased binding strength of restructured beef patties from 0.151 to 0.221 and 0.284 kg, respectively (Macfarlane, Turner, & Jones, 1986).

However, increased tumbling time does not always result in higher binding strength. Bedinghaus et al. (1992) reported that the binding strength of sectioned and formed pre-rigor ham tumbled for three hours was 25% greater than non-tumbled meat. Increasing tumbling time to 6 hours did not significantly increase binding strength. The binding strength reached a maximum and then plateaued out or reduced when tumbling time was increased. A quadratic effect with an increase followed by a decrease was shown between the breaking force of sectioned and formed ham against increasing time of massaging by Siegel, Theno, Schmidt, & Norton (1978). It appeared that the physical treatment of the meat should be limited so that the binding strength would remain sufficient and not decrease. Excessive tumbling apparently could result in the meat being broken within its structure rather than at the binding junction.

2.10. Conclusions and recommendations

i) Tumbling or massaging has been used extensively in meat processing for producing a wide range of reformed meat products. The operation parameters of tumbling are varied according to the characteristic of the product itself. Some products need a more vigorous tumbling action to apply more work to the meat, while for others sufficient work is applied with relatively delicate tumbling. The intensity of the work needed in the production of reformed meat products depends on these parameters:

   a. Time of tumbling
   b. Speed of tumbling
   c. Whether it is intermittent or continuous tumbling

ii) The work done could alter the structure of the meat and is dependent on the degree of tumbling carried out. The meat structure could become softer allowing the re-shaping of the meat pieces into new product and, more importantly, the work done could help to enhance the distribution of the brine into the meat via a pumping action.

iii) The brine consists of salt and phosphate as the main ingredients as they act synergistically with each other. Certain phosphates (TSPP is the most effective type) disassociate the actomyosin into actin and myosin while salt (NaCl is commonly used) solubilizes myosin. The pumping action, produced by tumbling, extracts these proteins to the meat surface to form sticky exudates.

iv) The amount of myosin that is extracted during tumbling under constant conditions depends on both animal species and muscle type.

v) Myosin is the main protein in the exudate extracted from meat that functions as a glue to stick neighbouring meat pieces together after appropriate cooking treatment and temperatures (65 to 70 °C).

vi) The mechanical work done during the tumbling process could influence the binding strength of the reformed meat, with the amount of work needing to be sufficient but not excessive.

Many studies have been carried out to optimize the quality of meat products by adjusting processing parameters, by selection of meat sources, and by taking into account other factors affecting reformed meat production. However, there have been no studies carried out to measure the total work done on the meat. Measures of the amount of total work applied could be a useful approach to investigating the mechanisms involved in changes occurring during tumbling and that could lead to improvements in reformed meat product quality in terms of characteristics such as the binding strength. Information on the amount of total work needed on the meat to extract the protein exudate, and its relation to binding strength, could facilitate the optimization of processing parameters and possibly the design of tumblers or massagers. In order to gain information on these subjects, the development of equipment with the ability to convey a known force to the meat, and to record the work done on the meat, is required. The results could provide

more accurate information on the nature of various relationships, such as that between the amount of protein extracted from the treated meat and the binding strength of combined meat pieces in relationship to the work imparted to the meat. Such a piece of equipment would also enable a study to be carried out that related the binding strength and amount of actin extracted as a function of how the work was applied, i.e. intermittent, continuous and rate of hitting (simulating rotation speed of the tumbler drum.

CHAPTER 3

Development of an Impact and Friction Mechanical Robot (IFMR) for use in quantifying the amount of work applied to meat samples by impact and/or friction

3.1. Introduction

Reformed meat is produced by applying physical treatments to meat pieces along with brine to extract the protein, which functions to bind adjacent meat pieces together when heated. Tumblers are used to treat the meat physically and provide the work required on meat in the production of reformed meat products (see Section 2.2: "Tumbling and massaging" for more information on tumblers). The amount of work done to the meat pieces in falling and hitting the tumbler floor, baffles or other pieces of meat in the tumbler, however, is unknown. This project had the objective of identifying a means of quantifying the work applied to the meat pieces. Equipment, named the Impact and Friction Mechanical Robot (IFMR), with the ability to hit the meat vertically in a repeatable way, and to measure the amount of work applied to the meat, was developed. The IFMR required features that would enable it to be operated automatically after a series of parameters had been set. These features were:

1. The number of hits on the meat sample could be pre-set.
2. The equipment could control the nature of each hit, either by specifying the maximum force exerted, or by specifying the maximum distance travelled by the Hitting Head (HH).
3. The time to complete a particular hit could be set.
4. The average time between hits could be varied (this time, together with the time per hit, will determine the frequency of hitting).
5. The force applied and depth of meat compressed could be recorded for every hit so that work applied to the meat could be calculated.

3.2. Design and Construction

3.2.1. Framework and compartments

In tumbling, the work applied to the meat in the container is a result of both abrasion and impact. The IFMR was designed to deliver both these forces. The rubbing action produces a friction force on the meat, which is an alternative method of extracting myosin (Knipe, 2004a). Dr Rory Flemmer was involved in designing the IFMR and contributing the basic software commands to connect two computers with the IFMR.

The framework of the IFMR was designed to provide platforms for both vertical hitting for the impact force and horizontal rubbing for the friction force. However, this study only investigated an aspect of the impact force and the friction force will be considered in future studies. The basic detail of the framework for the IFMR is shown in Figure 3.1. The framework consists of a base floor and three pillars. A stainless steel plate 630 × 260 × 13 mm, was used to build the base floor. Three aluminium posts, each 45 × 45 mm and 550 mm high, were attached to the floor in a triangular arrangement for the pillars. The same aluminium cross sectional material was attached to the top of the three posts so they were connected in a 'T' shape. A 10 mm thick layer of Perspex, which acts as a stabilizer for the cylinder arms during hitting and rubbing processes, was mounted between the three poles (see "Stabilizer" in Figure 3.1).

Two Nema 23 stepper motors Model M1233070 (LAM Technologies, Sesto Fiorentino, Firenze, Italy), were used to control the vertical and horizontal movements. Two arms made of cylindrical stainless steel tubes (25 mm diameter) were connected to the two stepper motors and then attached to the IFMR framework. The first vertical arm was connected to the vertical stepper motor that drives the hitting process (Figure 3.1A), while the horizontal arm was connected to the horizontal stepper motor and could be operated for the rubbing action (Figure 3.1D). The arms were connected together at the ends away from the stepper motors.

A limit switch (Figure 3.1A) was attached to a steel plate and positioned directly at the side of the stepper motor cam. Whenever the stepper motor was rotating, the cam pushed the lever of the limit switch from an open position to a closed position. The function of the limit switch was to act as a counter based on the number of hits. At the end of the connected arms, a stainless steel block (15 mm height and 38 x 38 mm cross sectional area) was attached as the component that hit or rubbed the meat (Figure 3.1C). This stainless steel block is referred to as the Hitting Head (HH). A 2 mm thick layer of Glass-Reinforced Plastic (GRP), glued to the surface of the HH was used to hit the meat sample, acted as an insulator to minimise transfer of heat between the HH surface and the meat and thus minimise the effects of temperature variation on extraction of myofibrillar and sarcoplasmic proteins.

The stepper motor, vertical arm, limit switch and the HH were all grouped in one special unit. This unit was equipped with a moveable screw which was used to manually lift or lower the whole unit (Figure 3.1A). The purposes of having this facility were to

assist placing the meat sample under the HH and to control the initial height of the HH above the meat piece before starting any hitting treatment.

An aluminium container was built with dimensions of 23 × 23 mm and 5 mm depth for placing the 20 × 20 × 20 mm meat samples. It was located directly under the HH used for hitting the meat sample (Figure 3.1B). Four holes 1 mm diameter with two each on opposite sides of the container wall were drilled 5 mm away from the centre of the container wall. Four pins 1 mm diameter and 7 mm long were prepared to pierce the meat through the holes. The purpose was to hold the meat down as hitting the meat could produce sticky exudate and the meat could be lifted up if not anchored to the container. The container was clamped on top of a steel plate with an area of 173 × 137 mm, and with a 45 mm long, 7 mm diameter, rod attached at the bottom which went down through a hole drilled on the base floor to a load cell.

A bending beam type load cell with maximum capacity of 25 lb. (111.206 N), Model LC509-025 (Omega, Stamford, CT, USA) was bolted at the bottom of the floor and connected directly to the rod from the steel plate (Figure 3.1C). Every time the meat sample was hit, the force was transferred from the meat to the steel plate and directly recorded by the load cell. An S-beam load cell with maximum capacity of 11 lb. (48.93 N), Model LCR-25 (Omega, Stamford, CT, USA) was attached between the end of the horizontal cylinder arm and stepper motor (Figure 3.1D). This load cell was not activated for the current operation but would be used to measure friction forces on the meat. The IFMR was placed inside a 4 °C chiller room with the controlling computers connected from outside the room through a special insulated hole to maintain temperature. The purpose was to minimise any temperature effects on the meat due to the hitting process, as these may affect extraction of proteins from the meat.

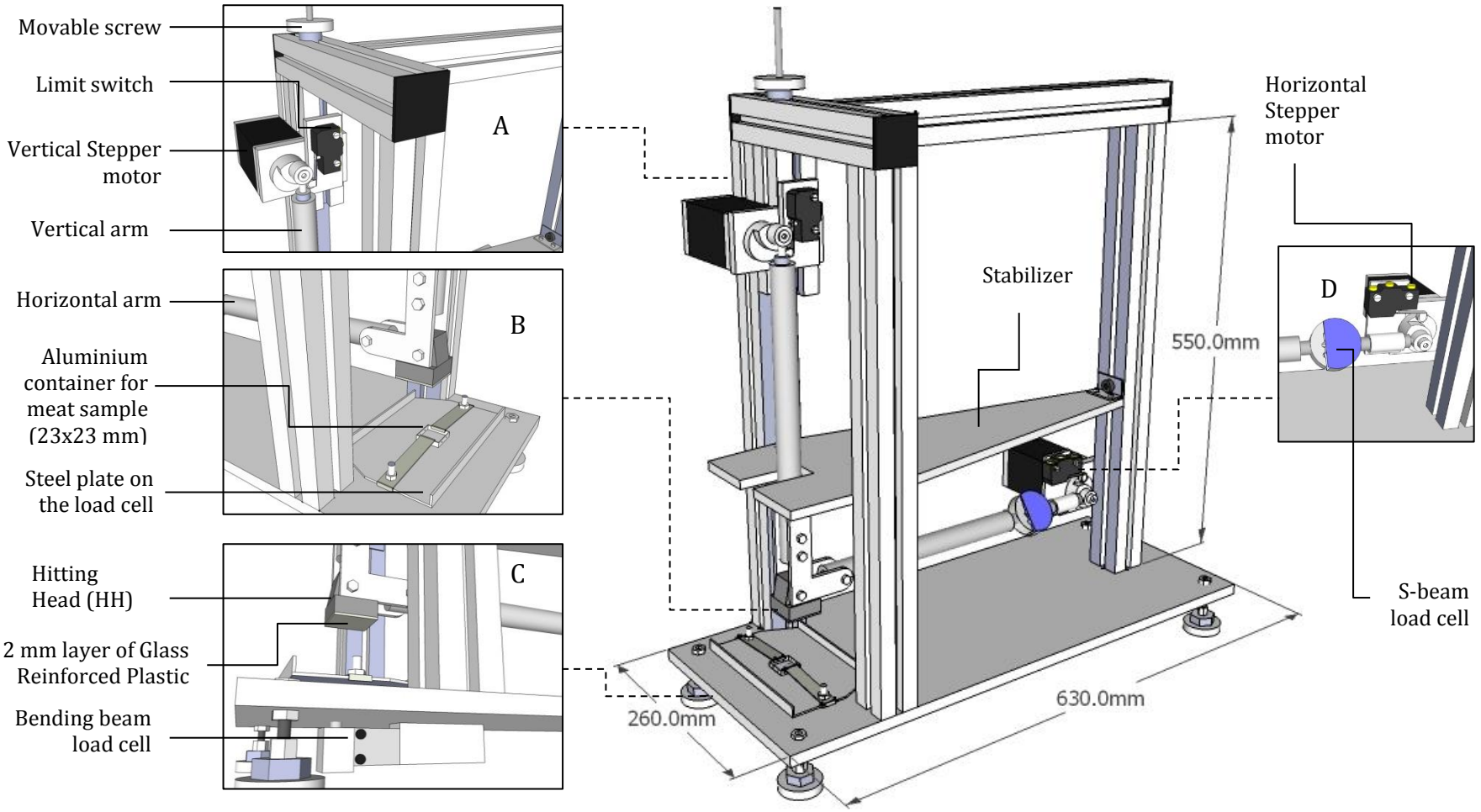

Figure 3.1. The framework and compartments of the IFMR equipment. A, vertical stepper motor, limit switch and movable screw; B, steel plate and aluminium container; C, Hitting Head (HH) used for hitting the meat sample and bending beam load cell; and D, horizontal stepper motor and S-beam load cell.

3.2.2. Computers and electronics

Two computers were set up to run the equipment. The first computer functioned as the master computer running Windows XP and was installed with the Visual Basic (VB) 6.0 program. Its function was to provide a platform for the user to give instructions to the equipment and to change the parameters for vertical hitting. The second computer was considered the slave computer. It was installed with the Disk Operating System (DOS) program and executed the commands and changes given to the equipment from the master computer i.e. it controlled the actuators and read the sensors. Two computers were required as the master computer was user-friendly with the Windows operating system but it could not control the hardware of the equipment *per se*, while the slave computer could operate the hardware using the DOS program.

Figure 3.2 shows the connection and wiring between the two sets of computers, the hardware in the box, and the equipment. Commands as VB code, from the master computer were sent to the slave computer. The slave computer then transferred each command through a CIO-DIO48 digital input/output board (Measurement Computing, Norton, MA, USA) to the stepper motor drivers (Model DS1044, LAM Technologies, Sesto Fiorentino, Italy) located inside the box, and powered by a 24 V power supply. The drivers ran the stepper motors. For the vertical stepper motor, the impact force produced on the sample, applied by the HH, was read by the bending beam load cell.

The powered load cell produced a low zero-load voltage signal of 0.5 V. When a specific force bent the load cell, the voltage signal increased the peak signal, which could reach up to 9.5 V. This voltage was scanned by a 12-bit analog-to-digital converter controlled by a PCI-DAS6025 board (Measurement Computing, Norton, MA, USA) with InstaCal™ version 5.74 driver software installed in the master computer.

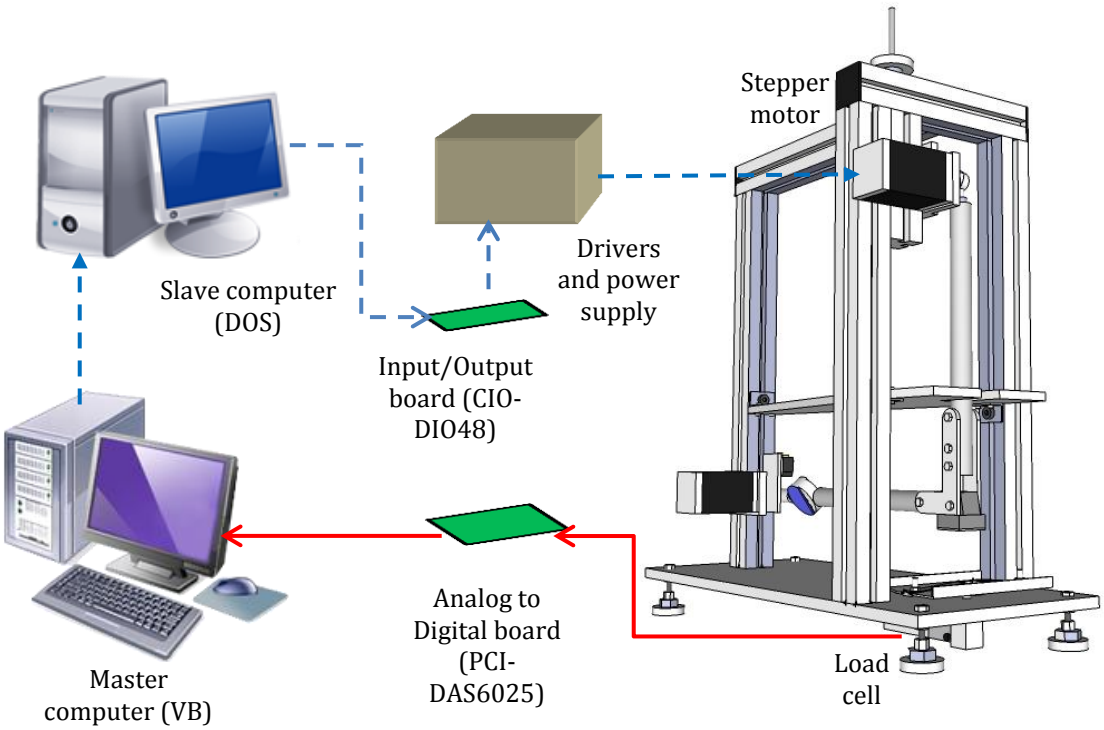

Figure 3.2. Connections and wiring set up between the computers and the equipment. Dashed arrows are commands from the master to the slave computer and then to the stepper motor drivers through the CIO-DIO48 board. Solid arrows are the forces recorded by the load cell, converted from analog to digital via the PCI-DAS6025 board and transferred to the master computer.

3.3. Code and functions

Running the IFMR was controlled by a VB 6.0 program in the master computer. There were also codes written with DOS commands on the slave computer to communicate with the stepper motor driver. These computer programs started the operation, managed the data collection, and varied the operating parameters. A flowchart outlining the commands used to run and control the IFMR are shown (Figure 3.3). Three basic VB commands set up the Graphical User Image (GUI) in the master computer started the operation. The 'Run' command started the movement of hitting the meat sample, the 'Pause' command stopped the movement during the hitting process, and the 'Home' command recalibrated the movement of the stepper motor cam so it returned to the initial starting point.

Two groups of commands were set up before running the hitting process. The first group was used to collect data, and depended on two criteria. First, the scanning rate of the data (S_R) (data/sec) determines the density of data points per hit. Second, the total amount of data to be scanned (S_T) determines the length of time for data to be scanned from the load cell per hit.. Both the scanning rate (S_R) and total data to be scanned (S_T) are explained in detail in Section 3.4: "Measuring the force and time".

The second group of commands had four criteria for the hitting parameters. First is the number of hits (n) for a particular run. The VB code specified a message box into which the number of hits was entered prior to every run. Second, the average gap time (s) between each hit (H_G). This was determined via the formula:

$H_G = {}^{x}/_{1000} + 0.665$ s, where *x* is the value input into the program.

Third, the length of time to complete a hit (H_T) (measured as the average time per step) controlled the speed of hitting the meat sample by a delay unit code in the DOS program in the slave computer (for more detail see Section 3.3.1: "Controlling the stepper motor movement").

Fourth, the method of hitting the meat was set up by choosing either: a) the fixed force method, which worked on the basis of a targeted maximum force level (F_T) for every hit or b) the fixed distance method, which functioned on the basis of a targeted maximum distance. In the fixed force method, the distance moved for each hit was automatically adjusted to achieve the required force (Figure 3.3). The fixed distance method was set up by applying a fixed distance for the hitting (Figure 3.4). The distance target (D_T) of the moving HH was determined by setting up the fixed step number (S_N) of the stepper motor before running the IFMR. This method meant the vertical arm was kept moving to the pre-determined distance no matter how much force was applied to the meat sample. The loop would repeat until it reached the pre-determined number of hits (n).

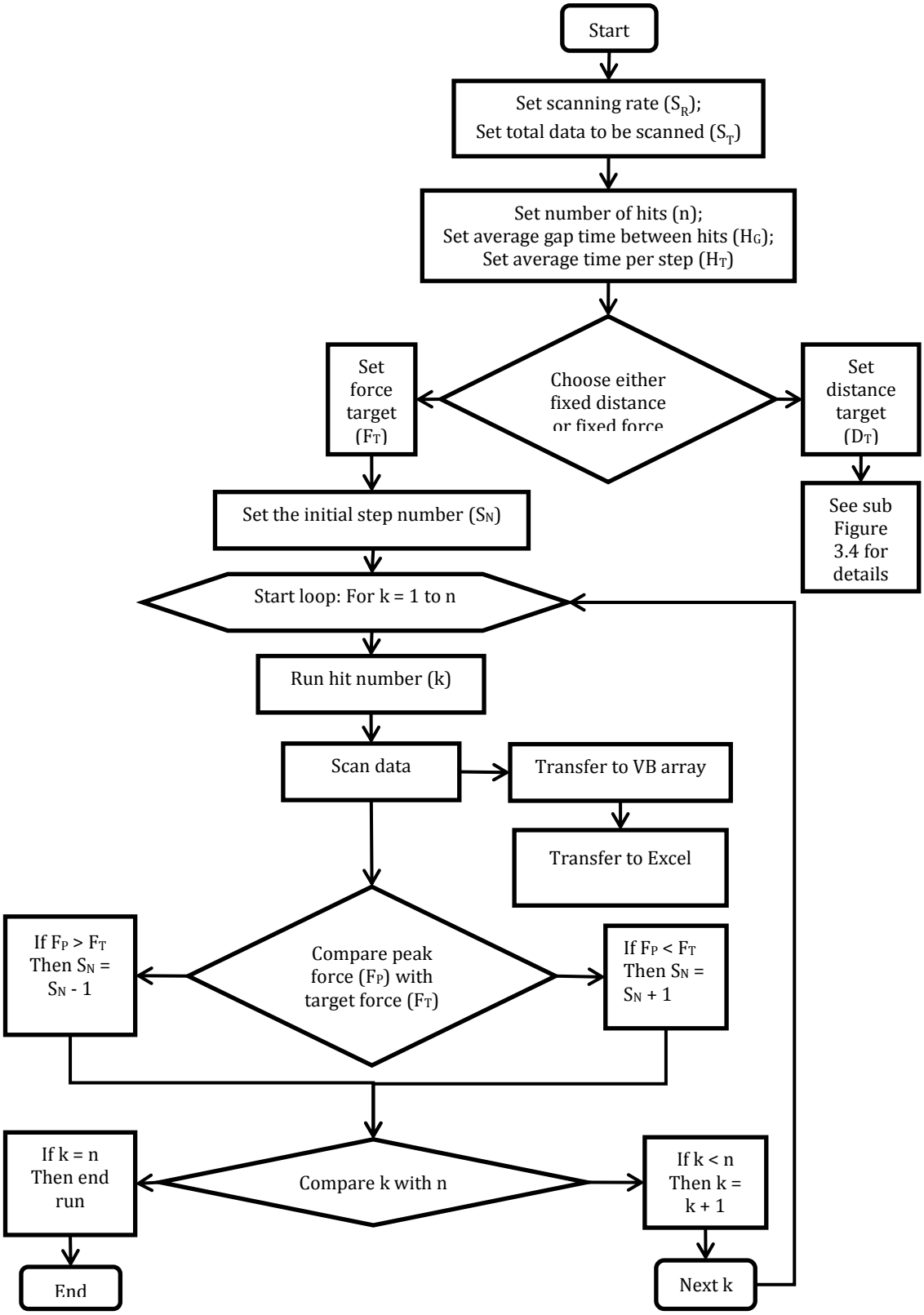

Figure 3.3. Flowchart for commands to operate the IFMR for the fixed force method. The increase or decrease of the step number (S_N) can be adjusted as required. Details for the fixed distance method are given in Figure 3.4.

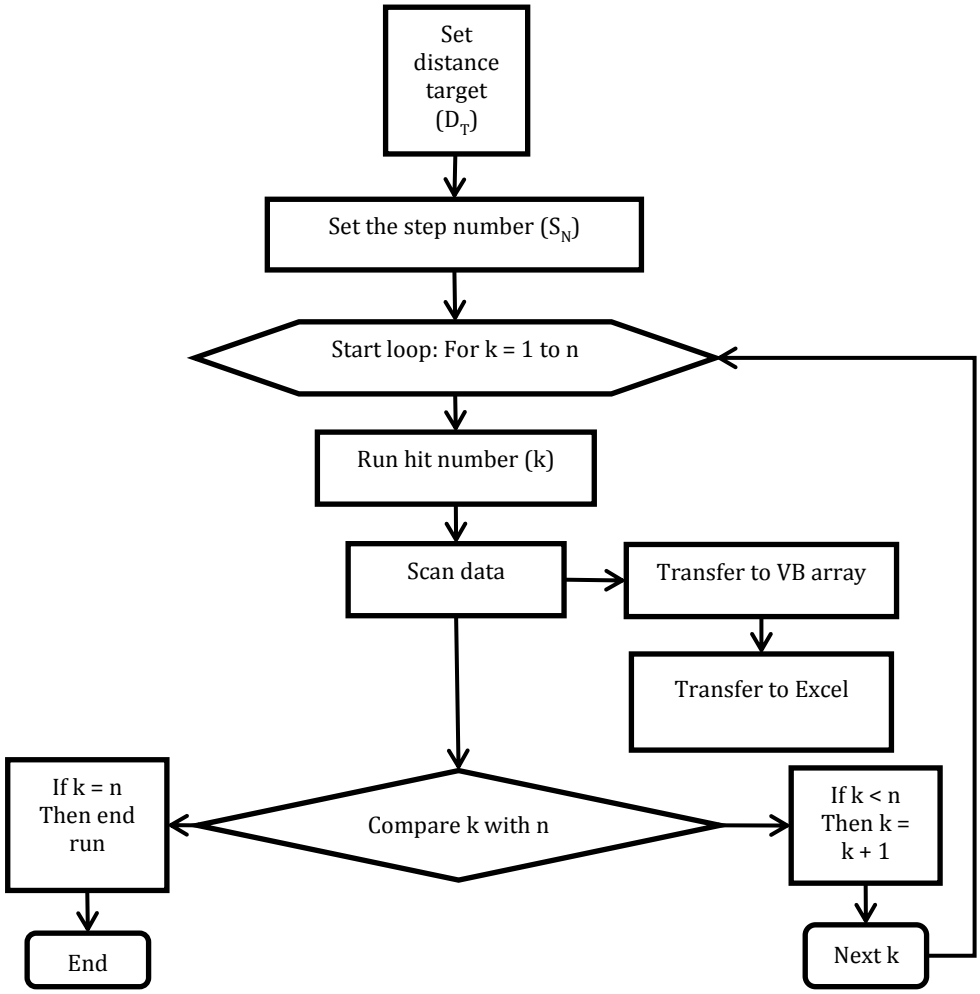

Figure 3.4. Flowchart showed commands to operate the IFMR for the fixed distance method.

The next sets of commands allowed the computer to make choices before proceeding with the hitting process. The commands were written inside the 'For-Next' loop that represented the cycle for hit k. The number of steps (S_N) was set before the loop. The step number within the loop was increased or decreased based on data, which was scanned and collected in the VB array before being transferred to an Excel workbook.

Then, the corrective code for controlling the force applied to the sample was written. The computer was asked to make a decision based on a comparison between the determined peak force (F_P) for hit k and the target force (F_T). If $F_P < F_T$ then the initial step number (S_N) would be increased one step for the next hit. If $F_P > F_T$, then S_N reduced by one step for the next hit.

The next step was to compare the cycle number (k) with the required number of hits (n). The computer once again was asked to decide if the k was less than n, then the k would increase by one hit and the loop would be repeated. However, if the k equalled n then the computer would end the treatment. These steps are summarised in Figure 3.3.

3.3.1. Controlling the stepper motor movement

The stepper motor movement was controlled by the stepper motor controller, which is a DOS program in the slave computer. This program was written by Dr Rory Flemmer and contained a specific formula to control movement of the stepper motor. The commands and their functions are explained, based on discussions with Dr Flemmer.

The commands were written in a For-Next loop, which asks the stepper motor driver to move the stepper motor by one step if the existing step number has not achieved the target required. The number of steps required is equivalent to the step number (S_N) set up earlier in the VB program. The For-Next loop was repeated several times before the stepper motor took a step. The number of loops taken for each step is different as specified in the formula written by Dr Flemmer. The formula smoothes the movement of the stepper motor so that when moving from step one to step S_N, the motor starts slowly, accelerates in the middle, and decelerates before stopping. The force curve read from the load cell also has the same effect by the movement of the stepper motor.

The variations in loops per step meant different times were needed per step movement. The time required to move every step was recorded and accumulated, and plotted against step number (Figures 3.5A and 3.6A). Figures 3.5B and 3.6B show accumulated time (ms) against accumulated distance (mm) of the vertical arm movement. Calculating the distance per step is explained in Section 3.5. These figures show that the stepper motor had the same movement pattern regardless of the number of steps, but the distance and time was greater if the number of steps is higher.

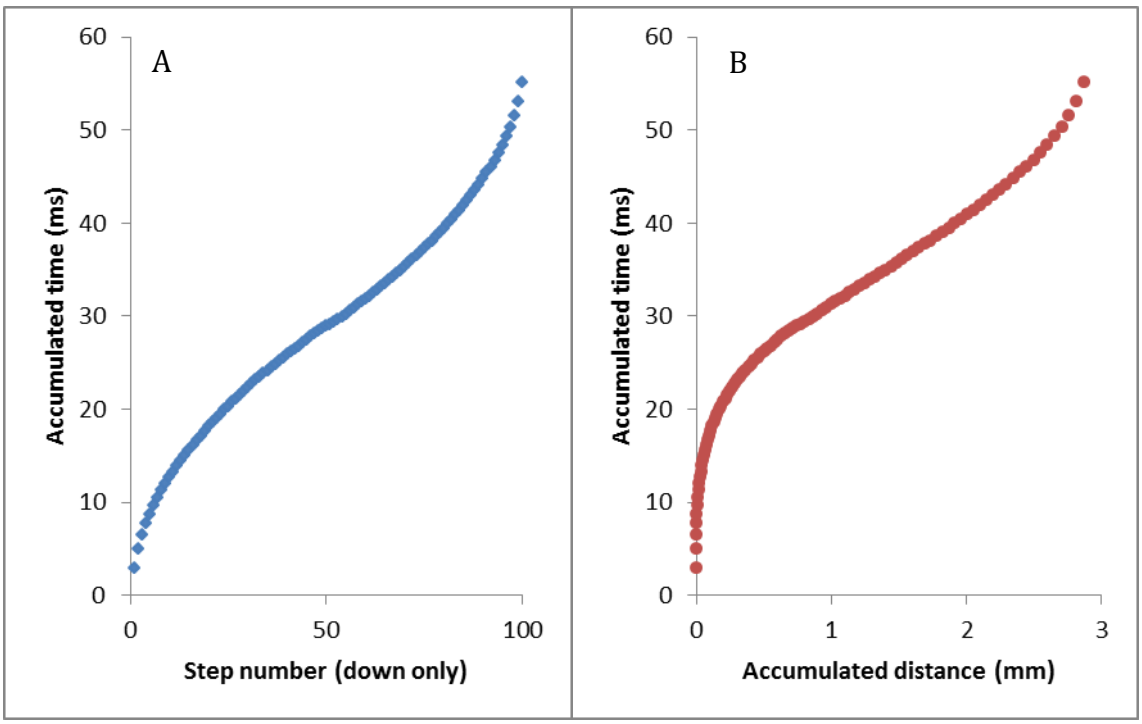

Figure 3.5. A) accumulated time against step number (100 steps down) and B) accumulated time against accumulated distance during the process (Section 3.5 for calculation of distance per step).

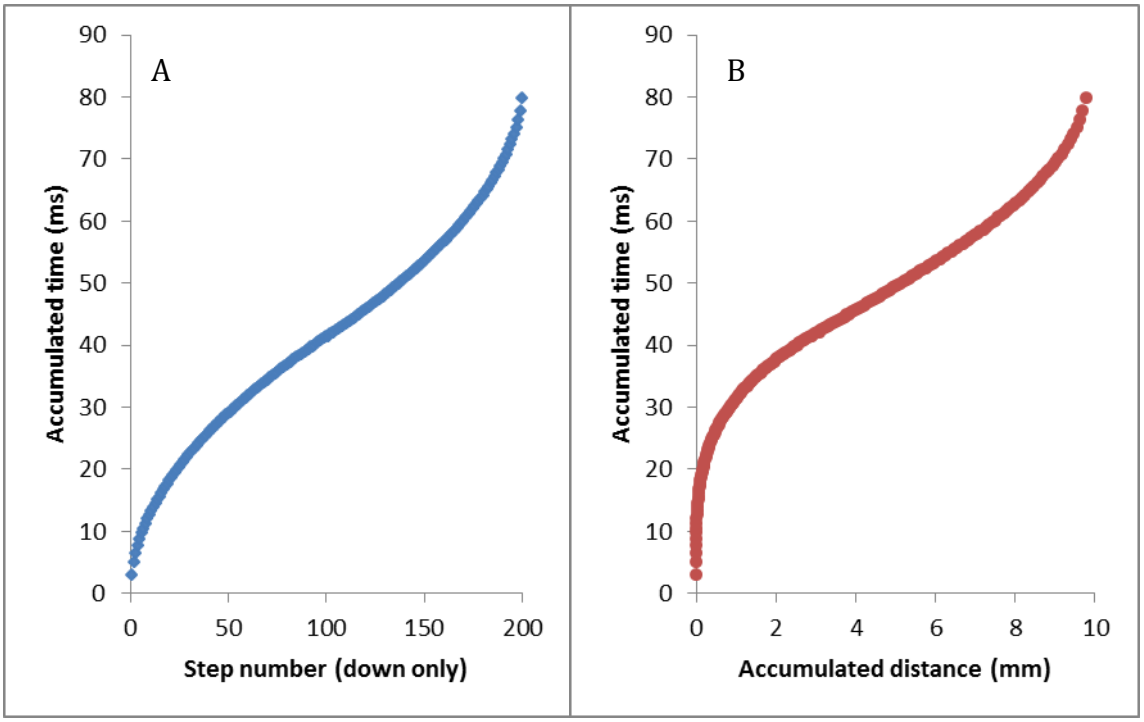

Figure 3.6. A) accumulated time against step number (200 steps down) and B) accumulated time against accumulated distance during the process.

3.4. Measuring the force and time

Force applied to the meat sample was measured by the load cell and then converted from analog to digital as a Binary-Coded Decimal (BCD) for easier execution of mathematical operations. The 12-bit analog to digital converter could produce BCD numbers ranging from 0 to 4096. Total data to be scanned per hit (S_T) and the scanning rate (S_R) could be altered. For example, if the delay unit was added to increase the time to complete a hit (H_T), more data were recorded for a particular hit on a meat sample. When the stepper motor moved faster, a fewer data points had to be scanned. For example, if 1500 data points were to be scanned per hit (St) at 2000 data point per second, then 1500 data points were loaded in Binary-Coded Decimal (BCD) from the load cell for every single complete down and up movement of the vertical arm, regardless of the number of steps. This meant that data point was scanned at 0.5 ms per point and array with 1500 data points required 750 ms for the scanning process.

Data collected in BCD were converted to force (N) in the VB program before being transferred to an Excel workbook. The load cell was calibrated by applying known weights that were converted to applied forces using the equation 3.1:

Equation 3.1**:**

$F = mg$, where F is the force (N), m is the mass or weight (kg), and g is the gravity (ms^{-2}). Ten weights ranging from 0 to 5.049 kg were placed singly on top of the load cell and the IFMR was run for 50 hits each, but with the actual hitting mechanism disconnected. All 1500 data points from the load cell were recorded and averaged. A calibration curve was plotted for load read in BCD against Force (N) (calculated from the weights used) resulted in a straight line with R^2 of 1 (Figure 3.7).

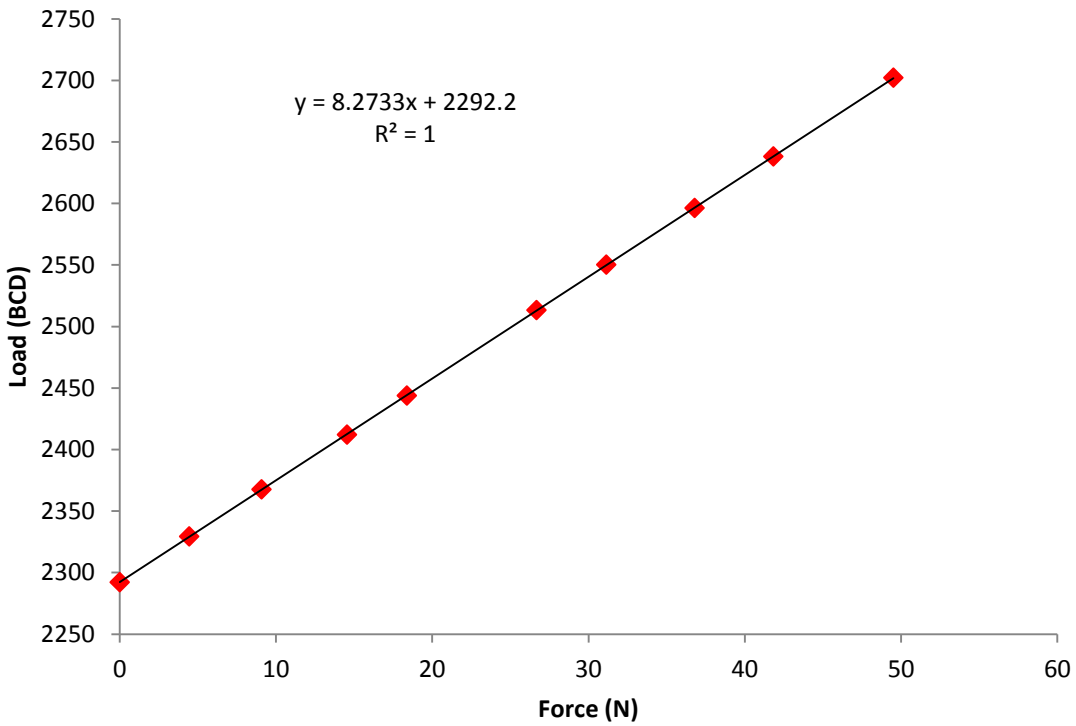

Figure 3.7. Calibration curve of reading from the load cell in Binary-Coded Decimal (BCD) and resultant Force (N).

Equation 3.2 was produced from the calibration curve.

Equation 3.2:

Force = Load − 2292.2/8.2733, where the load read in BCD and force is measured in newtons. This equation was inserted in the VB program in the master computer to convert the BCD reading collected for every hit on meat sample into force (N). The equation was also used to set up for the fixed force option by converting the pre-determined force in newtons into BCD before transferring to the VB program.

3.5. Calculating the distance

The level for the meat to be compressed in the fixed distance (D_T) method was set up by measuring the distance the HH travelled for each step. The distance also calculated the work done on the meat sample. The movement of the stepper motor cam was crucial to calculate the compressing distance. The stepper motor used for IFMR was a mechanical device equipped with a shaft that could rotate 360°. A 38.1 mm diameter cam was

attached to the shaft and the pivoting vertical arm was fixed at 0° with 9.79 mm distance from the centre of the cam. The stepper motor used 800 steps for a full rotation of the cam. Thus, the vertical arm moved downward for 400 steps, which equalled 180° rotation.

The stepper motor cam with the calculations used to relate the step number or degrees of rotation from the vertical position to the distance travelled by the vertical arm (Figure 3.8). The distance travelled was equal to the height ('a' in Figure 3.8) for an angle "B".

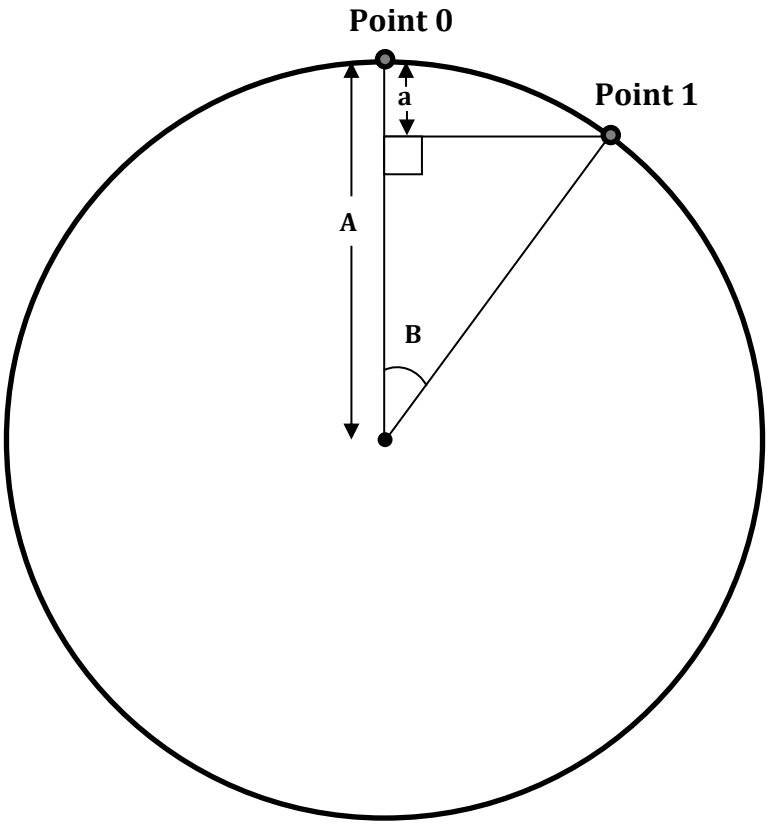

Figure 3.8. Stepper Motor rotation movement and its relation to distance travelled by the vertical arm and Hitting Head (HH).

The first step was to calculate the angle the cam moved through per step:

$$\frac{360°}{800 \text{ steps}} = 0.45° \text{ for every step}$$

For example, if 35 steps were needed to move the pivoting vertical arm from Point 0 to Point 1, it required $0.45° \times 35$ steps $= 15.75°$ of rotation angle of the stepper motor cam which is shown in Figure 3.7 as the 'B' symbol. The length of radius is 9.790 mm, marked as 'A'. The distance travelled for the HH which resulted from the movement of the stepper motor cam from Point 0 to Point 1 was marked as 'a'. It was calculated by formula:

a = A (1 – cos B), which is equal to 0.368 mm.

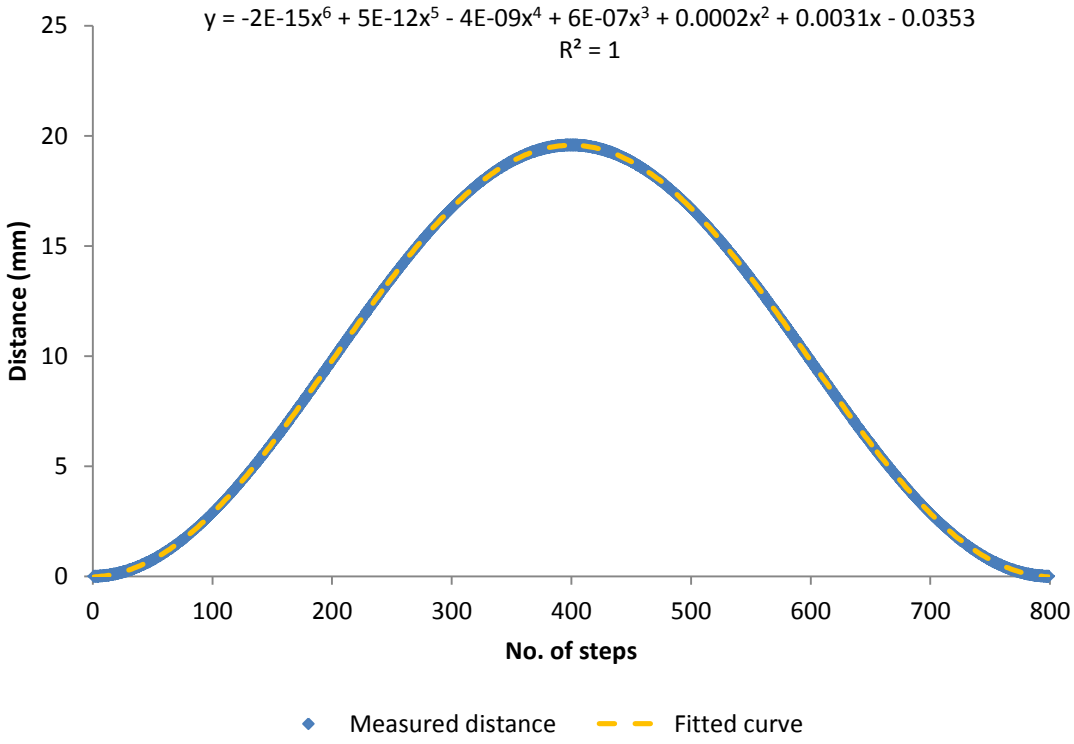

Figure 3.9. Accumulated distance travelled by HH with number of steps taken by the stepper motor. The steps number represents 400 steps down and 400 steps return. The fitted line (dotted line) was overlaid with R^2=1.

These series of formula and calculations were inserted into an Excel work book and the distance travelled by HH calculated for every step taken by the stepper motor cam. The relationship between number of steps and distance travelled was plotted for 400 steps

downwards and 400 return steps (Figure 3.9). The polynomial regression of the data point is shown in Equation 3.3 with an R^2=1.

Equation 3.3:

$$y = -2 \times 10^{-15} x^6 + 5 \times 10^{-12} x^5 - 4 \times 10^{-9} x^4 + 6 \times 10^{-7} x^3 + 0.0002 x^2 + 0.0031 x - 0.0353$$

Equation 3.3 was used to determine the number of steps that need be specified for attaining a fixed distance for the meat to be compressed by the HH. The gap between the HH and the meat surface before starting the hitting process needed to be accounted for in the fixed distance method. The usual practice was to place the meat surface 1 mm from the HH using a feeler gauge.

3.6. Calculating the work

An important aim of this study was to measure not only the force but also the work applied to the meat sample. Therefore, the distance the meat was compressed from its original height at every point a force measurement was taken during a hitting treatment needed to be measured to calculate the work done (Equation 3.4).

Equation 3.4:

$w = F \times d$, where, w is the work (J), F is the force (N) and d is the distance travelled (m) or in this study, the distance the meat sample is compressed.

Data were collected in BCD and Equation 3.5 was used to calculate the work done on the meat between sample points, where t=time.

Equation 3.5:

$$Work = \frac{(Force\ at\ t2 + (Force\ at\ t1))}{2} \times (Distance\ at\ t2 - (Distance\ at\ t1))$$

Total work done on the meat sample was calculated by summing the individual work increments calculated using Equation 3.5. To use this formula; the distance travelled needed to be simultaneously recorded with the force applied to the meat. Data collected from the load cell were converted into force (N). The scanning rate indicated the time for

each data point collected. Distance was determined from the number of steps taken during a particular hit. The time for every step taken also was recorded.

The time for scanning the force data and the time of the steps could not be matched directly as each parameter had different total data points. Therefore, the time for every step was matched with a similar time for the data force being scanned. Two methods were tested. In the first method, the time when the force was applied was matched with the time of the stepper motor movement using the look-up feature in Excel. The second method involved matching the time using the equation produced from the fitted functions of different distributions based on modelling the actual distance. These two approaches are considered separately in Section 3.6.1.

3.6.1. Matching time using Look-up feature in Excel

To tell the effectiveness of matching the time of the force being scanned and the real time of the hitting movement, a spring rather than a meat sample was used. The spring was hit once with 10 delay unit (equivalent to average of 0.65 ms per step). The time for every step the stepper motor took for 200 steps down and 200 steps return was recorded. The time then was rounded to zero decimal places or to have 0.5 digits at the end to match the scanning time, which had a rate of 0.5 ms per data. The data for rounded time were then paired with the distance and, by using the look-up feature in Excel, the force recorded was matched to the distance based on matching times for both distance and force. The distance (mm) and the force (N) plotted against time based on the look-up feature (Figure 3.10).

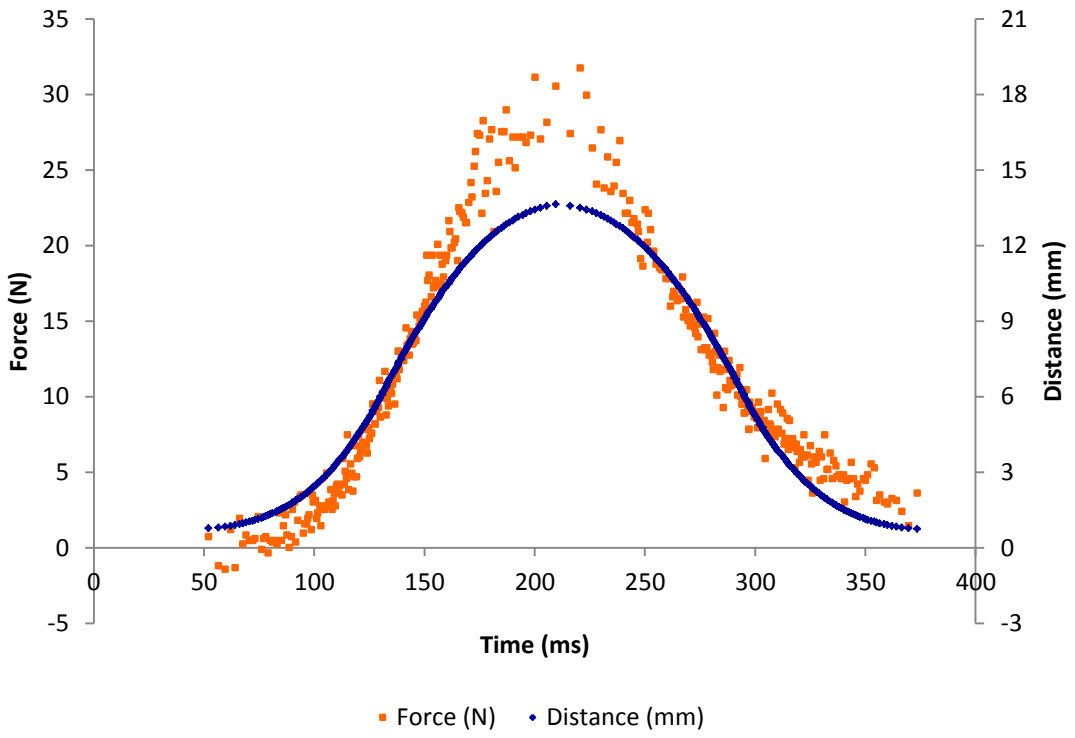

Figure 3.10. The force (N) recorded and distance travelled (mm) against time (ms) using Look-up feature on the spring. The spring was hit for 200 steps down and 200 steps return with average time of 0.65 ms per step.

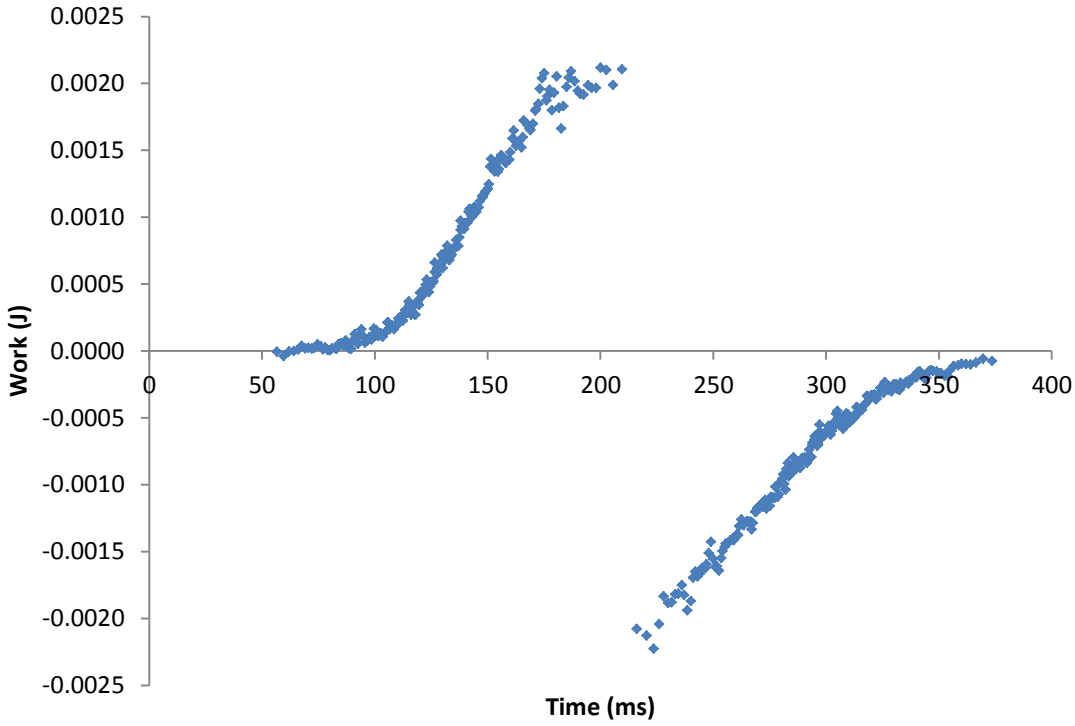

Figure 3.11. The calculated instantaneous work (J) done on the spring for a single hit. The spring was hit for 200 steps down and 200 steps return for an average of 0.65 ms per step. Total work was 0.0015 J.

The work (J) on the spring (Figure 3.11) showed two parts. The first, with positive values, is work done on the spring, and the second, with negative values, represents work returned by the spring against the HH used for hitting. The graph of the work shows a big gap between the positive and the negative work values because there was a time gap between the maximum step and the next step taken to return the hitting head (HH) back to step 0. This is where the HH stop for a while before return to home. The total work for this hit was 0.0015 J as calculated using Equation 3.5.

This method had lost most of the points from the graph that carried the information of the force as the total number of data of force plotted in Figure 3.9 was dependent on the steps number (S_N). The overall number of data to be scanned (S_T) for force was initially set up at 1500 data points but it had been reduced to only 400 data points corresponding to 200 steps down and 200 steps up. This Look-up feature method had affected the work calculated as shown in Figure 3.11, where the total work reading was low because of the reduction of the data points (from 1500 reduced to 400 points). The total work reading did not represent the real work done on the spring (or meat) and could lead to less accurate analysis.

3.6.2. Fitting the force with the distance

The second method for calculating work used the function of distance (mm) with time (ms) to determine the distance travelled based on the time of each force value. The similar hitting treatment was done on the spring, with 200 steps down and 200 steps return. Average time of each step was 0.65 ms. Distance travelled was calculated from the step number, and the distance (green line) plotted with time for the stepper motor to complete a hit (Figure 3.12).

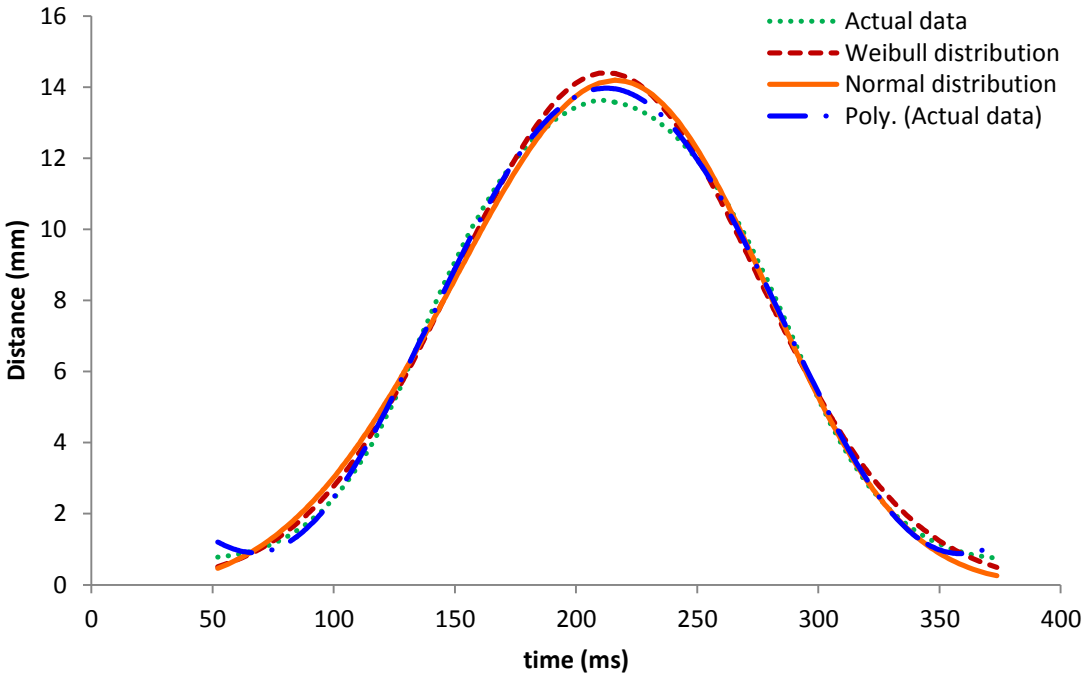

Figure 3.12. Modelling distance travelled using Weibull, Normal or Polynomial distributions. Data was collected from hitting the spring for 200 steps down and 200 steps return with an average step time of 0.65 ms.

The goodness of fit of the data generated was modelled using a Weibull distribution (red line), Normal distribution (orange line) and Polynomial distribution (blue line) (Figure 3.12). A stepper motor delay of approximately 52 ms compared with the load cell scanning time was built into the VB program. As none of the distributions gave an R^2 of 1, the general model Gaussian function (Matlab) was tried and gave the best fit with R^2=1(Figure 3.13) and had the following equation:

Equation 3.6:

$$y = a1 * exp(-((x - b1)/c1)\text{^}2) + a2 * exp(-((x - b2)/c2)\text{^}2) + a3 * exp(-((x - b3)/c3)\text{^}2) + a4 * exp(-((x - b4)/c4)\text{^}2) + a5 * exp(-((x - b5)/c5)\text{^}2)$$

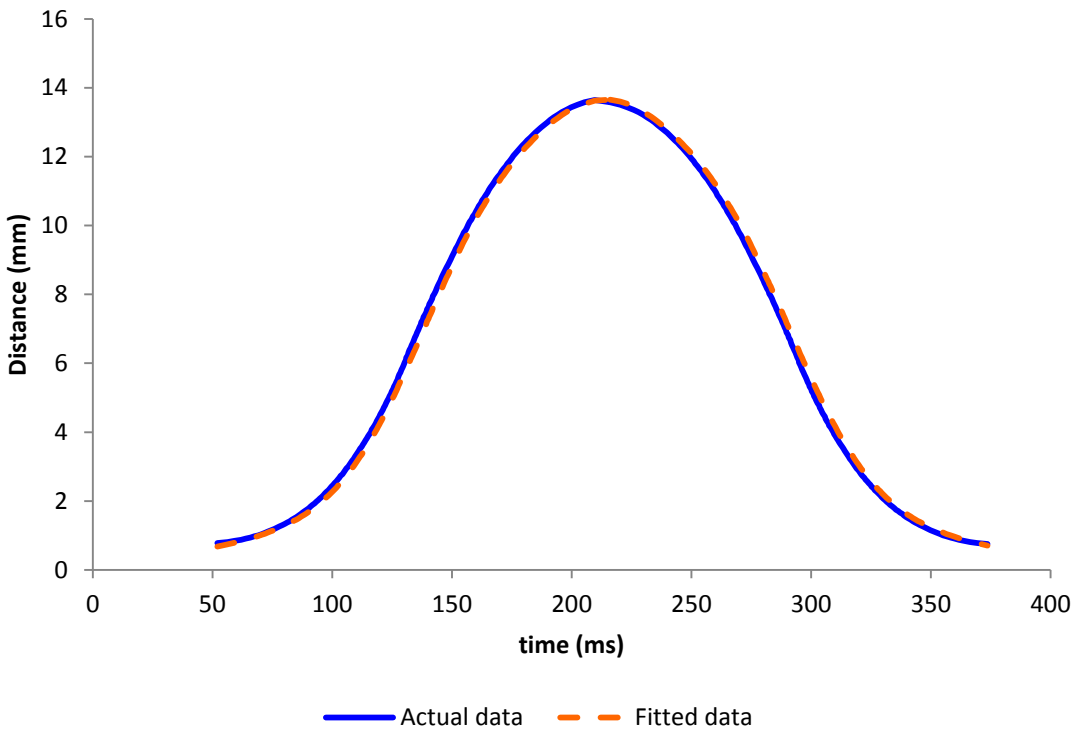

Figure 3.13. Comparison of curves between actual data and fitted data for distance travelled against time using the General model Gaussian (Mat Lab). The actual data was collected from hitting the spring for 200 steps down and 200 steps return with average time of 0.65 ms per step.

Equation 3.6 allowed the distance (mm) to be calculated from the scanning time of the force (N). Both distance and force were plotted against time (Figure 3.14) then the work (J) done on the spring was calculated and plotted against time (Figure 3.15). Total work was 0.0108 J which was about seven times higher than work calculated using the look-up feature.

For a hit on the spring (or meat sample), although the equation obtained using Mat lab fitted the data more accurately than the equation generated from the Excel look up feature, both methods took more than 5 minutes to calculate the total work. This project aimed to hit the meat sample at least 400 times. The IFMR needed to be able to record the force applied and distance travelled (depth of meat compression) automatically so the work could be calculated easily and in the shortest time. Therefore, another method of measuring the distance travelled was developed to reduce the analysis time and yet still enable collection of accurate data.

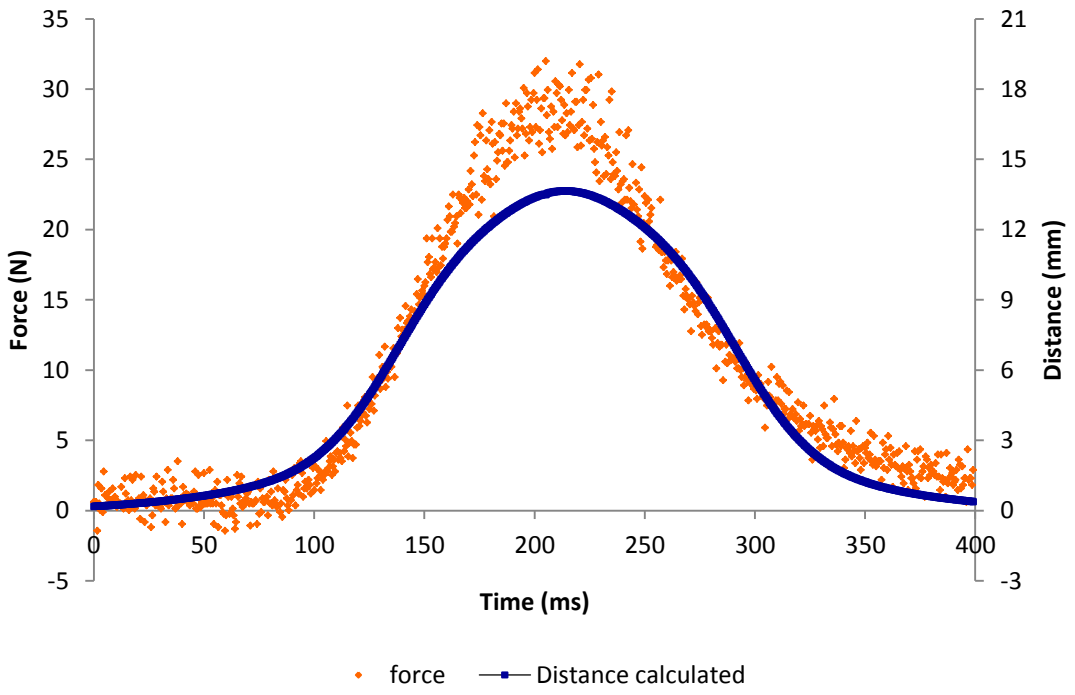

Figure 3.14. Force (N) and distance (mm) travelled when spring was hit for 200 steps down and 200 steps up with an average time of 0.65 ms per step.

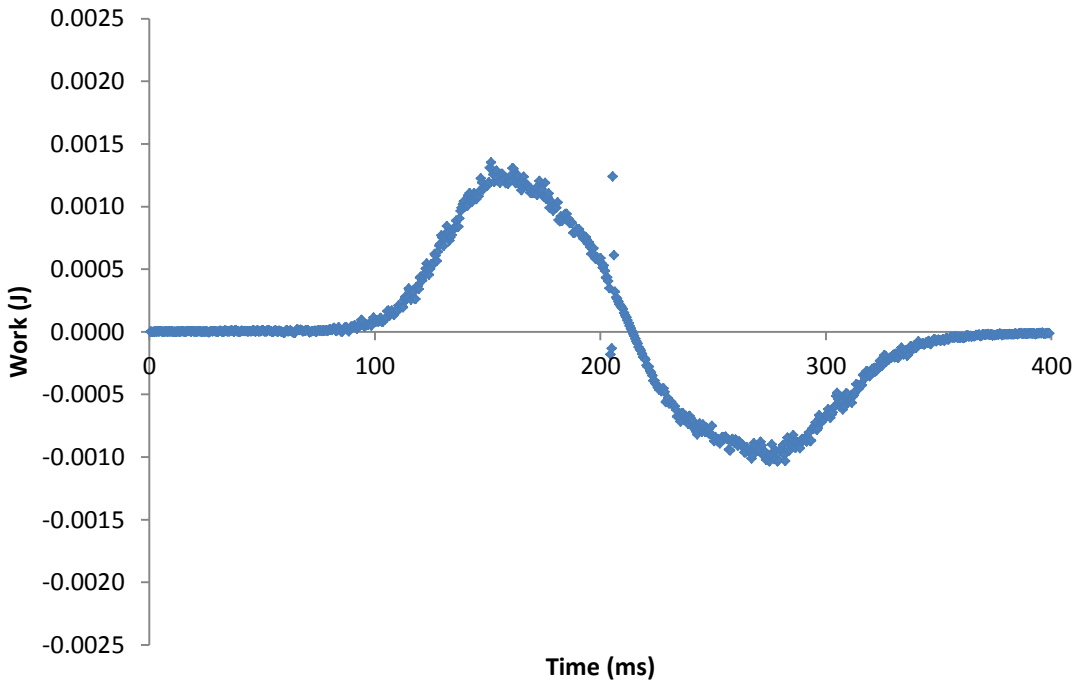

Figure 3.15. The work (J) done on the spring against time (ms) calculated based on an equation produced from modelling the distance fitted line. The spring was hit for 200 steps down and 200 steps return with average time of 0.65 ms per step. Total work was 0.0108 J.

3.7. Distance measurement by a potentiometer variable resistor

A potentiometer variable resistor produced a linear voltage when moved in a circular motion was fixed at the back of the stepper motor. Every time the stepper motor moved, the potentiometer also revolved. The potentiometer variable resistor was screwed through a small hole drilled into the back of the stepper motor (Figure 3.16).

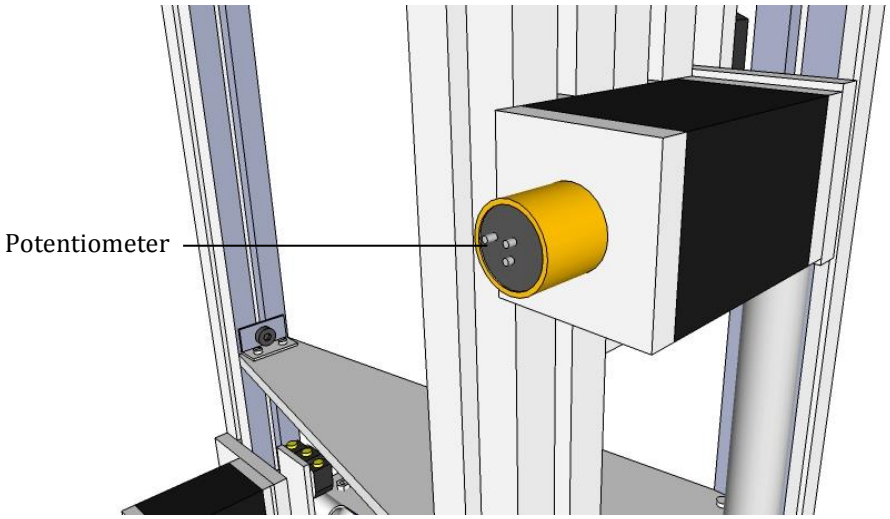

Figure 3.16. Detail of attaching potentiometer to the back of the stepper motor.

The potentiometer was wired to the channel in the same board as the load cell and the signal was read alternately into the same array as the load cell signal by including an extra command (Figure 3.17), in the VB to separate data from the load cell and the potentiometer before converting the data to Force (N) and Distance (m), respectively, and then calculating the Work (J).

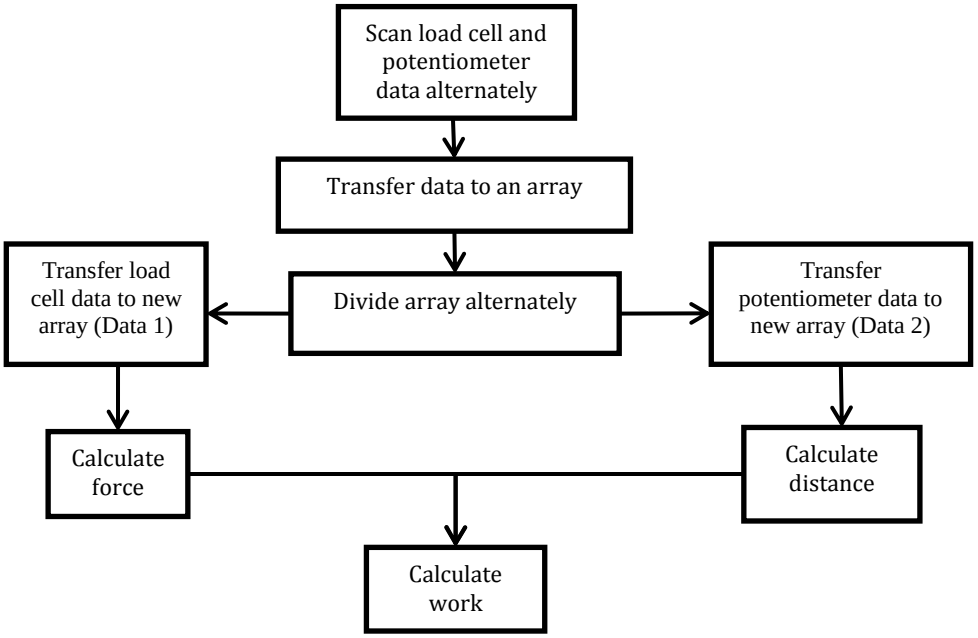

Figure 3.17. A flowchart showing commands in the VB program to collect the data from both load cell and potentiometer and converting them to force, distance and work.

A linear regression of the maximum BCD reading of the potentiometer for steps 0 to 400 (Figure 3.18), gave distance against step number. Thus, every step the stepper motor took automatically produced a reading of distance from the potentiometer value.

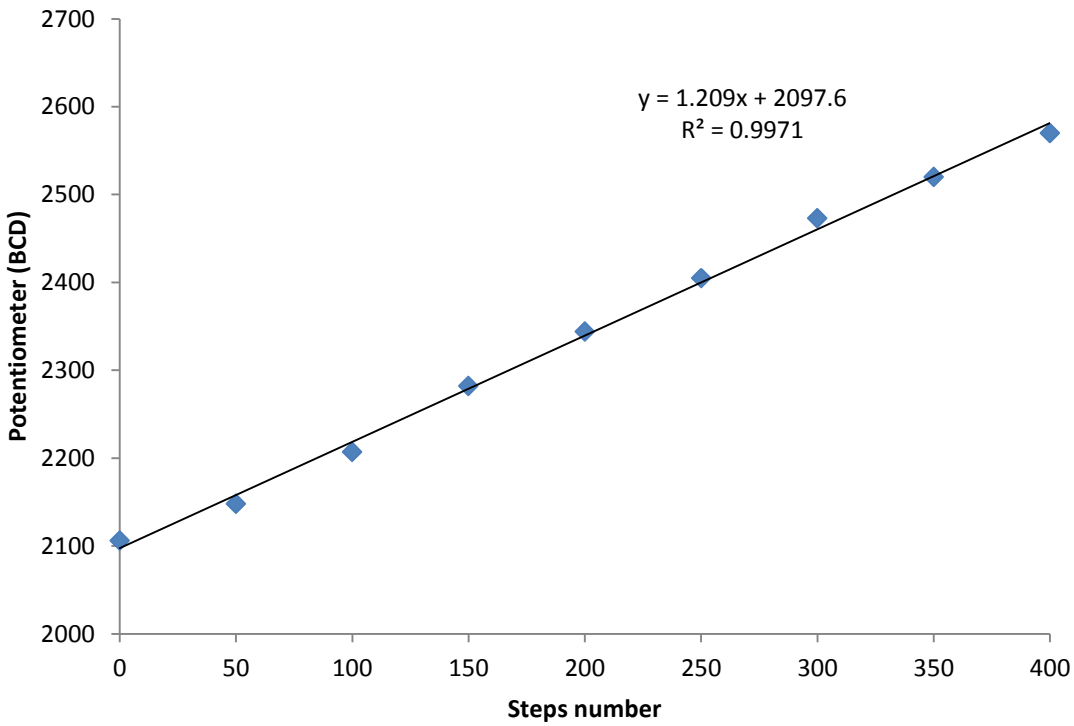

Figure 3.18. The calibration curve of the potentiometer output against step number.

A test was done to calculate total work done on a spring. Figure 3.19 shows the force recorded from the load cell and distance from the potentiometer for the fastest rate of hitting with a delay unit of 1, which was an average of 0.15 ms per step. The force and distance indicated by the circular motion of the stepper motor and potentiometer read up should be synchronized but the lines were mismatched with peak for force being later and broader than distance.

Delay units of 10 and 100 equal to averages of 0.65 and 5.89 ms per step, respectively (Figure 3.20 and 3.21). The peaks for force and distance were closer in Figure 3.20, while in Figure 3.21, both peaks reduced the differences. However, increasing the delay unit, substantially increased the time taken to complete a hit (Figure 3.21) with a total time of about 2150 ms. Data points to be collected per hit had to be increased as the delay unit increased, to allow all information of the peak to be recorded. Moreover, the peak value of the force increased as the delay unit increased, and the total work calculated resulted in negative values. For example, -0.00452 J for a delay unit of 100 even the peaks were perfectly overlaid (Figure 3.21). The total work with delay of 1 or 10 units (Figure 3.19 and 3.20) were not considered as the peaks for the force and distance did not overlap perfectly.

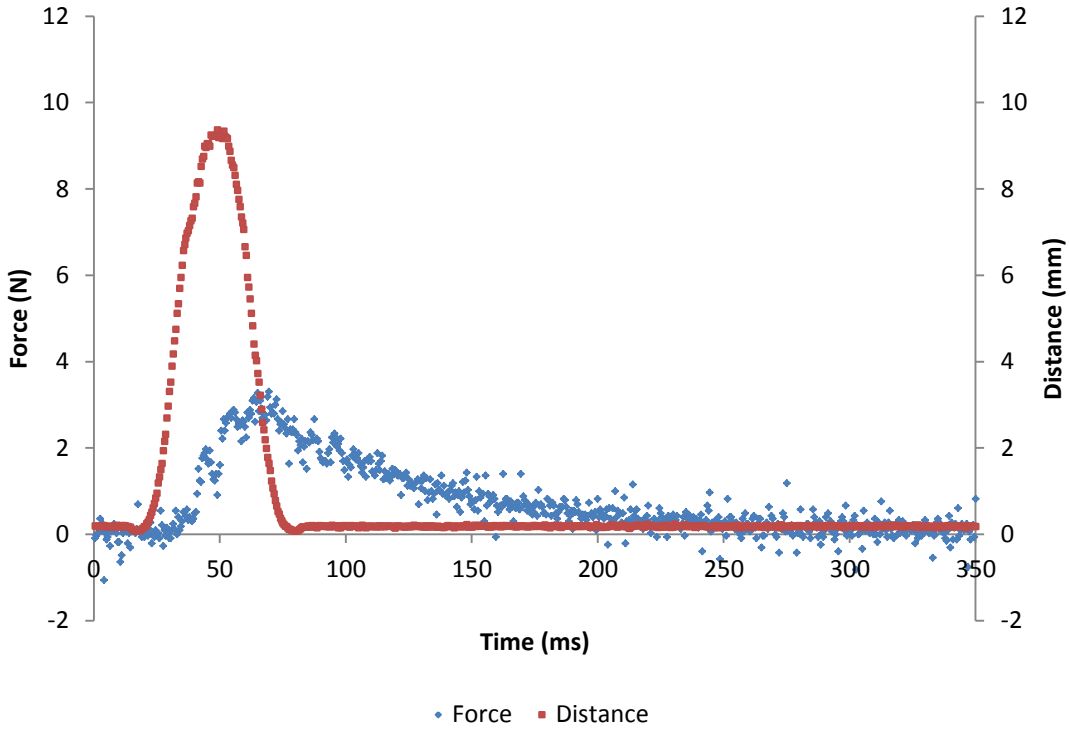

Figure 3.19. The force and distance against time for one hit on the spring sample with 1 delay unit.

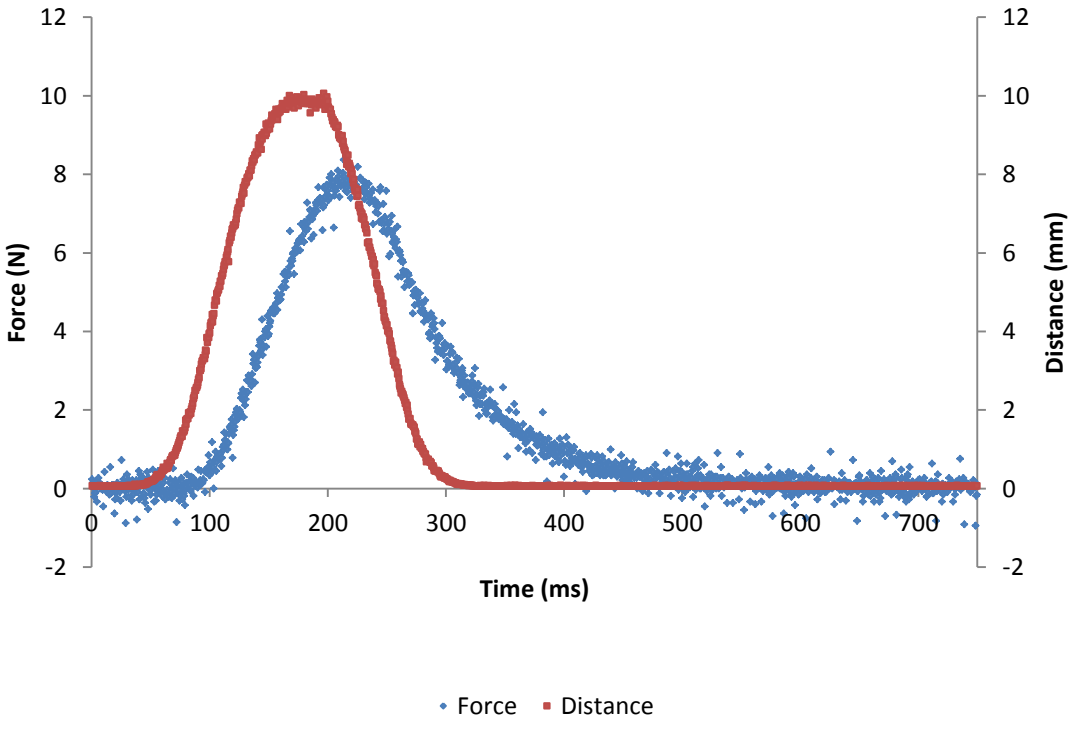

Figure 3.20. Modelling force and distance for one hit on the spring with delay of 10 units.

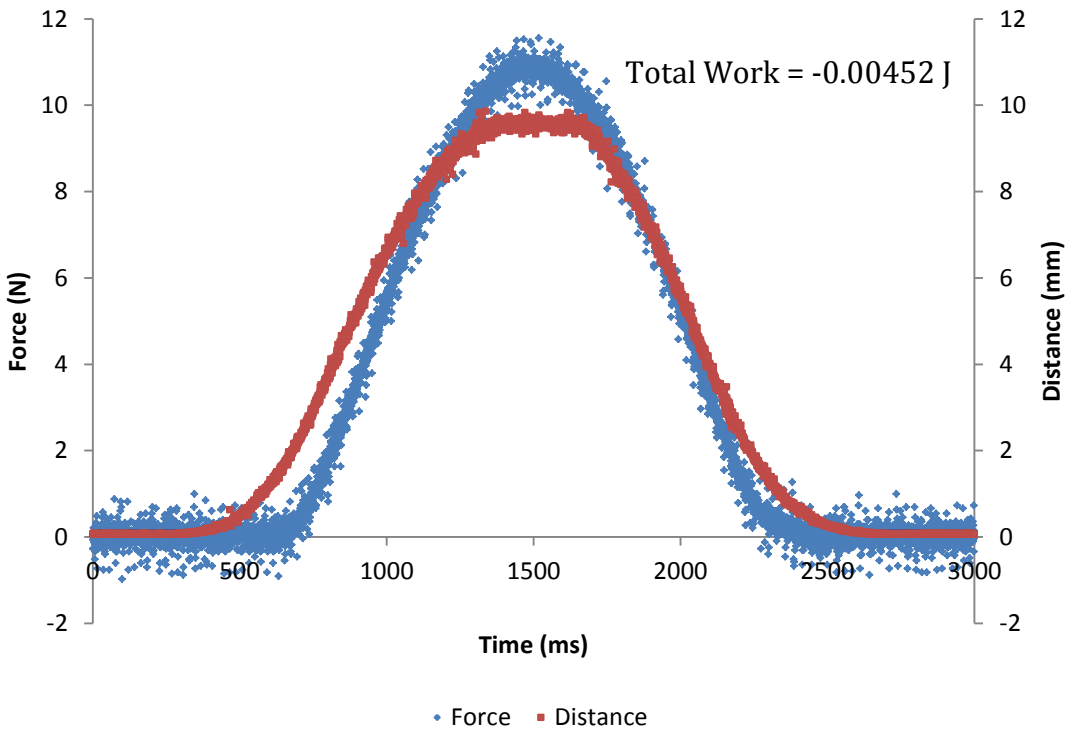

Figure 3.21. Modelling force and distance for one hit on the spring with delay of 100 units.

Further analyses were carried out to identify the cause of the differences between calculated and actual force and distance. A series of hits on the spring were done using delay units of 1, 5, and 10, and then by increases of 10 up to 150 delay units. The peak forces with small delay units were smaller than the bigger delay units (Figure 3.22). The peak force started to plateau only at delay units of 50, which is equivalent to 2.9 ms average time per step (Figure 3.26). It was concluded that the load cell response was too slow (3 ms) so data from the potentiometer and the load cell were not matched. This affected calculated total work and produced negative values, so it was necessary to investigate alternatives that would respond more rapidly than the load cell.

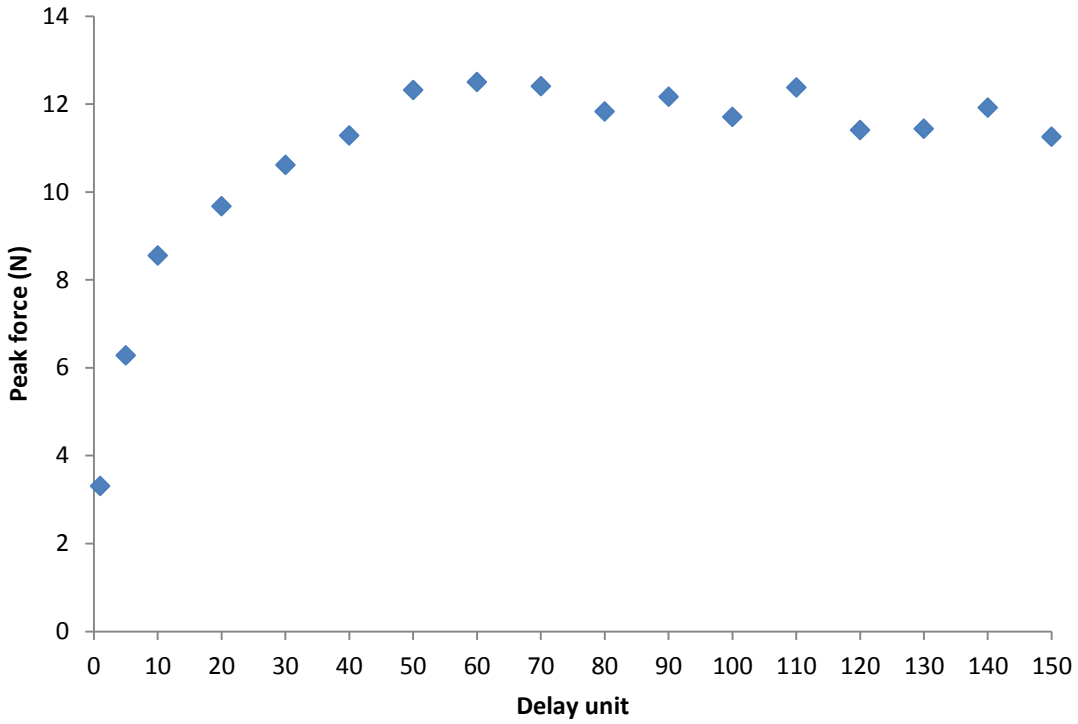

Figure 3.22. Peak force (N) against the delay unit number during hits on the spring.

3.8. Replacing the load cell with a hydraulic pressure sensor

To increase the responsiveness of the system, the load cell was replaced with a 0.5 bar hydraulic pressure sensor (MRH 21, MeasureX Pty Ltd, VIC, Australia), which had a response time of 1×10^{-6} seconds. The hydraulic sensor was fixed to the side of a 64 x 64 x 35 mm aluminium block (Figure 3.23). The container previously used to hold meat samples was attached to an aluminium plate (64 x 64 mm), which was attached to a steel cylinder with a rubber 'O' ring around it. The steel cylinder and attached container was fitted into a 25 mm hole drilled into the aluminium block filled with hydraulic oil. A bleeding process removed the air inside the block. Finally the block was attached directly under the HH used for hitting the sample.

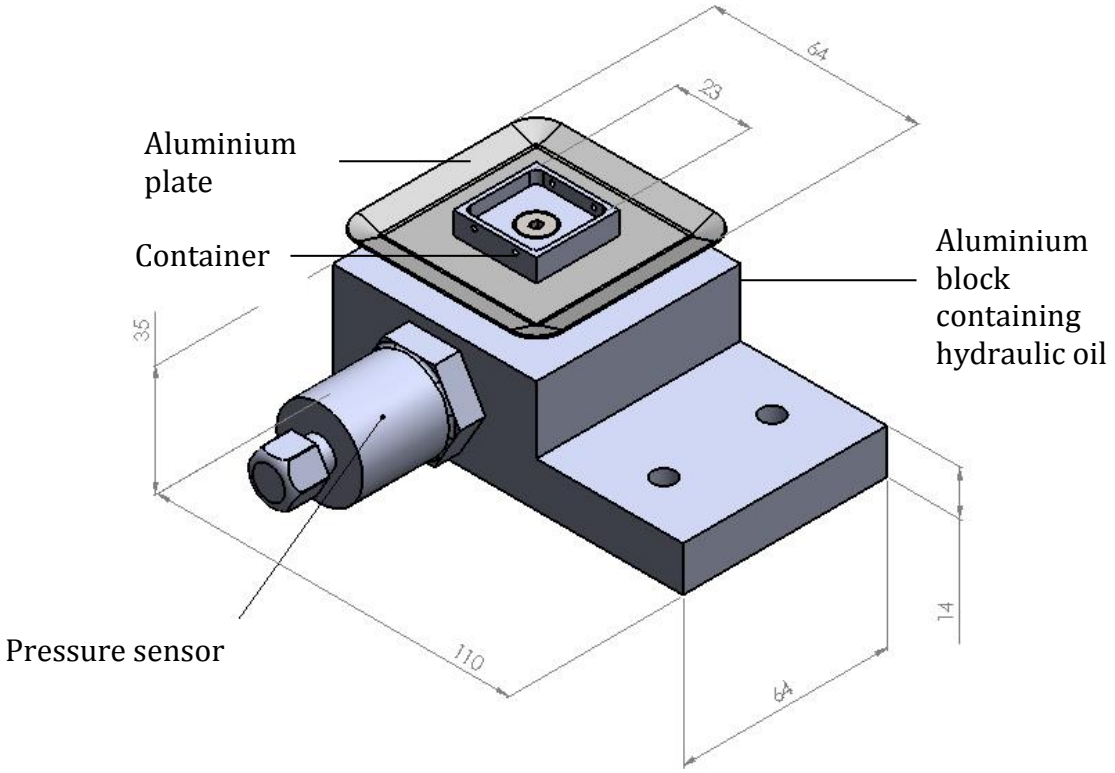

Figure 3.23. The hydraulic pressure sensor attached to the aluminium block containing hydraulic oil.

Six different weights ranging from 0 to 1.827 kg were placed on top of the plate and the IFMR was run with 50 "hits" for each weight without the hitting mechanism being connected. The readings were averaged and the load (BCD) was plotted against the force (N) to produce a calibration curve with R^2 of 0.9978 (Figure 3.24). The equation was then put into the VB command to allow the pressure reading in terms of BCD values to be automatically converted to newtons.

To test the response of the hydraulic pressure sensor, the IFMR was run using the spring and delay units of 1, 5, 10 and 25, and then increases of 25 units up to 150. The maximum force for each delay unit was very similar (Figure 3.25) and demonstrated the hydraulic pressure sensor could handle the output data, even at the fastest speeds.

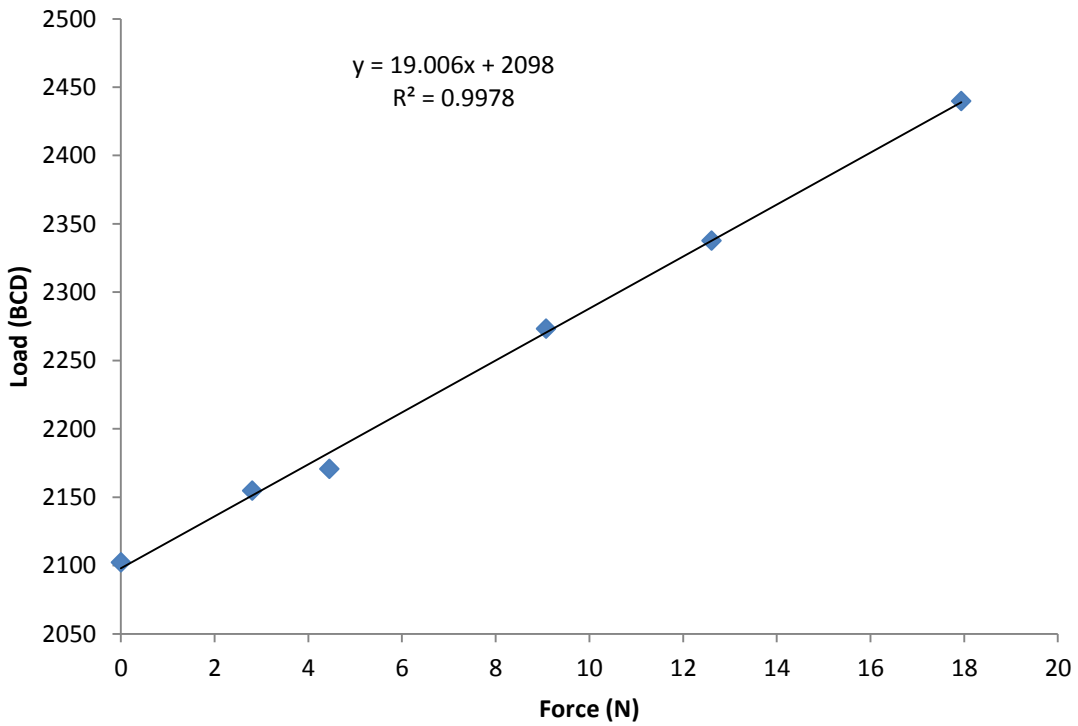

Figure 3.24. Calibration curve for relationship between reading from pressure sensor in BCD and Force (N).

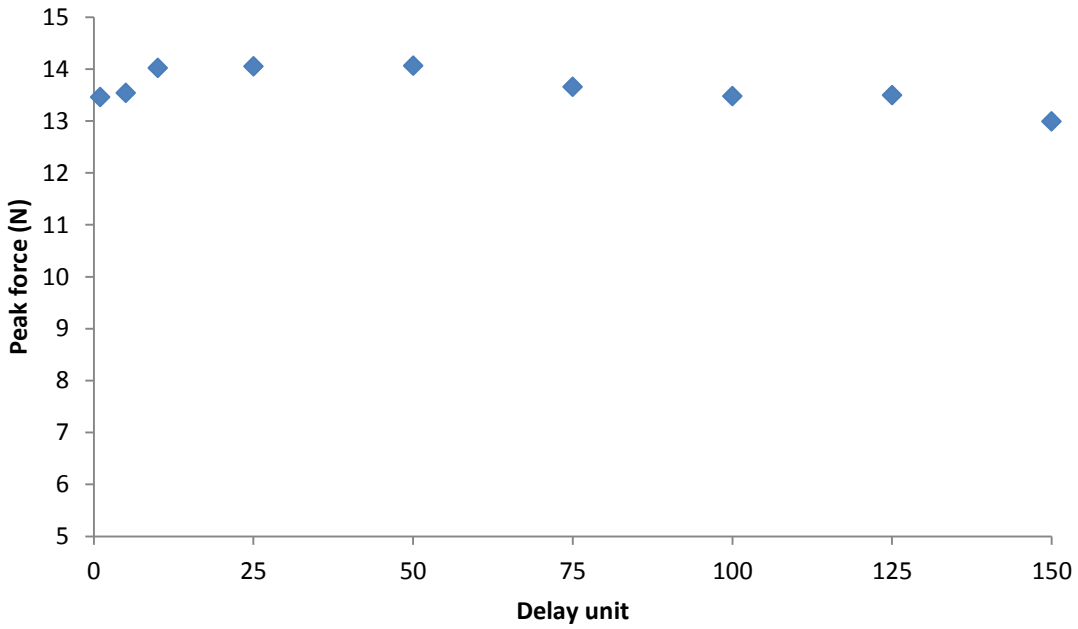

Figure 3.25. The peak force (N) against number of delay units during hits on spring.

3.9. Tests of IFMR performance using meat samples

Meat cubes were prepared from beef *semitendinosus* (eye of round) muscle bought from Taylor Preston Limited (Palmerston North, New Zealand). The whole muscles generally were cylindrical and weighed between 1.5 to 2.0 kg. The 20 mm cubes were cut with fibre direction parallel to the cutting point and excluded the external epimysial layer. Approximately 60-70 meat cubes were produced from a single muscle depending on shape of the muscle and amount of connective tissue. The meat cubes were kept in a closed container in the -20 °C freezer until used. Prior to every analysis, the meat cubes were thawed in a 4 °C chiller. For each test, a meat cube was placed with fibres perpendicular to the HH in the aluminium container (Figure 3.1B). Four pins (5 mm long) were placed through small holes drilled at the side of the container and pushed into the meat. The HH was adjusted using the moveable screw to give a 1 mm gap measured with a feeler gauge, between the HH and the meat sample. The stepper motor was set at step zero.

Different numbers of hits were tested up to a maximum of 2000 hits. No problems occurred during the hitting process. However, the stepper motor had to be brought back to the initial starting point (step zero) every time a new set of hits was done to ensure the HH travelled the same distance for every treatment.

The delay unit was set up in DOS to control the time to complete a hit. As every step taken has a unique time (Figures 3.5 and 3.6), the average time per step (ms) was calculated and plotted against the delay unit set (Figure 3.26) to express delay unit in ms. This meant, the time to complete a hit could be pre-determined before running the IFMR. A second unit, called the gap unit was also set up in the VB program to control the gap between each hit. The gap unit is from the end of a hit to the start of the next hit and allowed the meat to bounce back after every hit before taking another hit. The relationship of average gap time between hits and gap unit value is shown in Figure 3.27. The gap time needed will depend on the meat characteristics such as level of thawing, meat hardness, meat size, fibre orientation and many others features.

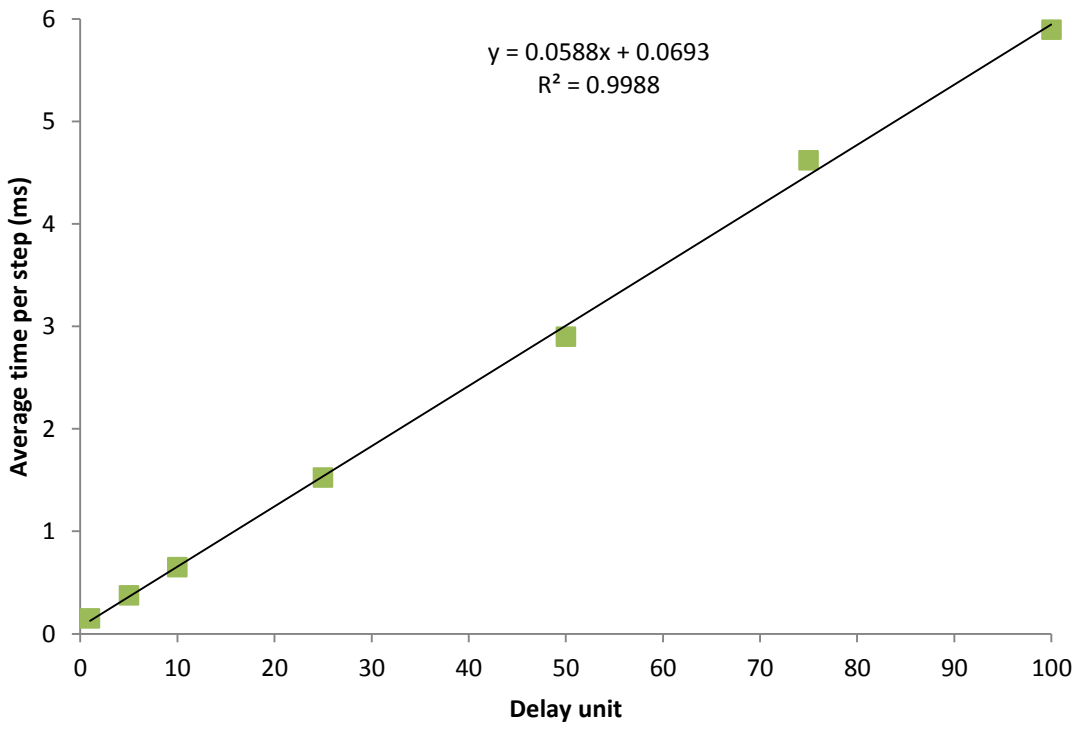

Figure 3.26. Average time per step (400 steps) against delay unit value as set up in DOS.

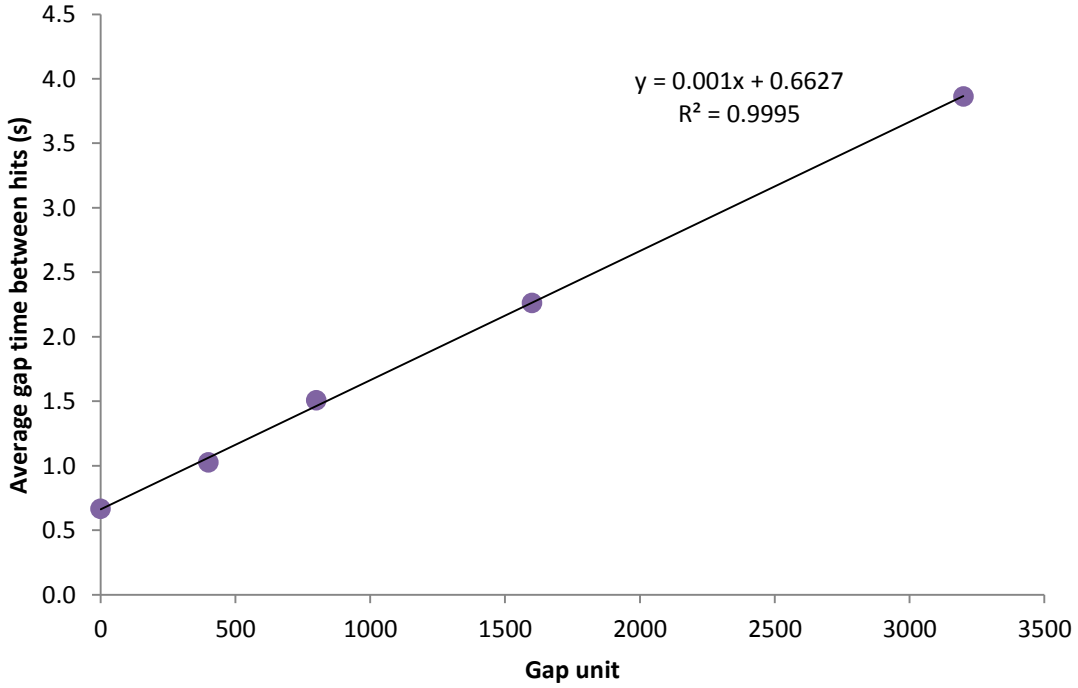

Figure 3.27. Average gap time (s) between each hit with the gap unit set in VB command.

A meat sample was treated with a series of hits set at a 10 N target force with average step time of 0.15 ms and an average gap time of 0.665 sec. The force and distance for one hit (Figure 3.28) show the potentiometer and pressure sensor were functioning well. The total work done on the meat for the same hit, calculated automatically from the force and distance recorded was 0.0066 J (Figure 3.29). The information from the work calculated for each hit in a series of hits is totalled up to give total work.

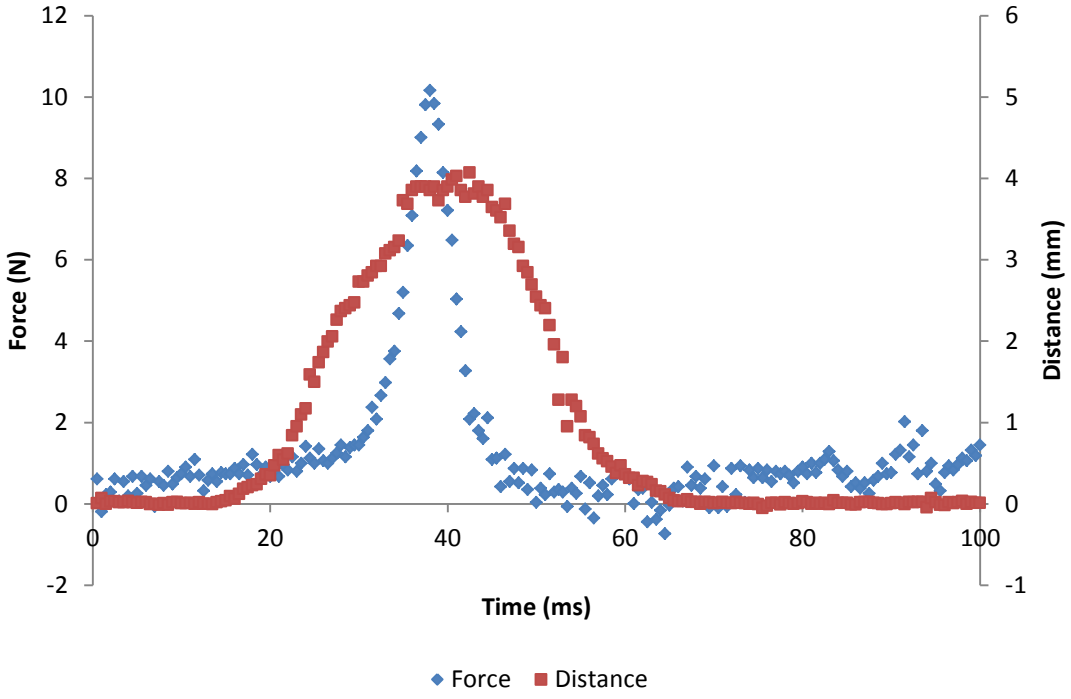

Figure 3.28. Force and distance travelled from a single hit on a meat sample hit with 10 N target force with average step time of 0.15 ms and average gap time of 0.665 sec

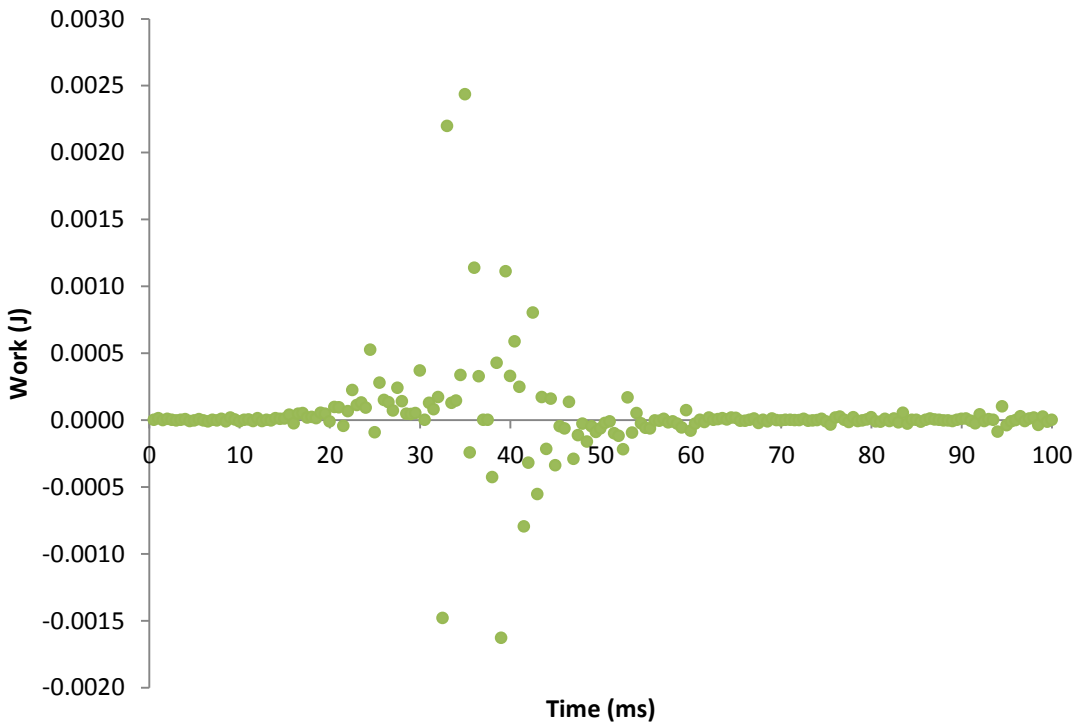

Figure 3.29. Work (J) done on meat sample calculated from a single hit as depicted in Figure 3.28. Total work was 0.0066 J.

Two basic analyses were carried out to test the two different methods of hitting a sample by the IFMR. The first analysis used the fixed force method on a meat sample. The 10 N target force was obtained using the calibration curve in Figure 3.24. The IFMR was run for 100 hits on the meat sample using the fastest average time per step and the average gap time between hits of 0.15 ms and 0.665 sec, respectively.

Figure 3.30 shows the pattern of maximum force per hit plotted against hit number. The maximum force per hit for hit numbers 1 to 35 was increased until it reached 10 N. The initial number of steps was set low at 100 steps. The number of hits needed to achieve the targeted force could be reduced by increasing the initial maximum step number (S_N).

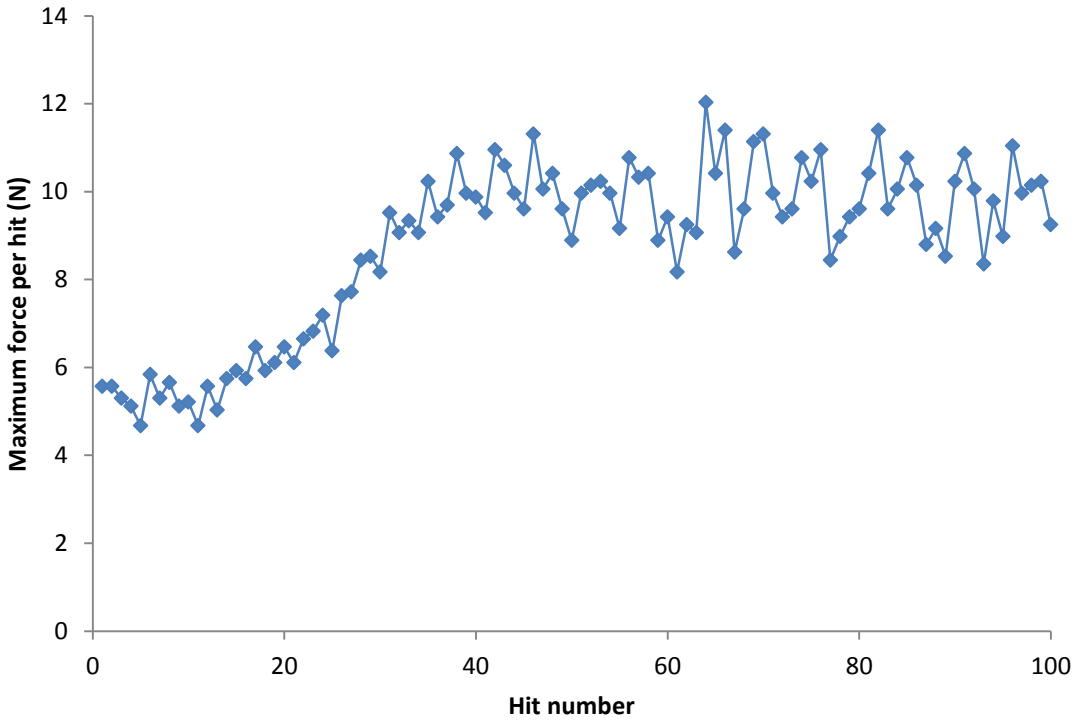

Figure 3.30. Pattern of maximum force recorded for 100 cycles of hitting with fixed force at 10 N on meat sample. The initial step number (S_N) was set up at 100 steps and the increase/decrease of step number was set up at 1 unit.

When the load cell measure of the maximum force was lower than the targeted force, the stepper motor increased 1 step for the next hit. This procedure was continued until the load cell read the target force of 10 N. When the maximum force was more than the target force, the number of steps was reduced by 1 step for the next hit. The stepper motor would automatically re-increase 1 step after that because the force reading was lower than the targeted force. This change in the number of steps produced the wave-like pattern of maximum force from hit number 36 until 100 (Figure 3.30). The wave-like pattern might also be due to characteristics of the meat being hit. The step number to be increased or decreased between adjacent hits could be adjusted as required for a run. The total work calculated was 0.460 J for the 100 hits.

The second analysis used the fixed distance method, where distance setting was determined using the equation in Figure 3.9. A fixed 200 steps was chosen for the test, which is equivalent to 8.46 mm with additional 1 mm gap between the HH and the meat. This procedure was carried out for 100 hits on a meat sample.

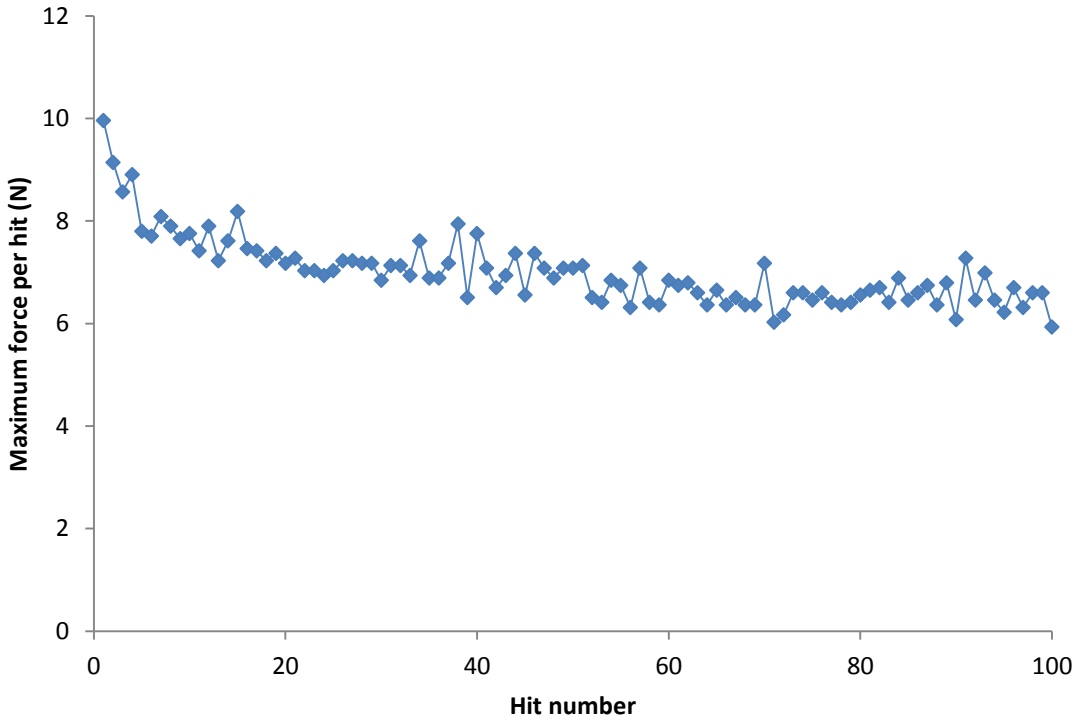

Figure 3.31. Pattern of maximum force recorded for 200 cycles of hitting the meat with a setting of fixed distance on the meat cube rather than to a fixed maximum force. The initial step number was fixed at 200 steps.

Figure 3.31 shows the pattern of maximum force against hit number. The first two hits produced a higher maximum force (9.95 N and 9.14 N) compared to the rest. Maximum force recorded then started to reduce with number of hits. This phenomenon was due to changes in the meat structure. The early phase was when the meat structure was still firm and high forces were needed to break down the structure. In the later phases, the meat structure started softening and the maximum force reduced. The distance between the HH and the meat sample became larger as the number of hits increased. The total work for the 100 hits was calculated to be 0.277 J.

3.10. Conclusions

The IFMR successfully recorded the force involved when a meat sample is impacted, and the work done could be estimated using the fixed-maximum-force method. A fixed maximum distance method was also developed. These two methods produced different patterns of change in force and work with increasing hit number up to 100. The

IFMR was successfully able to hit the sample with various ranges of maximum force and the number of hits per run could be changed, along with the time taken to complete a hit and the gap time between hits. The IFMR could be further improved with more parameters but the current version can be used to analyse the effect of various factors on extraction of proteins from the meat and the binding strength between two pieces of meat.

CHAPTER 4

Comparison of methods for harvesting exudate from the meat surface

4.1. Introduction

The mechanical work applied to meat pieces during tumbling or massaging with the addition of salt and phosphates breaks down the structure of the meat and extracts proteins to the meat surface (Theno et al., 1978b). The amount of protein extracted is usually related to the total work (due to length and speed of tumbling) applied to the meat pieces and concentration of added salt and phosphates (Theno, Siegel, & Schmidt, 1978a). Myosin in the proteins extracted, will influence binding of meat pieces (Macfarlane et al., 1977; Siegel & Schmidt, 1979). Therefore, data on work applied to meat, and its relationship to the amount of total protein and myosin extracted, is very important.

The IFMR developed (Chapter 3) can deliver impact forces to the meat with the desired parameters and the total work done can be calculated. A standard method for collecting protein exudate from the meat surface is needed so the total protein and myosin extracted from the meat can be measured. Therefore, the objectives of this chapter are to:

1) test the capability of an existing washing method for collecting protein exudate from the meat surface and then determine the amount of total protein and myosin.
2) develop a new scraping method to collect protein exudate and to compare its effectiveness with the existing method.

4.2. Preparation of meat cubes

The meat cubes were prepared as described in Section 3.9.

4.3. Meat soaking

The brine for soaking was prepared using a formula provided by Abbott Deane NZ Limited, and consisted of 1.5% sodium chloride (NaCl), and 0.35% phosphate (containing 75.11% sodium tripolyphosphate (STPP), 15.86% tetrasodium pyrophosphate (TSPP) and 9.03% sodium hexametaphosphate (SHMP)). Individually weighed meat cubes were placed in a known position in a plastic container. The brine was poured into the container

to give a ratio of 1.5:1 of brine to meat cubes, and ensuring that the brine covered all the meat cubes. The container was kept at 4 °C for 72 hours prior to being used in the hitting experiments.

4.4. Meat hitting

The meat cube was removed from the soaking container and placed on a paper towel for one minute before the hitting treatment. The meat was then weighed and placed inside the container under the hitting head (HH). Four pins were skewered through the container to hold the meat in place. The HH was adjusted to be positioned 1 mm above the meat cube by using a feeler gauge. The meat was hit a specified number of times at a fixed force level of 10 N with an average time of 0.15 ms per step, and 0.665 sec between each hit (see Section 3.9). The force and displacement (distance) for each hit was recorded and the work was calculated automatically.

4.5. Collecting and analysing protein from meat

This washing and the scraping methods for collecting protein exudate from the treated meat surface were compared. The total protein and myosin in the protein exudate was determined by using the Bradford method for protein and by electrophoresis, respectively.

4.5.1. Washing methods

4.5.1.1. Short term washing

The washing methods described by Itema-Sancoueze (2012) were used. Exudate from the hit-treated meat (approximately 20 x 20 x 20 mm cubes of beef semitendinosus muscle) was collected by washing the meat surface with 5.8% NaCl. The meat piece was placed in a 23 x 23 x 50 mm deep stainless mesh container while being washed (Figure 4.1). A 50-mL beaker was filled with 15 mL of 5.8% salt solution and a magnetic stirrer.

The surface area of the hit-treated meat was measured and then placed face down inside the mesh container. Only the bottom 2 mm of the meat cube could be washed by the salt solution. The salt solution was stirred for 10 min and was then collected and stored in a 15-mL screw cap plastic container at -70 °C to preserve the samples until being assayed for total protein and myosin.

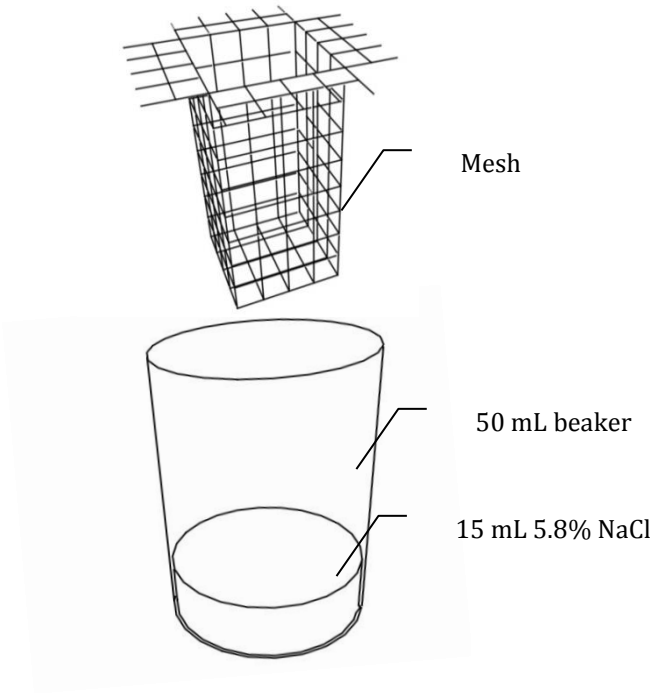

Figure 4.1. The mesh containing the meat cube was inserted into the 50 mL beaker containing 15 mL 5.8% salt solution.

4.5.1.2. Long term washing

The same method as described in Section 4.5.1.1 was used but washing was extended to 48 hours. A 1-mL sample was sampled at 3, 6, 9, 24 and 48 hour and replaced with an equal value of 5.8% NaCl. Result was reported as total protein extracted from the meat sample by multiplying the protein in the 1-mL sample by 15. The dilution effect of adding fresh sample solution was calculated using Equation 4.1. Any additional volume coming from the meat or being absorbed by the meat, during the washing process was not taken into account.

Equation 4.1:

$$Total\ Protein\ (mg/mm^2) = \frac{a + b}{Washed\ area\ (mm^2)}$$

where, a is the protein (mg) measured in 15 mL washing solution at the current measurement time and b is the sum of the amounts of protein (mg) in each of the previous 1-mL samples that had been taken. The washing solutions were stored in 15-mL screw cap plastic container at -70 °C until analysed for protein and myosin content.

4.5.2. Scraping methods

Protein exudate from the hit meat was scraped from the meat surface using the back of a No 22 scalpel blade that had been ground straight. The blade was placed at the edge of the meat cube surface at an angle of about 60° with the scraping direction. The exudate scraped off the surface, was washed in 1-mL of 5.8% NaCl solution inside a 15-mL screw cap test tube by stirring for 10 sec with a vortex mixer. The scraping was done carefully to avoid cutting meat from the meat cube. The scraping method was repeated a further four times on the same meat surface but only washed after the last scrape. Transferring the exudate from the blade to the solution in the screw cap test tube was successfully carried out with no visible exudate remaining on the blade after completing the vortex process. Protein collected was frozen at -70 °C before analysis for protein and myosin.

4.5.3. Bradford protein assay

The Bradford protein assay (Bradford, 1976) was used to determine the amount of protein in the scraped or washed-off exudate. One part of Protein Assay Dye Reagent Concentrate (catalogue number 500-0006), (Bio-Rad Laboratories, Hercules, CA) containing dye, phosphoric acid and methanol was diluted with four parts Milli-Q water. The solution was filtered through Whatman #1 filter paper to remove particulates and could be used for up to 2 weeks at room temperature.

The scraped exudate solution was first thawed in a 4 °C chiller, and then centrifuged at 13,000 g for 2 minutes. Then 0.1 mL of supernatant was diluted with 0.9

mL water (10 times dilution) and then 0.1 mL combined with 5 mL diluted dye reagent, stirred for 30 sec on a vortex mixer, and allowed to settle for 15 min. The solution then was transferred to a 1-cm disposable plastic cuvette and the absorbance read at 595 nm using a spectrophotometer (SPECTROstar Nano, BMG LABTECH, Germany). If the reading exceeded 1000 μg/mL, the sample was diluted to ensure linearity of the standard curve.

The calibration curve was obtained by 200 to 900 μg/mL bovine serum albumin (BSA) (Sigma Aldrich, St Louis, MO). A BSA standard was prepared by weighing 100 mg of lyophilized BSA into a beaker and adding 99.9 g of Milli-Q water. The solution was mixed to dissolve BSA and then divided into small aliquots of 1.5 mL that were kept at -70 °C. The total protein calculated allowed for any additional volume from the scraped exudate. The volume of exudate collected was determined by measuring the differences of the solution before and after washing the blade. The total protein obtained from the spectrophotometer reading was converted to total protein (mg/mL) using the standard curve and the dilution factor over the area of scraping using Equation 4.2.

Equation 4.2:

$$Protein\ (mg/mm^2) = \frac{\left[\frac{a\ (AU) - b\ (AU)}{m\ (AU/mg)}\right] \times \left[Dilution\ factor\ (\frac{mL}{mL})\right]}{Area\ of\ scraping\ (mm^2)}$$

a= spectrophotometer reading

b= intercept = 0.0235 (from Figure 4.2)

m= slope = 1.0711 (from Figure 4.2)

Dilution factor= (volume of washing solution (mL) + volume of scraped exudates (mL))/0.1

AU= Absorbance Units

4.5.4. Electrophoresis (SDS-PAGE) analysis

The chemicals used for preparing the gel are given in Table 4.1.

Table 4.1. List of chemicals for preparing the gel.

Chemicals	**Brand**
Acrylamide	Sigma Aldrich St Louis, MO
N,N'-Methylene-bis-acrylamide (bis)	Biorad Laboratories, Hercules, CA
Glycerol	Sigma Aldrich St Louis, MO
Tris (hydroxymethyl) aminomethane (Tris)	Biorad Laboratories, Hercules, CA
Sodium dodecyl sulphate (SDS)	Biorad Laboratories, Hercules, CA
Tetramethylethylenediamine (TEMED)	Biorad Laboratories, Japan
Ammonium persulfate	Biorad Laboratories, Hercules, CA
Ethylene diamine tetra acetic acid (EDTA)	Biorad Laboratories, Hercules, CA
Glycine	Sigma Aldrich St Louis, MO

The gel electrophoresis method of Ngapo (1994) with several modifications, was used. The gel to load the sample, consisting of the casting gel and the stacking gel, was cast in a Hoefer Dual Gel Caster (SE 245, Hoefer Scientific Instruments, San Francisco, U.S.A.) with a 1.0 mm thick, 10-well comb. The chemical solutions for the gels were prepared as follows.

The acrylamide/bis solution was prepared using a 100:1 ratio of acrylamide:bis. The concentration of the acrylamide/bis solution was based on the weight per overall volume (w/v) of the formula. Acrylamide/bis with 6.5% concentration was used because it was suitable for separating high molecular weight proteins such as myosin. A stock solution of 6.5% acrylamide/bis was prepared by dissolving 4.0223 g acrylamide and 0.0402 g bis with Milli-Q water in a 25-mL volumetric flask and made to volume. This solution contained 16.25% of acrylamide/bis. Two mL of acrylamide/bis of stock solution contained 0.325 g of acrylamide/bis over 5-mL overall volume equals to6.5% when diluted.

A stock solution of 4.5% acrylamide/bis for the stacking gel was prepared by dissolving 2.7847 g acrylamide and 0.02785 g bis with Milli-Q water in a 25-mL volumetric flask and making to volume to give 11.25% of acrylamide/bis and 1.2 mL (in 0.135 g of acrylamide/bis) was made over 3 mL (w/v) to produce 4.5% in the gel. Both stock solutions were filtered and stored in brown bottles at 4 °C for up for up to a month.

The 2.0 M Tris/glycine stock solution for the casting gel was made by dissolving 3.0286 g of Tris and 5.630 g of glycine in Milli-Q water in a beaker and then adjusting the pH to 8.8 using 1.0 M NaOH. This solution was then transferred to a 50-mL volumetric

flask and made to volume with Milli-Q water. Another set of 2.0 M Tris/glycine stock solution was prepared using a similar method but with 1.0 M HCl instead of NaOH to adjust pH to 6.8. The stock for the 50% glycerol solution was prepared by adding 25 mL of glycerol to a 50 mL volumetric flask and adding Milli-Q water to volume. A stock solution of 2.5% SDS/2.5 mM EDTA was prepared by dissolving 1.25 g SDS and 0.0366 g EDTA in Milli-Q water in a 50-mL volumetric flask and making to volume. The stock 1% TEMED solution was prepared by dissolving 0.1 g of TEMED with Milli-Q water in 10 mL volumetric flask made up to volume and stored at 4 °C. The 10% ammonium persulfate solution was prepared fresh on the gel-making day by dissolving 1 g with Milli-Q water in 10 mL volumetric flask and making to volume.

Different formulas were used for the casting and stacking gels (Tables 4.2 and 4.3). The casting gel was prepared first according to the formula in Table 4.2. Part 1 solutions were mixed properly and bubbles were removed. Then, part 2 solutions were added and mixed properly. The casting gel solution was immediately poured into the gel caster before the gel set. The gel was left to set for 45 to 90 minutes. The stacking gel was then prepared (Table 4.3). Part 1 was prepared first and then part 2 was added. The stacking gel solution was poured on top of the set casting gel and the 10-well comb was placed in the stacking gel to produce 10 wells for samples. The stacking gel was left to set for 45 to 90 minutes.

Table 4.2. Formula for one layer of casting gel.

Part	Solutions	Volume (mL) of gel
Part 1	6.5% Acrylamide/Bis	2.00
	2.0 M Tris/ Glycine (pH 8.8)	1.00
	50% Glycerol	0.50
	2.5% SDS/ 2.5 mM EDTA	0.20
	Milli-Q water	0.90
Part 2	1% TEMED	0.20
	10% Ammonium persulfate	0.20

Table 4.3. Formula for one layer of stacking gel.

Part	Solutions	Volume (mL) of gel
Part 1	4.5% Acrylamide/Bis	1.20
	2.0 M Tris/ Glycine (pH 6.8)	0.60
	50% Glycerol	0.30
	2.5% SDS/ 2.5 mM EDTA	0.12
	Milli-Q water	0.54
Part 2	1% TEMED	0.12
	10% Ammonium persulfate	0.12

4.5.4.1. Sample preparation

Chemical solutions for preparing the samples were prepared as follows. The stock solution for concentrated heating buffer was prepared by dissolving 0.30284 g of Tris (25 mM), 0.56304 g of glycine (75 mM), 0.05844 g of EDTA (2 mM) and 2.0 g of SDS (2%) in a 100-mL volumetric flask with Milli-Q water and made to volume. Stock solution for 0.02 M dithiothreitol (DTT) was prepared by dissolving 0.07713 g DTT with water in a 25-mL volumetric flask and made to volume. The 25% sucrose/0.0025% bromophenol blue solution was prepared by dissolving 25 g sucrose and 0.0025 g bromophenol blue in a 100 mL volumetric flask with Milli-Q water and made to volume.

The sample of protein exudate collected by the scraping method (Section 4.5.2) was mixed with the prepared solution (Table 4.4) in an Eppendorf tube and stirred for 30 sec on a vortex mixer. Then, it was heated in a 50 °C water bath for 20 minutes. The Eppendorf tube was removed from the water bath and 0.1 mL of 25% sucrose/0.0025% bromophenol solution was added. The Eppendorf tube was stirred again for 30 sec by using the vortex mixer. The Eppendorf tube was then centrifuged for 2 minutes at 13,000 g and the sample was ready to be loaded onto the gel.

Table 4.4. Sample preparation formula.

Solutions	Volume (mL)
Concentrated heating buffer	0.02
DTT	0.02
Milli-Q water	0.16
Sample	0.20

4.5.4.2. Gel electrophoresis

The following reagents were prepared for running the gel electrophoresis. A stock solution of reservoir buffer of 200 mM Tris/Glycine, 5% glycerol and 0.1% SDS was prepared by dissolving 6.06 g of Tris, 11.26 g of Glycine, 50 mL glycerol and 1 g SDS with Milli-Q water. The pH of the buffer was adjusted to 8.8 and made to 1000 mL in a volumetric flask. The staining solution was made by preparing 1 g of Coomassie Brilliant Blue, 500 mL of isopropanol and 200 mL of acetic acid in a 2-L volumetric flask. The destaining solution was made by preparing 100 mL isopropanol and 100 mL acetic acid in a 1-L volumetric flask. Both solutions were dissolved with Milli-Q water and made to volume in each volumetric flask. These solutions can be reused up to three times but need to be filtered through Whatman filter paper each time.

A Hoefer Mighty Small II Slab Gel Electrophoresis (SE 250, Hoefer Scientific Instruments, San Francisco, U.S.A.) was used to run the gel electrophoresis. The upper and lower chambers were filled with reservoir buffer. Then, 10 µl of the Precision Plus Protein Standards Unstained (Bio-Rad Laboratories, Hercules, CA) was loaded at the first and tenth well and used as the reference. Ten µl of sample was loaded for each well from the second to the eighth well. The gel was run at 150V with Power Pac Universal (Bio-Rad Laboratories, Hercules, CA) for 45- 90 minutes or until the samples lines reached three quarter of the distance along the gel.

The gel was transferred into a container filled with the staining solution and left on a rocker for 90 minutes. The gel was then rinsed once with destaining solution by adding destaining solution to the gel and leaving it on the rocker for 45 minutes. The used destaining solution was poured out and replaced with Milli-Q water. The gel was then stored in the Milli-Q water before being read by a densitometer (Bio Rad). The reading of the myosin by densitometer was in arbitrary units (AU) and reported as total protein per

unit area of scraped meat surface to account for any dilution factor. For example, the myosin readings were multiplied by 40 (for 10 μL sample injected from 0.4 mL prepared samples), by 2 (for 0.2 mL sample prepared with 0.2 mL of other solutions) and by stock exudates volume divided by 0.2 (for 0.2 mL sample taken from stock exudates, which consisted of 1 mL washing solution + volume of scraped exudate).

4.5.5. Identifying the suitable method for collecting exudate

The first analysis compared the short term and long term washing methods, and then the washing methods were compared with the scraping method for collecting protein exudate for total protein determination. The pre-soaked meat cube was hit for 800 times and the protein exudate was collected from the treated surface using the short term washing method for 10 minutes. The same meat cube was washed for 3, 6, 9, 24 and 48 hours (Section 4.5.1.2). For the scraping method, a meat cube was also hit for 800 times and the protein exudate collected by the scraping method. The scraping method was repeated with five meat samples. Protein exudates were analysed for total protein by using the Bradford method.

The second analysis compared the washing and scraping method in collecting protein exudate for determining myosin. Two batches of meat cubes were hit for 0, 400, 800, 1200, 1600 and 2000 times. The protein exudate was collected from the treated meat surface using the short term washing method (10 minutes) for the first batch, and the scraping method for the second batch. The solution (15 mL) used for washing method was freeze-dried and then made to a 1 mL solution before being analysed for myosin. The electrophoresis method was used to determine the relative amount of myosin in arbitrary unit (AU), in total protein (mg) collected. Data was analysed as collection method from meat cubes treated for different number of hits.

4.6. Results and discussion

The standard curve for the Bradford protein analysis (Figure 4.2) was developed from absorbance at 595 nm of bovine serum albumin (BSA). This standard curve was used to calculate total protein collected from the meat surface.

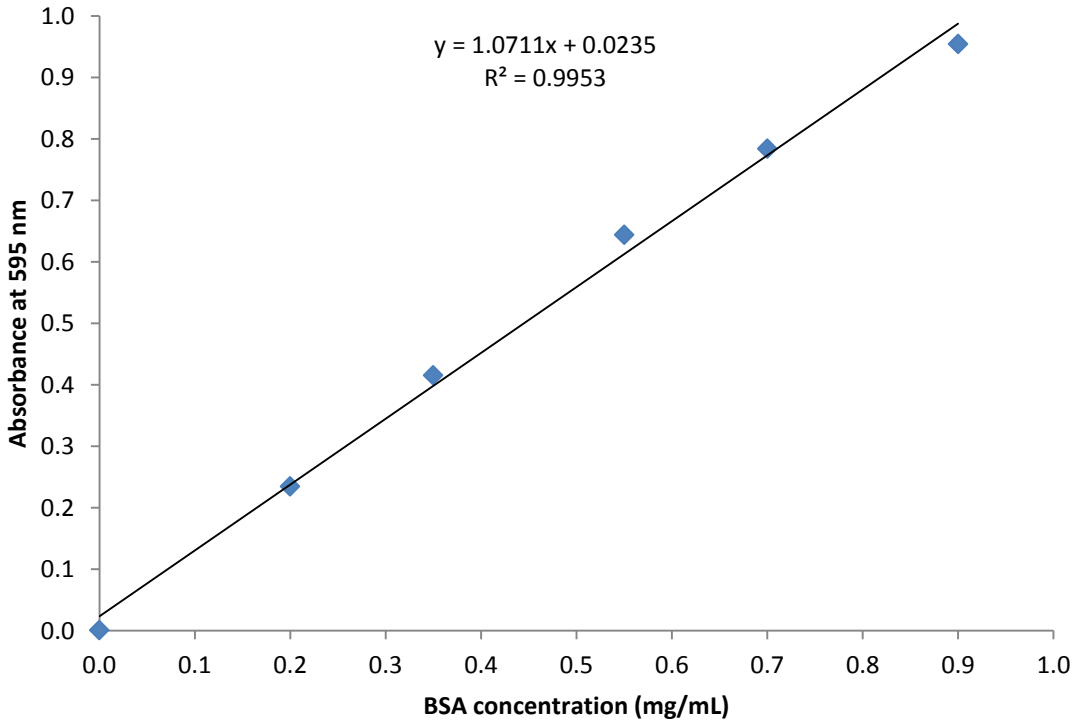

Figure 4.2. Standard curve for the Bradford protein assay.

Hitting the meat for 800 times was chosen as this produced a visible protein exudate on the meat surface but little protein exudate on the sides of the meat cube. Analysis of the difference of short and long term washing method showed that total protein produced decreased exponentially with time of washing with R^2 of 0.968 (Figure 4.3). Total protein per unit area at 10 minutes of washing was 0.0136 mg/mm^2. After washing for 3, 6 and 9 hours, total protein per unit area were 0.0179, 0.0200 and 0.0217 mg/mm^2, respectively.

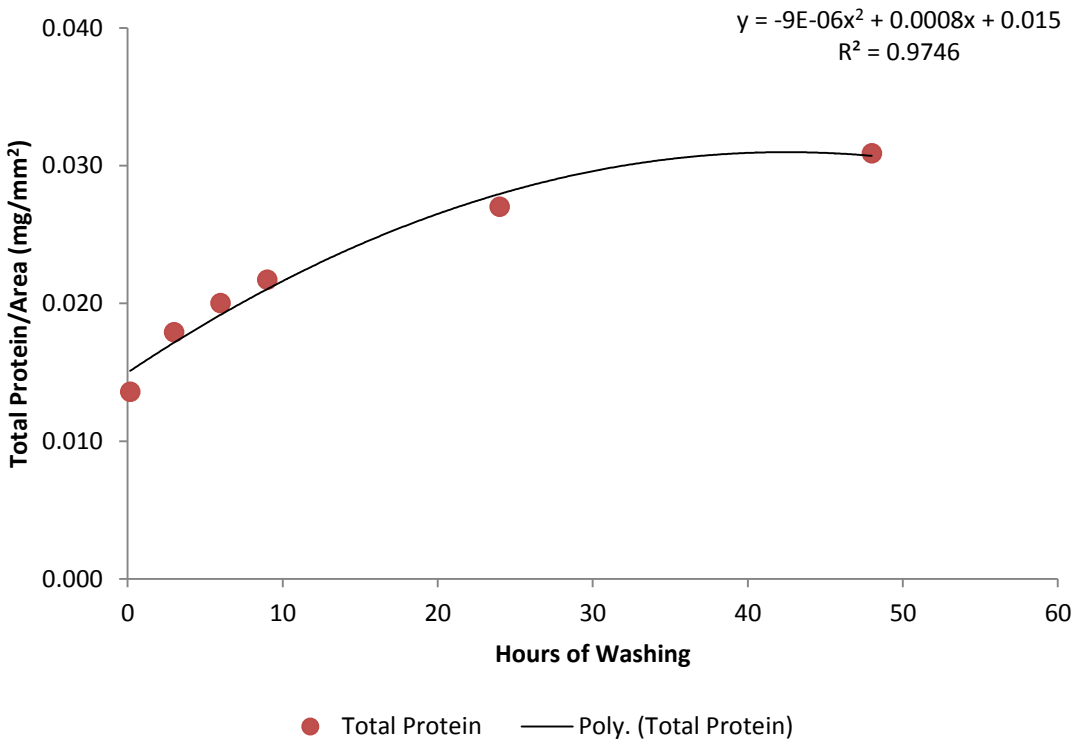

Figure 4.3. Total protein (mg/mm^2) in the exudate collected from meat surface treated with IFMR (800 hits, fixed force at 10 N, average time of 0.15 ms per step, and average of 0.665 sec between each hit) against time of washing (hours).

Extending the washing to 24 or 48 hours increased total protein per unit area to 0.0270 and 0.0309 mg/mm^2, respectively. Surfaces that had been washed for 24 hours or longer were much paler than meat cubes washed for 10 min, 3, 6 or 9 hours indicating that sarcoplasmic and possibly myofibrillar protein proteins had diffused into the washing solution, which had a very high molarity (~1.0M) (Offer & Trinick, 1983). The discoloration of the meat cubes immersed in the washing solution was also more than 2 mm, which also indicated that more proteins had transferred to the washing solution.

Analysis by using the scraping method following an identical hitting treatment produced an average of 0.0120 ± 0.005 mg/mm^2 total protein from five replications. The data show the total protein obtained from the scraping method was comparable to washing for 10 minutes. The scraping method on the other hand only needs about one minute to be performed.

The effectiveness of the two methods for collecting myosin was investigated. Different numbers of hits were applied to demonstrate that the myosin content was based on the method for collecting the protein exudate and not by the total work done on the

meat. There was no myosin in protein exudates collected via 10 min of washing method. Protein exudate was still visible on the surface of the treated meat after washing for 10 min, indicating that its sticky characteristic held the extracted protein to the meat surface and that stirring with a magnetic stirrer did not transfer protein into the washing solution within 10 minutes.

Myosin was detected in samples obtained by the scraping method (Figure 4.4). Lanes 8 and 9 were ignored as they involved different samples. The concentrations of the bands were inconsistent with more hitting resulting in a less concentrated myosin band. This might be because the figure was the first gel used to detect myosin using the scraping method. The scraping method then was improved and standardized to be used to collect the exudate. Nevertheless, this gel indicates that the scraping method could transfer myosin from the meat surface to the solution.

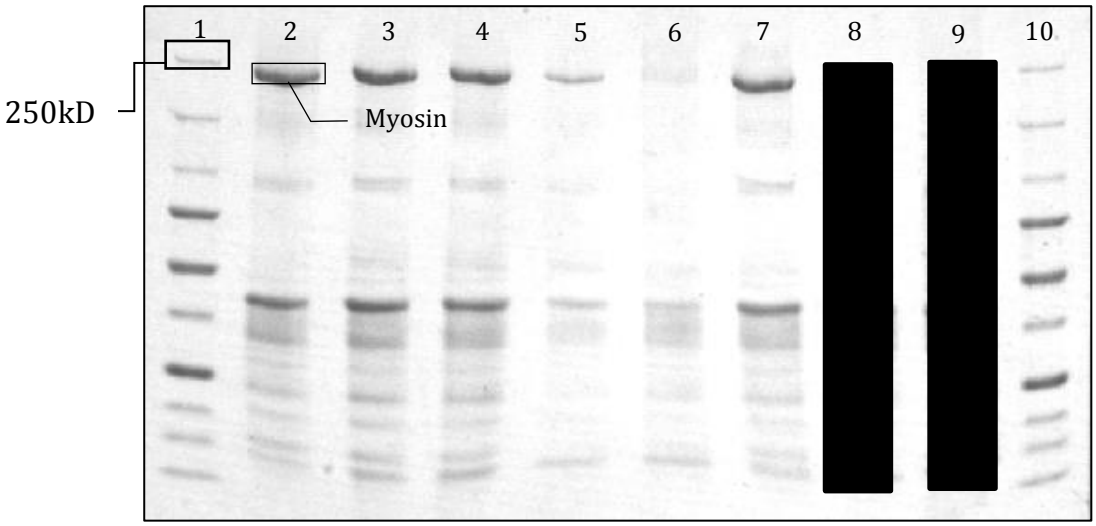

Figure 4.4. Typical gel from SDS-PAGE electrophoresis of scraped exudate from meat samples with different numbers of hits. Lane 1 and 10 were standard with the 250 kD molecular weight for the highest band. Lane 2, 3, 4, 5, 6 and 7 were meat samples treated with 0, 400, 800, 1200, 1600 and 2000 hits, respectively. The highest band for each meat samples lane was the myosin with a molecular weight detected from 190 to 220 kDa.

4.7. Conclusions

Based on the results from both the washing and scraping methods, the latter showed better results and consumed less time to perform when collecting protein exudate for total protein analysis. In addition, the scraping method collected myosin from the meat surface where clear bands of myosin were shown on the gel. In terms of the time taken, the scraping method was better since the scraping took only about one minute. Furthermore, longer washing times could produce inaccurate results as protein could be extracted from other parts of the meat. Therefore, the scraping method was chosen to collect exudate on the treated meat samples for total protein and myosin analysis. The binding strength of meat pieces could be influenced by either the total protein or the myosin in the exudate on the treated meat surface as it is important to be able to measure both protein and myosin.

CHAPTER 5

Cooking and testing protocols for testing binding strength between meat pieces

5.1. Introduction

In the production of reformed meat, the meat pieces after tumbling or massaging are covered with a protein exudate (Theno et al., 1978b). An appropriate weight of tumbled meat is then usually filled into a smoke-permeable fibrous casing, with or without defatted, pre-treated skin. The fibrous casing is then pulled to a specific pressure and clipped. The pressure forces adjacent pieces of extremely pliable meat together. When the meat is cooked to a specific end-point temperature, the mass has a homogenous appearance with barely visible seam lines and enhanced binding strength. The pressure also forces air from the mass so that there are no holes in the pressed product (Hong, Park, Kim, & Min, 2006; Macfarlane, McKenzie, Turner, & Jones, 1984).

Some processing plants still use metal moulds to form the product. This process involves placing a specific weight of product in a mould lined with plastic film. A spring-loaded lid is then used to apply a uniform force to all meat pieces in the mould. The stainless steel moulds are then placed in a water bath and cooked (Dikeman & Devine, 2004). Proteins (mainly myosin) gel and bind the neighbouring meat pieces together and form a reformed meat product. The cooking temperature and pressure applied influence binding strength. To compare different treatments, a standard cooking and pressure protocol is required, along with a method of measuring binding strength of the cooked meat pieces. Therefore, the objectives of this chapter were to develop:

1) A protocol for combining two cubes of meat, prepared as described in Section 3.9, and of cooking the combined cubes.

2) A method for determining the tensile adhesive strength (binding strength) of the combined meat pieces after cooking.

5.2. Meat cooking and adhesion testing

5.2.1. Wrapping the meat cubes

Two thawed untreated meat cubes were placed surface-to-surface with the fibre direction of both meat cubes perpendicular to the junction and then wrapped firmly with a layer of Glad plastic food wrap and rolled and folded 10 times. The plastic wrap was then

sealed at every opening using sticky tape (Sellotape®) to prevent water ingress during cooking. Wrapped samples were kept at 4 °C for 24 hours before cooking.

5.2.2. Mould

A mould was developed to simultaneously retain the meat shape during cooking and guide the weights applied to the meat. This mould, consisted of 16 steel 22x22 mm by 60 mm high tubes attached to a 185x185x2 mm steel plate (Figure 4.2). The weights were made from steel and consisted of Part A and Part B (Figure 4.3). Part A (the base) was a steel cuboid with 21.8 x 21.8 mm x 62.5 mm high with a steel rod at the top (110 mm high and 5 mm diameter) with total weight of 250 g, which functioned to hold additional weights from part B. Part B consisted of steel cylinder with a 5 mm diameter hole through the centre and weighed 250 g and could be added to the rod (Part A) to achieve maximum overall weight of 1000 g.

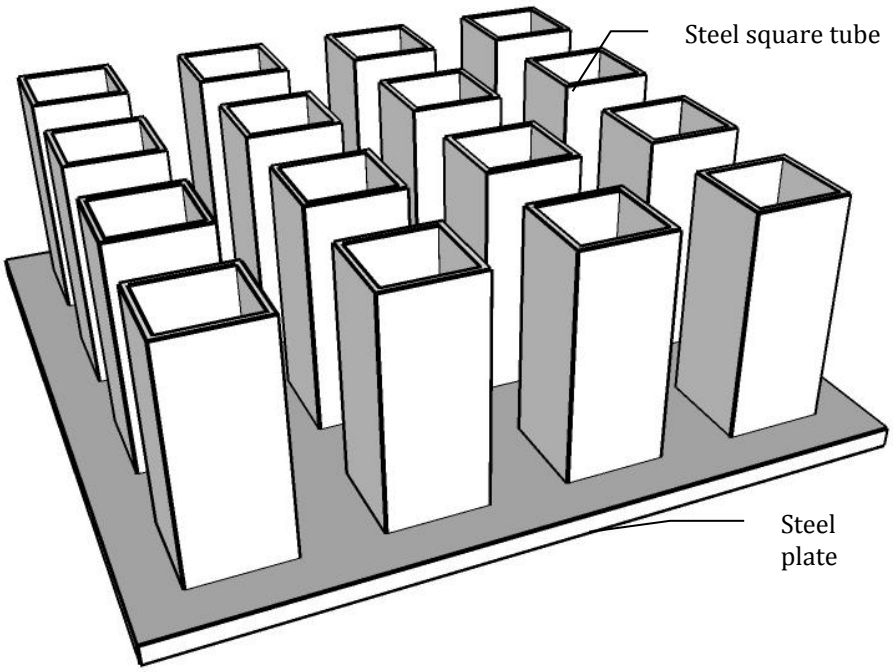

Figure 5.1. The mould made of steel to put meat cubes wrapped in pairs to cook. This mould is immersed in a water bath for cooking.

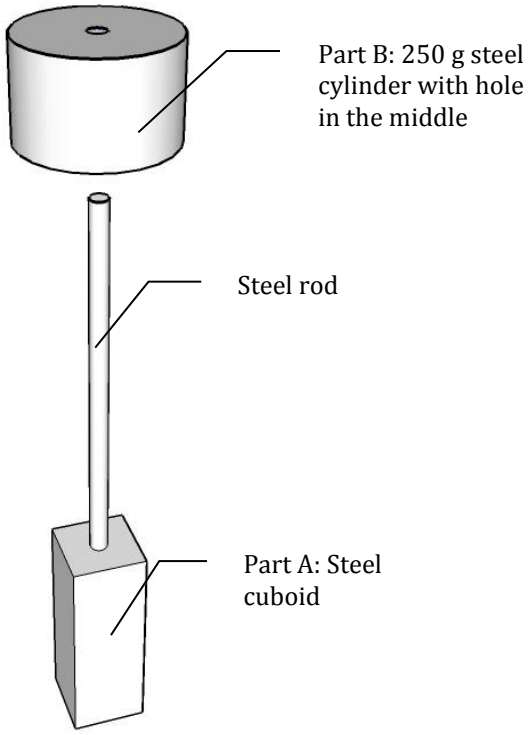

Figure 5.2. The weights for the meat with Part "A" as the base and Part "B" is the additional weight.

5.2.3. Cooking procedure

Meat pieces that had been combined were placed inside the square tubes and weights (0, 250, 500, 750 or 1000 g) added. The mould containing the combined meat pieces was immersed and cooked for 1 hour. The wrapped meat samples were then removed from the mould and placed under cold running water for 10 minutes to cool. The wrap was then removed and the meat samples were stored at 4 °C overnight before the binding strength analysis. Samples procedures were cook at temperatures of 60, 70 and 80 °C.

5.2.4. Tensile adhesive strength (TAS) analysis

Binding strength analysis was done with a texture analyser TA-XT2 (Stable Micro Systems, Surrey, England). Two identical clamps (Figure 5.3) were built to hold the meat pieces. One clamp was screwed to the floor of the texture analyser and the other was bolted to the moveable arm of the texture analyser. Each clamp consists of two plates; the

static plate and the moveable plate connected by a hinge. A square plate was attached 90° to the static plate to hold the meat. Three 9-mm long pins, with 4.9 mm between adjacent pins, were attached 5 mm above the square plate through the sides to spike the meat sample at both ends. The movement of the pulling was 0.1 mm/s. The highest peak was recorded and measured as the tensile adhesive strength (TAS).

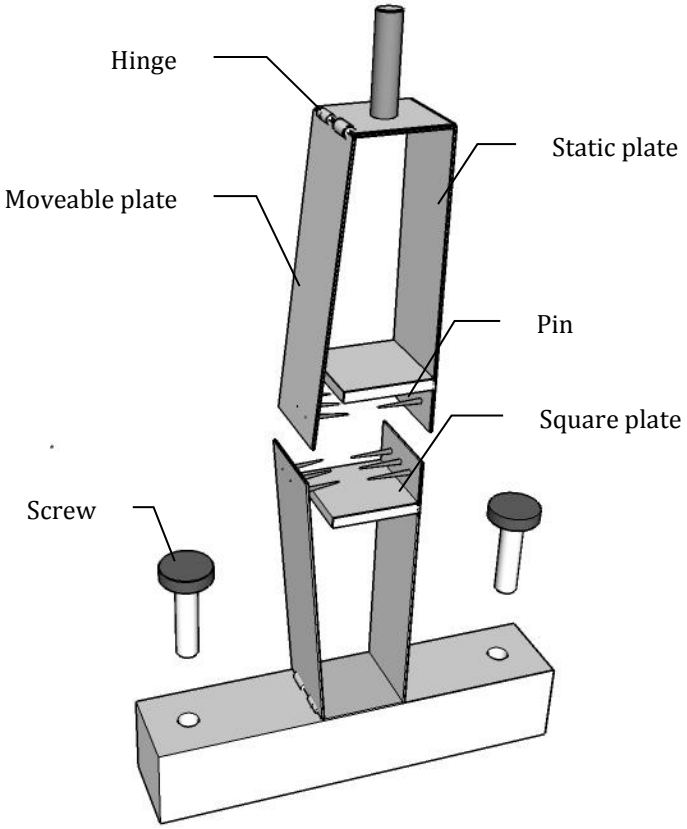

Figure 5.3. Diagram showing clamps used in the TA-TX2 to hold a pair of 20 X 20 X 20 mm meat cubes to measure the tensile adhesive strength.

5.2.5. Experimental design and statistical analysis

A 3x5 factorial experimental design was used to determine the effect of five weights and three cooking temperatures (Table 5.1). The PDIFF option within the SAS 9.2 statistical software (SAS Institute, Carey, NC, USA) was used to analyse differences between cooking temperatures and weights. The raw data are in Appendix 1. The treatment was replicated eight times. The average TAS was obtained by dividing the peak force by the surface area of the meat cube.

5.3. Results and discussion

There was a highly significant temperature effect, with increases noted between 60 °C and 70 °C ($p<0.0001$) and between 70 °C and 80 °C ($p<0.0001$) (Figure 5.4). At 60 °C, myosin is the main protein for binding the meat pieces but it is still not fully aggregated and has not formed a complete network. At 70 °C the myosin reaches its strongest gelation (Morita & Yasui, 1991). At 80 °C the collagen component will be undergoing gelation and contributing to the bond (Wu, Dutson, & Smith, 1985). When cooled, the solubilized collagen formed gels and at the same time added more binding strength to the existing myosin gelation.

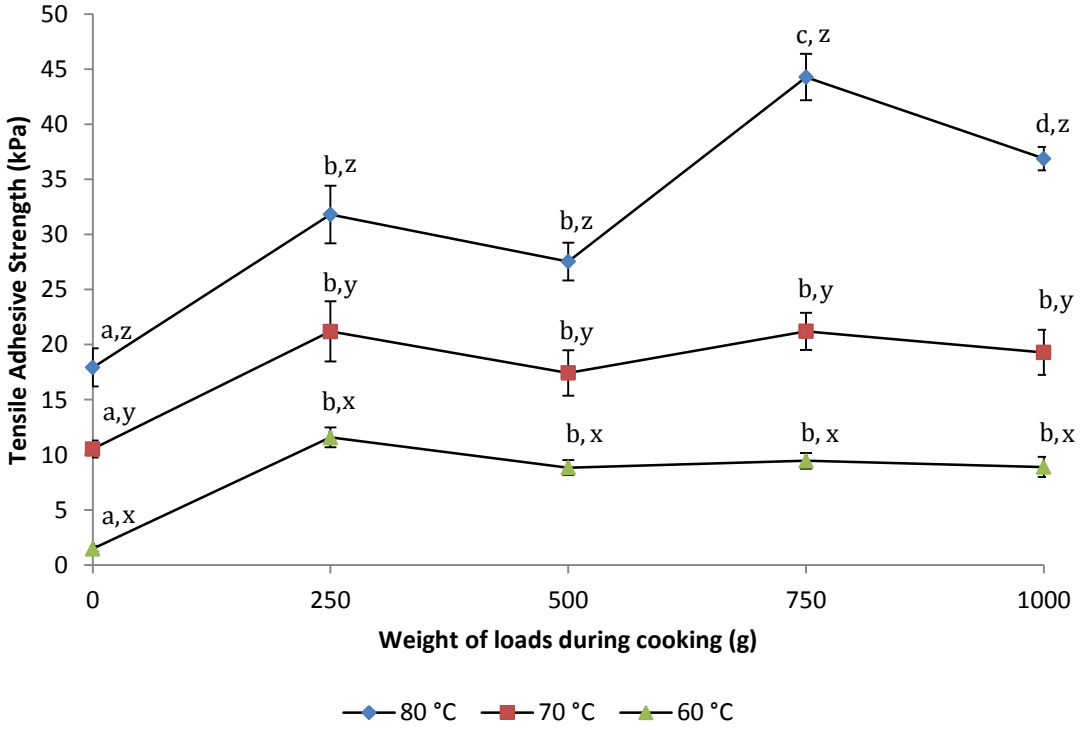

Figure 5.4. Tensile Adhesive Strength for combined pairs of meat cubes for different presser weights and cooking temperatures. Eight replicates were carried out for each condition. Error bars are the standard error, (a, b, c and d are symbols for significance of differences between weights within each temperature and x, y and z are symbols for significance of differences between temperatures at each weight (different letters show significant differences at $P < 0.05$).

The effect of applied cooking load was significant (p values ranged from <0.0001 to 0.0036 for individual loads when compared against the no load condition) at all cooking

temperatures, once sufficient load had been applied to ensure good contact at the joint. The suspected reason was that the meat cubes without applied loads had air pockets between the two meat surfaces, which prevented the protein-protein interactions (Wiebe & Schmidt, 1982). Applying a weight removed the air and the two meat cubes had full contact at the joining surfaces.

Cooking at 80 °C significantly increased TAS ($p<0.0001$) when the weight was increased from 250 or 500 g to 750 g. However, when the load was increased to 1000 g it resulted in a significantly lower ($p=0.0019$) TAS value than the 750 g result (44.3 c.f. 36.9 kPa). It is suggested that increasing the applied weight from 750 to 1000 g at a cooking temperature of 80 °C resulted in the gelatinized collagen being squeezed out of the joint. This collagen was observed as a visible bead around the join before tensile testing. Despite the increased bonding strength at a cooking temperature of 80 °C, cooking of reformed or restructured meat at this temperature is not practical in industry due to higher cooking losses and the added effect of collagen gelatinization, hence 70 °C was chosen as the cooking temperature for the protocol. Since the effect of applied load was not statistically significant at 70 °C, a loading of 250 g was chosen to press the meat cubes together yet minimize leakage of solubilised myosin that may occur due to the applied weight. The 250 g weight is seen as being sufficient to produce a good bond with a firm contact being formed with the gelatinized protein covering the edge of the binding junction.

5.4. Conclusions

Adding weights to the meat cubes during cooking significantly increased TAS compared to meat cubes without any weight applied within the respective cooking temperature groups. Increasing cooking temperatures significantly increased TAS irrespective of added weights. Good bind strengths were obtained at cooking temperatures of 70 and 80 °C. Cooking at 80 °C with heavier applied weights resulted in significantly higher TAS compared to 70 °C probably due to the gelation of solubilized collagen. However, cooking at 80 °C is impracticable in an industrial process, so, cooking at 70 °C with an applied weight of 250 g was chosen for the standard method.

With both the method of collecting the exudate for protein analysis, and the method for cooking and TAS measurement developed, further analyses were carried out to relate the total work applied to meat by the IFMR against the amount of protein and myosin extracted and the binding strength of two attached meat pieces.

CHAPTER 6

Effects of mechanical work and pre-soaking on exudate and binding strength of meat pieces

6.1. Introduction

In Chapter 4, the method for collecting protein exudate from the meat surface so total protein and myosin could be measured was described. In Chapter 5, the cooking protocol and method to measure binding strength between two cooked cubes of meat was described. In this chapter, the effect of work applied to a meat cube and different pre-soaking conditions on total protein and myosin in the exudate on the meat surface will be reported. The relationship between work done on two cubes of meat and binding strength of the resulting join will also be determined.

The hypothesis being tested is that increased work via impacts on meat that has been soaked in brine will extract different amounts of total protein and myosin. The protein and myosin in the exudate on the meat surface will influence the binding strength of meat pieces held together during cooking. Therefore, the objectives of this chapter are to determine:

1) the effect of brine concentrations (ionic strength) on the soaking gain and also the effects of ionic strengths and total work applied to the meat on hitting loss, cooking loss and firmness of the meat.
2) the amount of protein and myosin in the exudate on the surface of meat cubes pre-soaked with brine concentrations and treated with different total work done.
3) the tensile adhesive strength (TAS) of attached meat pieces pre-treated with brine concentrations and subjected to different total work.
4) the effect of the protein and myosin amount in the exudate collected on the treated meat surface on the tensile adhesive strength of the combined meat cubes.

6.2. Materials and methods

6.2.1. Meat soaking

Three brine formulations with varying concentrations of salt and phosphate were prepared (Table 6.1). Brine SP0.396 was prepared using a formulation provided by Abbott Dean NZ Limited*. Brines SP0.713 and SP1.146 were based on brine SP0.396 where the concentration of salt was used as the primary factor and the concentration of phosphate relative to salt was kept constant at 0.23. Three different types of phosphates sodium tripolyphosphate (STPP), tetrasodium pyrophosphate (TSPP) and sodium

hexametaphosphate (SHMP) were mixed together in the same proportion as the Abbott Deane NZ Limited brine formulation. The ionic strength represents the sum of molarity of salt and phosphates in the brine and was calculated using Equation 6.1.

Equation 6.1 (Freiser & Freiser, 1992)

$$I = \frac{1}{2}\sum C_i z_i^2$$

where C_i is a molar concentration of i^{th} ion present in the solution and z_i is its charge.

Table 6.1. Formulations used for making the brine consisting of salt (NaCl) and phosphates and the brine ionic strength.

Brine	NaCl (% w/v)	Phosphate (% w/v)	STPP[1] (% of total phosphate)	TSPP[2] (% of total phosphate)	SHMP[3] (% of total phosphate)	Ionic strength of brine (mol/L)[4]
SP0.396	1.5	0.35	75.11	15.86	9.03	0.396
SP0.713	3.5	0.82	75.11	15.86	9.03	0.713
SP1.146	5.8	1.35	75.11	15.86	9.03	1.146

[1] sodium tripolyphosphate
[2] tetrasodium pyrophosphate
[3] sodium hexametaphosphate
[4] the ionic strength concentration was the sum of the salt and all the phosphates

*The formula was acquired from the production of reformed meat by Abbott Deane NZ Limited. There was no official document on this formula.

Meat cubes were soaked in brine SP0.396, SP0.713 or SP1.146 (Section 4.3). Throughout this chapter, meat cubes soaked in brine SP0.396, SP0.713 or SP1.146 are referred to by the ionic strength of the brine used, as 0.396, 0.713 or 1.146 (mol/L) respectively. The non-soaked meat control is termed as 0 mol/L.

6.2.2. Meat hitting

The hitting method (Section 4.4) was applied to meat cubes for zero, 400, 800, 1200, 1600 or 2000 hits. The IFMR was programmed to produce hits with a constant force of 10 N, an average 0.15 ms per step and an average of 0.665 sec between each hit. The hitting rate was different for each sample. For example, meat cubes hit with 200 steps down and 200 steps return took approximately 0.06 sec for each hit. One minute of hitting time, involved hitting the meat cube about 82 times (including the gap time between hits). The number of hits in one minute could vary because meat toughness would decrease as number of hits increased, which subsequently increased the number of steps required to reach the pre-set force. The work from each hit was calculated. The total work was the sum of work for the individual hits (Section 3.6).

6.2.3. Weight gain after soaking

The weight gain from soaking in brine was calculated using Equation 6.2:

$$Soaking\ gain\ (\%) = \frac{Weight\ after\ soaking - Weight\ before\ soaking}{Weight\ before\ soaking} \times 100$$

6.2.4. Hitting loss measurement

The weight loss after hitting was calculated using Equation 6.3:

$$Hitting\ loss\ (\%) = \frac{Weight\ before\ hitting - Weight\ after\ hitting}{Weight\ before\ hitting} \times 100$$

6.2.5. Cooking loss measurement

The cooking loss was calculated using Equation 6.4:

$$Cooking\ loss\ (\%) = \frac{Weight\ before\ cooking - Weight\ after\ cooking}{Weight\ before\ cooking} \times 100$$

6.2.6. Firmness

Firmness (often called shear value) of the meat cubes was measured by applying a TA-42 knife blade with a sharp 45° chisel end to the cooked meat cube to cut the cube across the meat fibre direction at 0.5 mm/sec. Firmness was calculated as peak force per unit area (N/m^2) (Lyon & Lyon, 1998; Taiwo & Baik, 2007).

6.2.7. Measuring TAS of intact meat

The strength of intact (un-cut) meat was determined by cutting 2 x 2 x 4 cm samples beef semitendinosus muscle meat fibres was aligned with the long edge. Samples were pre-soaked in brine SP0.396, SP0.713 or SP1.146 for 72 hours. The control was unsoaked meat. All samples were cooked at 70 °C for 1 hour and immediately stored at 4 °C for 24 hours before the TAS analysis was done. The experiment was replicated three times.

6.2.8. Total protein and myosin analysis of hit-treated meat cubes and tensile adhesive strength of the junction of attached meat cubes

Meat cubes from each hitting treatment with the IFMR were analysed for total protein and myosin in the exudate on the hit surface and for the TAS. The exudate on meat surfaces of samples given identical treatments to samples used for measuring the adhesive strength was collected using the scraping method (Section 4.5.2). Total protein was then analysed by Bradford protein assay (Section 4.5.3) and myosin by gel electrophoresis (Section 4.5.4).

To measure the TAS, meat cubes that had been subjected to hitting treatments were combined (Section 5.2.1), and then cooked at 70 °C in the meat moulds with a 250-g weight for 1 hour (Section 5.2.3). The TAS method is given in Section 5.2.4.

6.2.9. Design and statistical analysis

Meat cubes were pre-soaked in brine SP0.396, SP0.713 or SP1.146 or non-soaked and hit for 0, 400, 800, 1200, 1600 or 2000 times (Table 6.2). Two replicates of hitting treatments were done. Exudate collected from one set was kept in 15 mL screw cap plastic containers at -70 °C until analysed for protein and myosin.

Table 6.2. Design of the hitting treatment applied to the meat cubes.

Number of Hits	Control	Brine[1]		
0	Non-soaked	SP0.396	SP0.713	SP1.146
400	Non-soaked	SP0.396	SP0.713	SP1.146
800	Non-soaked	SP0.396	SP0.713	SP1.146
1200	Non-soaked	SP0.396	SP0.713	SP1.146
1600	Non-soaked	SP0.396	SP0.713	SP1.146
2000	Non-soaked	SP0.396	SP0.713	SP1.146

A one-way ANOVA was used to test the Tensile Adhesive Strength (TAS) of intact meat (n=3) compared to attached meat cubes (n=2) with both sample groups being pre-soaked in brine SP0.396, SP0.713, SP1.146 and non-soaked, but no mechanical work was applied. This test was to determine the minimum and maximum TAS of meat that had not had a hitting treatment. A one-way ANOVA was also used to analyse the effect of brine concentrations on TAS of intact meat and attached meat cubes. TAS analysis was carried out with two pairs of attached meat cubes for each of the 6 hit-number treatments for each soaking concentration, which gave duplicate results at each hit-number by soaking-condition combination. For weight gain after soaking and cooking loss analysis, 24 samples (12 pairs) were used for each brine treatment (non-soaked, brine SP0.396, SP0.713 and SP1.146). For firmness analysis, 12 samples were used for each brine treatment.

The effect of ionic strength on weight gain after soaking was analysed using a one-way ANOVA. The hitting loss, cooking loss and firmness were plotted against total work (J) within ionic strengths. The total protein, myosin and TAS were plotted against total work (J) applied to the meat cubes. The TAS was also plotted against the total protein and myosin. These results were analysed statistically with the General Regression routine.

Type 1 sums of squares were used to allow the items in the model to be fitted and tested for significance sequentially. Statistical analyses were done to evaluate quadratic and linear effects for individual or grouped factors. The results were reported either linearly or quadratically based on which gave the lowest p value and highest R^2 value. Statistical analyses were done using Minitab 16 (Minitab Inc., State College, PA, USA) software.

6.3. Results and Discussion

6.3.1. TAS of intact meat

The failure of the intact meat under tension occurred where the meat was held by the pins rather than in the middle of the meat. The failure of joined meat cubes that had been pre-soaked in brine but not hit occurred at the join between the meat cubes (Figure 6.1). The brine concentration for pre-soaking the meat pieces had no significant effect on TAS of jointed meat ($p=0.053$). A similar result was also obtained with the attached meat cubes where no significant difference in TAS ($P=0.58$) between different soaking concentrations. However, the TAS of intact meat was significantly higher than the joined meat cubes ($P<0.001$). These results were used as an indicator of the minimum and maximum expected TAS results for bound meat pieces.

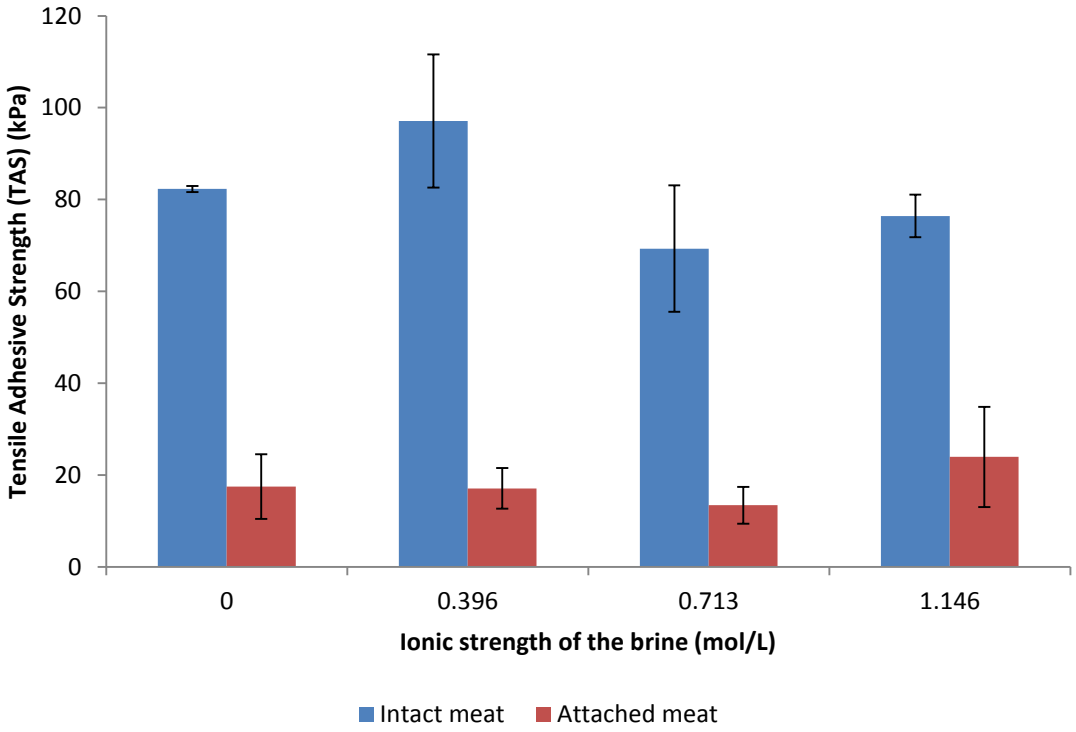

Figure 6.1. The Tensile Adhesive Strength (TAS) intact meat cuboids (n=3) and joined meat cubes (n=2) at each ionic strength of the brine (mol/L). Samples were not treated with any mechanical work.

6.3.2. Effects of brine strength and total work on weight gain, hitting loss, cooking loss and firmness of 20 mm cubes of beef semitendinosus muscle.

Table 6.3. The effect of ionic strength of the brine on weight gain of meat cubes.

Ionic Strength (mol/L)	N	Mean weight gain (%)	Standard Deviation
0.396	24	9.197[a]	5.365
0.713	24	24.392[b]	3.916
1.146	24	31.381[c]	3.717

Meat cubes soaked in brine gained weight (Table 6.3). The higher the ionic strength, the greater the weight gain ($p<0.001$). Salt and phosphates in the brine react with the meat structure and alter the physicochemical properties (Anjaneyulu, Sharma, & Kondaiah, 1989; Lee, Hendricks, & Cornforth, 1998). This is very important, especially in the myofibrils, because most of the water is being held in the meat structure; between the thin and thick filaments, within and between the myofibrils, and between myofibrils and

the sarcolemma (Huff-Lonergan & Lonergan, 2005; Toldra, 2003). Salt expands the space within the myofibrils. For example, no changes occur in the myofibrillar structure of chicken breast fillet for salt concentrations up to 0.5 M (Xiong, 2005) but 0.6 M salt significantly swelled the myofibrils and increased protein extraction. Higher protein solubilisation also affects the degree of swelling of muscle proteins (Stanley, Stone, & Hultin, 1994) with water uptake reaching a maximum for 0.8 M salt.

Tetrasodium pyrophosphate (TSPP) in the brine is the most effective phosphate for separating actomyosin into actin and myosin. Chloride ions from NaCl produce electrostatic repulsions to further push apart the thick and thin filaments, creating larger spaces and thus allowing more water to be absorbed (Offer & Trinick, 1983). For example, adding phosphates, either STPP or TSPP, to 0.5M NaCl swelled myofibrils more than treatment without phosphates. Greater extraction of myosin occurred with 0.6 M NaCl with phosphates (Xiong, 2005).

In this trial, meat cubes were pre-soaked in brine before the hitting treatment. The salt and phosphates swelled the surfaces of the meat cubes and gave average weight gains of 9.2, 24.4 and 31.4% for brine SP0.396, SP0.713 and SP1.146, respectively (Table 6.3). The difference in weight gained between SP0.396 and SP0.713 was bigger than between SP0.713 and SP1.146. Bertram, Kristensen, & Andersen (2004) suggested that the structural constraint is eliminated for lower to medium ionic strength (0.319 and 0.713 mol/L in this study), which allows greater water absorption. The medium ionic strength and higher ionic strength of brines (0.713 and 1.146 mol/L) on the other hand almost reached the saturation of swelling. Therefore, less additional water uptake was recorded.

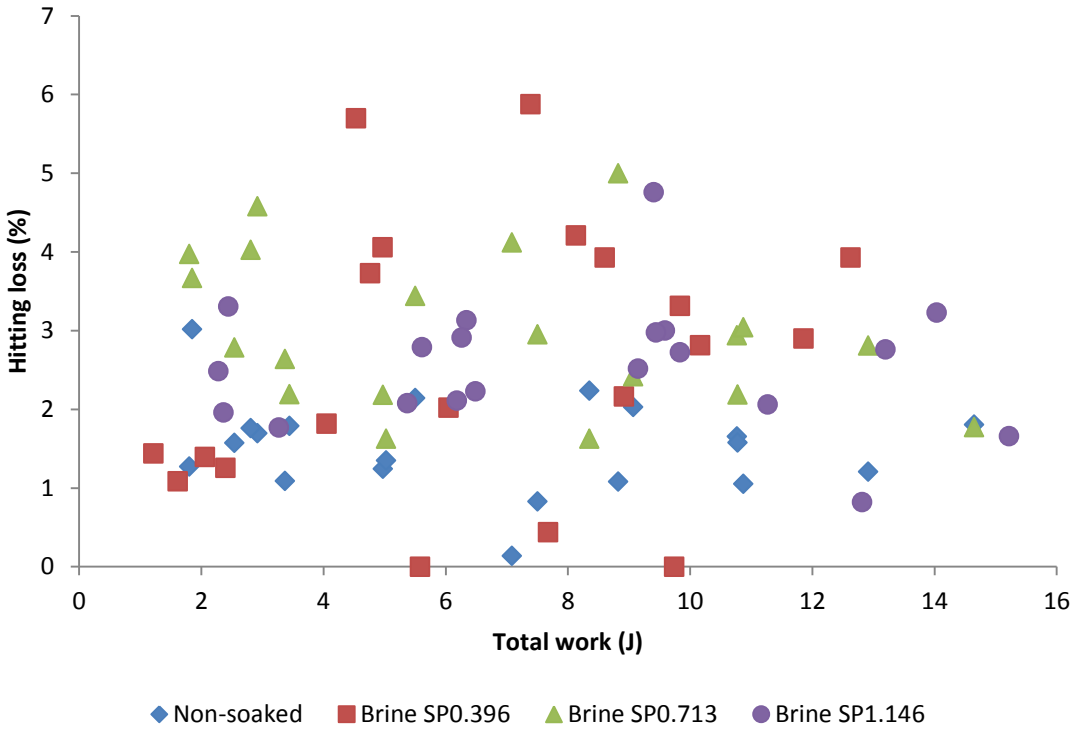

Figure 6.2. The hitting loss (%) of meat cubes measured against the total work (J) following soaking in four concentrations of brine. Control (non-soaked), Brine SP0.396=0.396 mol/L, Brine SP0.713=0.713 mol/L and Brine SP1.146=1.146 mol/L.

Increasing the total work (J) applied to the meat cubes did not affect the hitting loss percentage ($p=0.938$) (Figure 6.2). The hitting processes used in the study did not totally crush the meat. Increasing total work, however, did make the treated meat surface become visibly beaten and compressed but no meat particles were detached from the hit meat cubes.

Spots of liquid on the plate used to hold the meat cube during the hitting process presumably had come from the bottom of the meat cube. The surface of the meat cube did accumulate exudate but this exudate remained on the surface due to its sticky nature. The weighing process following hitting, did not affect the exudate on the meat surface. Therefore, weight loss from the meat pieces was mainly due to the liquid coming from the bottom of the treated meat after hitting.

The ionic strength of the brine used to soak the meat influenced the hitting loss percentage ($p=0.004$) (Figure 6.2). Water gained during soaking affected hitting loss. Non-soaked meat cubes had a significantly lower hitting loss percentage ($p=0.001$)

(1.53±0.60 %) compared to pre-soaked meat cubes (2.6±1.73, 3.0±0.98 and 2.56±0.81 for SP0.396, SP0.713 and SP1.146, respectively).

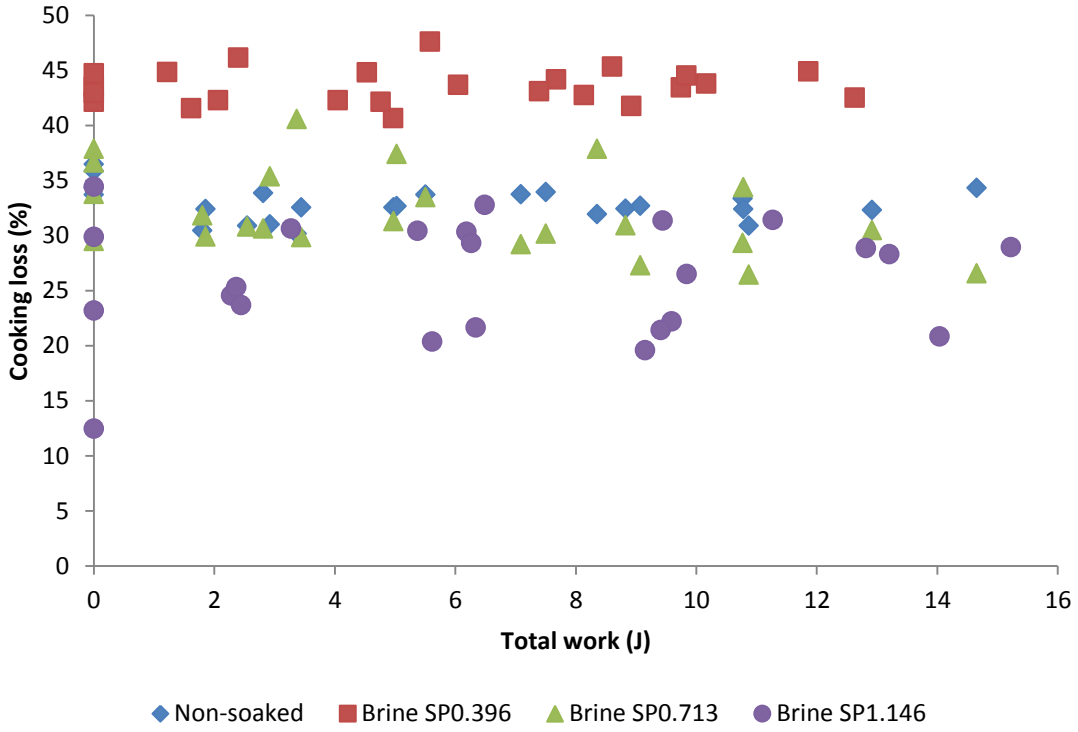

Figure 6.3. The cooking loss (%) of meat cubes measured against the total work (J) applied within the four brine-soaking treatments. Control (Non-soaked), Brine SP0.396=0.396 mol/L, Brine SP0.713=0.713 mol/L and Brine SP1.146=1.146 mol/L.

Total work on the meat cubes did not significantly affect cooking loss ($p=0.075$) (Figure 6.3). However, ionic strength of the brines did significantly affect cooking loss ($p<0.001$) (see Table 6.4 for equation). Siegel, Theno, Schmidt, & Norton (1978) also reported that massaging time for sectioned and formed ham did not affect cooking loss, but the adding of phosphate in the salt brine reduced the cooking loss because water holding capacity had increased. Increased salt and phosphate concentrations work synergistically to reduce cooking loss in restructured pork (Schwartz & Mandigo, 1976). In this study, the cooking loss percentage decreased at higher ionic strength of the brine. The meat cubes showed higher cooking loss when pre-soaked in brine SP0.396 than brine SP0.713 ($p<0.001$) and brine SP1.146 ($p<0.001$).

The increased brine absorption with increased ionic strength (Table 6.3) was negatively correlated (-0.798) to the cooking loss (Figure 6.3). This is consistent with

previous studies where cooking loss percentage decreased despite the increased absorption of brine (Siegel, Theno, Schmidt, & Norton, 1978). However, a contrasting result was reported by Pietrasik and Shand (2003) in their study with beef rolls, where 50% of brine added produced a higher cooking loss compared to 25% of brine addition. The contradictory outcomes could be due to the effects of cooking and types of meat products. The meat cubes used in this study consisted of a whole muscle that had been cut into small cubes. The muscle fibres of these cubes shrank along with the connective tissue due to the heating treatments which reduced the meat weight after cooking (Honikel, 1998). The higher the brine ionic strength used, the more swollen was the meat due to the water absorbed and subsequently reduced the cooking loss. Meanwhile, beef rolls, a meat product with a ground meat base, that is a high emulsion product, when heated, a protein gel network that traps the water is created leading to a reduced cooking loss (Tornberg, 2005). However, the protein matrix cannot hold excessive water added to the meat and this can result in higher cooking losses (Pietrasik & Shand, 2003).

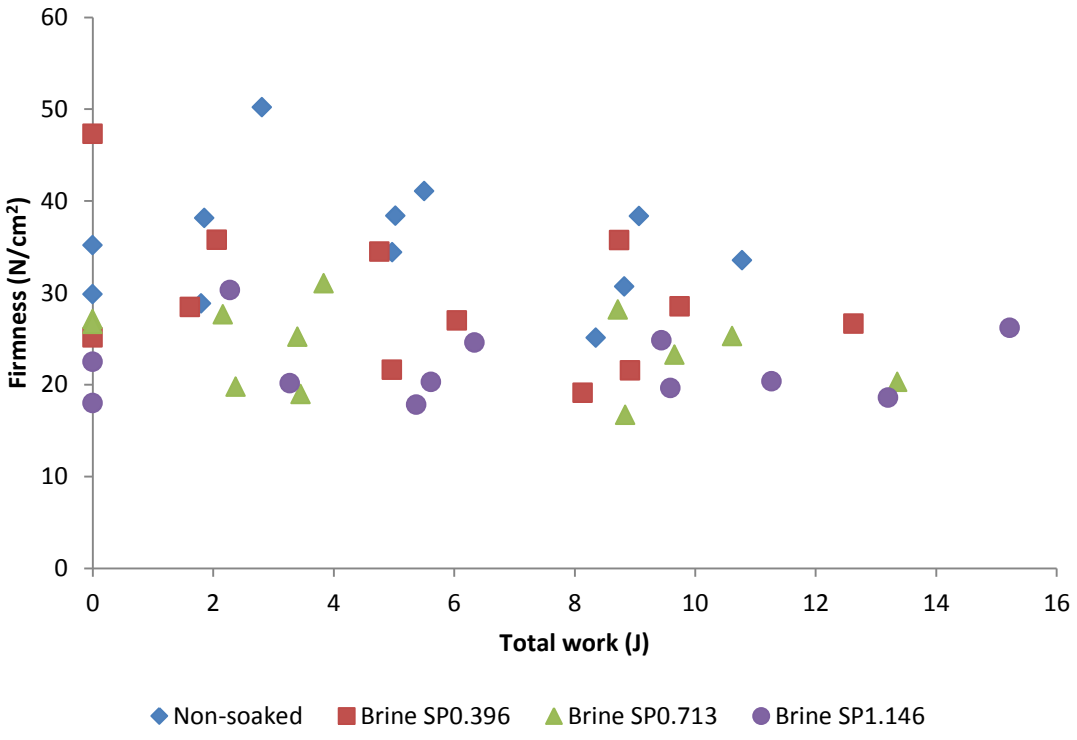

Figure 6.4. The firmness (N/m²) of meat cubes measured against the total work (J) done within their soaking conditions. Control (non-soaked), Brine SP0.396=0.396 mol/L, Brine SP0.713=0.713 mol/L and Brine SP1.146=1.146 mol/L.

Meat cubes firmness decreased linearly with increased total work ($p=0.0247$) on the meat cubes and with increased brine ionic strength ($p<0.001$) with the R^2 of 45.41% (Figure 6.4) (see Table 6.4 for the equation). Lachowicz, Sobczak, et al. (2003) and Zochowska-Kujawska, Lachowicz, Sobczak, Gajowiecki, et al. (2007) also showed meat firmness decreased with increased massaging time. Meat cubes pre-soaked in brine were softer meat due to the water absorbed. The higher the ionic strength of the brine, the greater the water absorbed and the softer the meat cubes. Meat structure, especially at the myofibril level would have also changed due to the actions of salt and phosphate. Increasing the total work alters and damages the meat fibres thus loosening the meat structure (Cassidy et al., 1978, Theno et al., 1978). These factors all contribute to reducing meat firmness (Figure 6.4).

Table 6.4. The linear or quadratic equation produced with R^2 and Relative Standard Deviation (RSD). The factors were fitted similar to the sequence in the equation. The significance levels of the items involved are given at the relevant places in the text.

Figure	Equation	R^2	Significant value		RSD
			Total work	Ionic Strength	
6.2	Hitting Loss = 2.01045 - 0.0152931 Total Work + 0.924218 Ionic Strength	10.10%	p=0.938	p=0.004	1.17 (%)
6.3	Cooking Loss = 38.4214 - 0.00809504 $(\text{Total Work})^2$ - 8.71528 $(\text{Ionic Strength})^2$	40.16%	p=0.075	p<0.001	5.58973 (%)
6.4	Firmness = 35.8182 – 0.2921 Total work – 11.4819 Ionic Strength	45.41%	p=0.0247	p<0.001	5.8440 (N/cm^2)

6.3.3. Effects of brine concentration and applied work on the exudate and binding strength of a pair of cooked 20 mm cubes of beef semitendinosus muscle

The protein and myosin content of the exudate on a meat cube surface is influenced by the total work done on the meat cubes. The TAS could also be affected by the total work applied during the hitting process. Relationships between TAS and total protein and myosin in the exudate could indicate which factor contributes most to binding meat cubes. The following graphs and results are presented as a basis for discussing these possibilities.

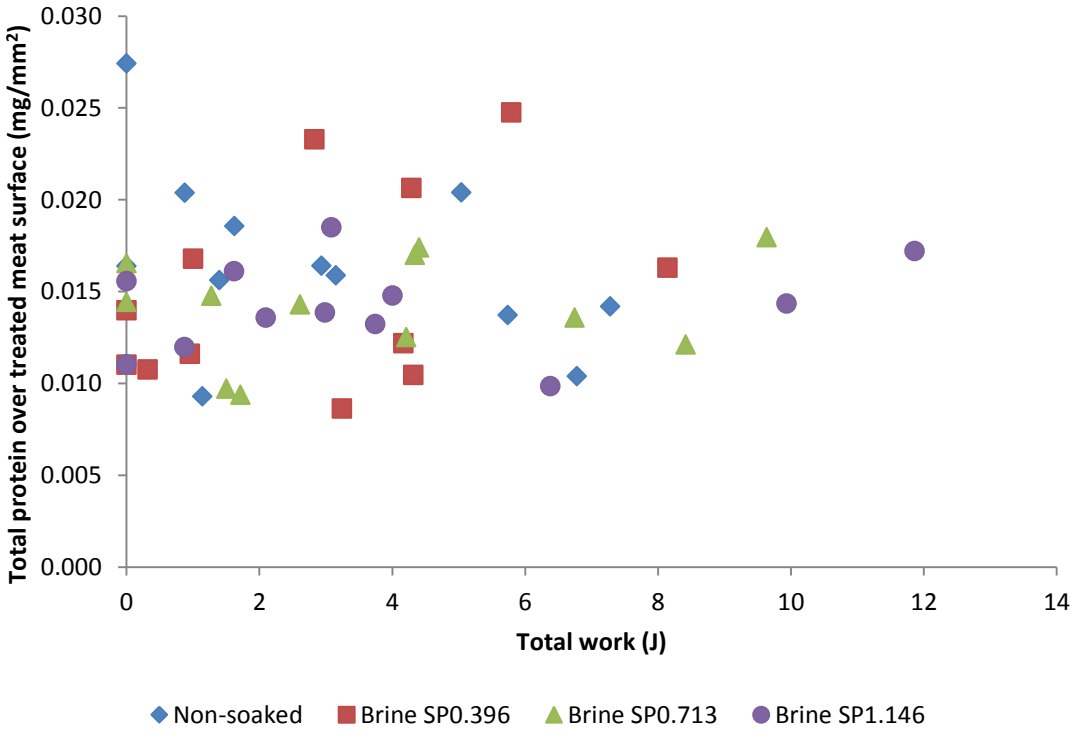

Figure 6.5. Total protein (mg/mm²) in the scraped-off exudate from the meat cube surface, plotted against total work (J) applied to the meat for meat cubes subjected to four brine treatments. Control (non-soaked), Brine SP0.396=0.396 mol/L, Brine SP0.713=0.713 mol/L and Brine SP1.146=1.146 mol/L.

The total protein (mg/mm²) in the exudate collected from the treated meat surface was plotted against total work (J) done on the meat (Figure 6.5). Total work done on the meat ($p=0.764$), and ionic strength of the pre-soaking brine ($p=0.123$), did not affect total exudate protein (refer Table 6.7 for equation). These results were compared to other

studies but protein percentage in the exudate was used to report the results. For example, these results are similar to the study by Motycka & Bechtel (1983), who showed that increasing tumbling time from 4 to 24 hours did not significantly increase protein percentage in the exudate collected from pork semimembranosus and biceps femoris muscles. Increasing salt concentration in brine up to 22% also did not affect the protein content in the exudate collected from tumbled pork semimembranosus muscle (Bombrun, Gatellier, Carlier, & Kondjoyan, 2014). However, Ghavimi et al. (1987) reported that increased tumbling speed, which increases the work done on the meat, increased the protein. The exudate protein was also reported to be significantly higher against the time of mixing when preparing sectioned and formed beef steak (Booren, Mandigo, Olson, & Jones, 1982). In addition, in another study, the protein percentage collected from the exudate of massaged ham resulted in a hyperbolic pattern against the time, which started to show a reduction at 12 hours of massaging (Siegel, Theno, Schmidt, et al., 1978).

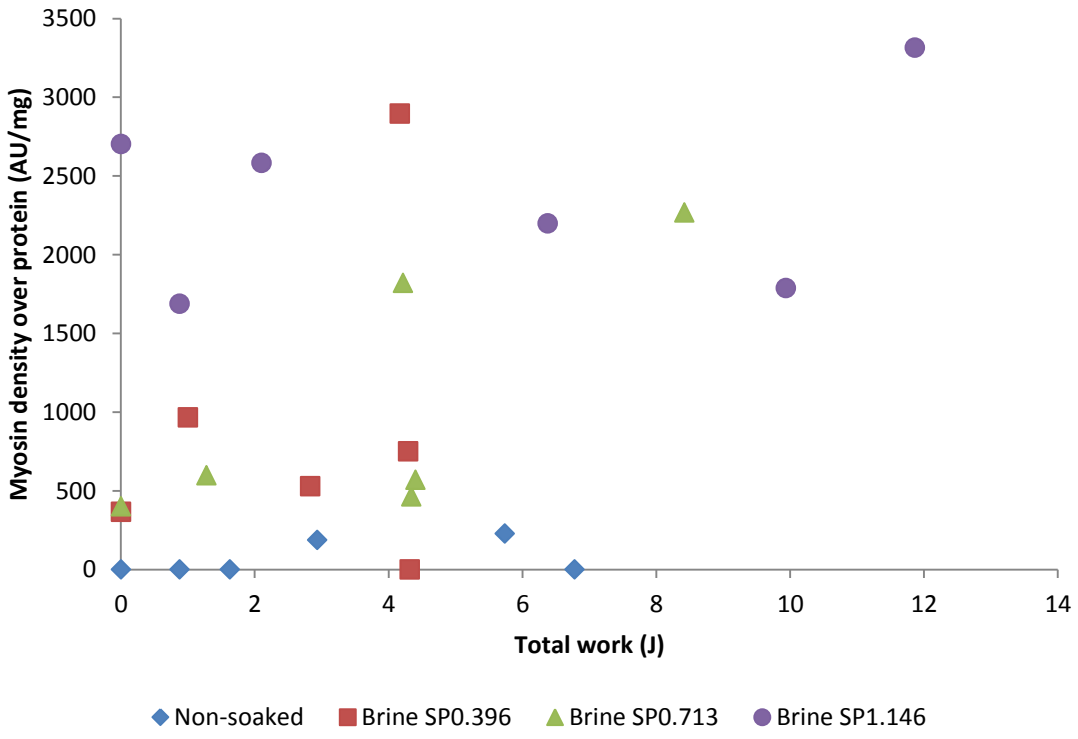

Figure 6.6. Myosin density over protein (AU/mg) in the scraped-off exudate from the meat cube surface, plotted against total work (J) applied to the meat for meat cubes subjected to four brine treatments. Control (non-soaked), Brine SP0.396=0.396 mol/L, Brine SP0.713=0.713 mol/L and Brine SP1.146=1.146 mol/L.

Myosin density significantly increased linearly (R^2=61.50%) with total work done (p=0.006), and with increases in ionic strength of the brine (p<0.001) (Figure 6.6) (refer Table 6.7 for equation). Solomon & Schmidt (1980) also showed increasing the mixing time increased crude myosin extracted from muscle homogenate of post rigor beef samples. Turner, Jones, & Macfarlane (1979) reported that yield of crude myosin showed a quadratic pattern with tripolyphosphate (TPP) concentration. Higher salt concentration increased the amount of crude myosin. In contrast, Bombrun et al. (2014) showed that myosin measured in protein exudate was not affected by increased salt content of brine (0, 1.92, 2.82 and 3.76 M NaCl). This result was in agreement with the study by Siegel, Theno, & Schmidt (1978) regarding changes in myosin in exudates with increased salt concentration and massaging time of sectioned and formed ham.

The salt concentration has an important role in solubilising myosin (Section 2.7). In this trial, the lowest ionic strength used for pre-soaking was 0.26M salt. Phosphates were included in all the brines prepared. Offer & Trinick (1983) report that the myofibril diameter shrink slightly when 0.1M of NaCl is used and observed slight changes when salt concentration was increased by 0.1M to 0.5M. At 0.6M the myofibril diameter had increased substantially with the centre of the A-band being extracted and Z-line not visible. When phosphate was included, the A-band had already started to be extracted at 0.4M salt. With 1M salt (the highest concentration used) nearly all the A-band was extracted, which indicates maximum myosin solubilisation (Knight & Parsons, 1988; Offer & Trinick, 1983). Therefore, the use of high salt and phosphate concentrations, along with applying more work to the meat surface, released more myosin into the exudate (Figure 6.6).

Results from published studies of total protein and myosin in exudates were inconsistent on the effect of increased processing time and/or brine concentration. The inconsistent results could be due to variation in meat samples, production method and different types and concentrations of the brine. In this study, the results for total protein and myosin were not parallel. Total exudate protein did not change with increasing the work done and increasing ionic strength. The myosin concentration in the total protein, however, increased both with increased work and brine ionic strength. The total protein will include proteins other than myosin in the exudate, especially sarcoplasmic proteins such as myoglobin. Red-coloured liquid was seen in abundance in the exudate collected from the surface of meat cubes treated with lower total work, and which were non-soaked or pre-soaked in lower brine ionic strengths. In contrast, meat cubes that had been pre-soaked in higher brine ionic strengths and treated with more hits (greater total work)

produced stickier, pink-coloured exudates, which were myosin extracts. This observation was supported by the higher readings of myosin density for exudate collected from meat treated with higher brine ionic strengths and greater total work. The sarcoplasmic proteins could possibly have been squeezed out from the treated meat surface whereas the myosin could have been extracted more and have stuck within the exudate as more total work was applied to the meat cubes resulting in no significant changes in total protein.

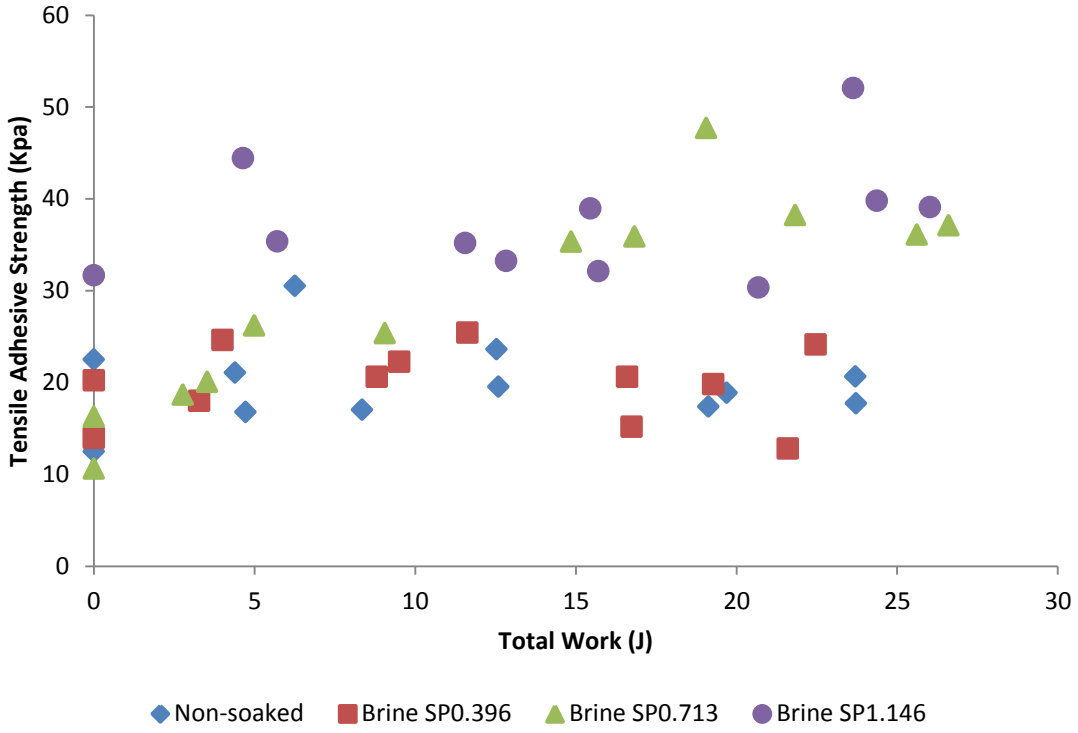

Figure 6.7. Effect of brine concentration and work on TAS between pairs of meat cubes. Control (Non-soaked), Brine SP0.396=0.396 mol/L, Brine SP0.713=0.713 mol/L and Brine SP1.146=1.146 mol/L.

TAS increased linearly (R^2=54.04%) with total work (J) done on the meat pieces (Figure 6.7) ($p=0.0001$) and also with increased ionic strength of the brine ($p<0.001$) (refer Table 6.7 for the equation). The results agree with work by Gillett et al. (1981) and Booren et al. (1981) (Section 2.9), who showed that increasing the mixing or massaging time increased binding strength of the meat products. The reason was related to the alteration of meat structure when work was applied to the meat cubes. Published work (Section 2.5) show continuous and increasing impact forces can break the sarcolemma and reduce fibre integrity of the meat (Cassidy et al., 1978, Theno et al., 1978b). Brine

used for soaking, contains salt and phosphate, which with the work done on the meat, causes myofibrils to be more fragmented and fibres to shred (Theno et al., 1978b). Therefore, the texture of the meat surface that has been impacted becomes softer and more pliable. Data from the trials showed that brine ionic strength and more applied work reduced firmness of the meat cubes (Figure 6.4). This condition could allow the meat to more easily attach together in full contact without any air pockets, thus increasing the TAS.

Table 6.5. The linear equations produced with R^2 and Relative Standard Deviation (RSD). The factors were fitted in the sequence shown in the equations. The significance levels of the items involved are given at the relevant places in the text.

Figure	Equation	R^2	Significant value		RSD (%)
			Total work	Ionic strength	
6.5	Total Protein = 0.0158808 + 9.93743e-005 Total Work - 0.00219963 Ionic Strength	5.40%	p=0.764	p=0.123	0.0000404 (mg/mm^2)
6.6	Myosin = -159.145 + 72.7324 Total Work + 1752.99 Ionic Strength	61.50%	p=0.006	p<0.001	701.923 (AU/mg)
6.7	TAS = 12.9989 + 0.42578 Total Work + 14.1499 Ionic Strength	54.04%	p=0.0001	p<0.001	6.95043 (kPa)

TAS values were plotted against total protein in the exudate, with pairs of values being chosen based on a similar or near total work values applied to the meat cubes as shown in Table 6.5. This was carried out because each hitting treatment produced a unique amount of total work (J). Figure 6.8 shows that the increase of the TAS was not influenced (p=0.421) by the total protein in the exudate collected from the treated meat surface. However, the ionic strength of the brine used for soaking did influence (p=0.01) the TAS, where the higher the ionic strength of the brine used, the stronger the TAS measured (refer Table 6.8 for the equation). The increased ionic strength could determine

how swollen the meat cubes became after soaking (Offer & Trinick, 1983). This was related to the firmness and pliability of the meat thus suggesting that the texture of meat did influence the binding strength of meat pieces. Whereas, the total protein released did not affect the binding, although this result could be at least partly because the total protein detected did not change much as shown in Figure 6.5.

Table 6.6. Matched pairs of values for TAS and total protein that have been paired because they were associated with similar or near applied averaged total work (J) ± SD values (4 values for TAS and 2 values for total protein).

Brine Ionic Strength (mol/L)	Similar or near value of total work (J) done for TAS and total protein analysis		Value of the TAS and total protein	
	Work (J) from TAS analysis	Work (J) from protein analysis	TAS (kPa)	Total Protein (mg)
0	0.00	0.00	17.48	0.022
	2.28±0.23	1.51±0.16	18.92	0.017
	3.65±1.48	3.04±0.15	23.78	0.016
	6.28±0.04	5.91±1.23	21.57	0.015
0.369	0.00	0.00	17.09	0.013
	1.82±0.52	1.89±1.33	21.31	0.017
	4.58±0.49	5.05±1.04	21.43	0.018
	7.09±3.61	6.16±2.81	20.30	0.014
0.713	0.00	0.00	13.43	0.015
	1.57±0.54	1.39±0.16	19.35	0.012
	3.51±2.88	3.03±1.86	25.77	0.013
	3.51±2.88	3.51±1.27	25.77	0.016
	7.92±1.39	9.03±0.86	35.60	0.015
	10.22±1.96	9.03±0.86	42.95	0.015
1.146	0.00	0.00	23.94	0.013
	2.59±0.76	2.59±0.70	39.88	0.016
	6.09±0.90	6.46±4.91	34.20	0.014
	7.79±0.18	7.80±5.37	35.52	0.015

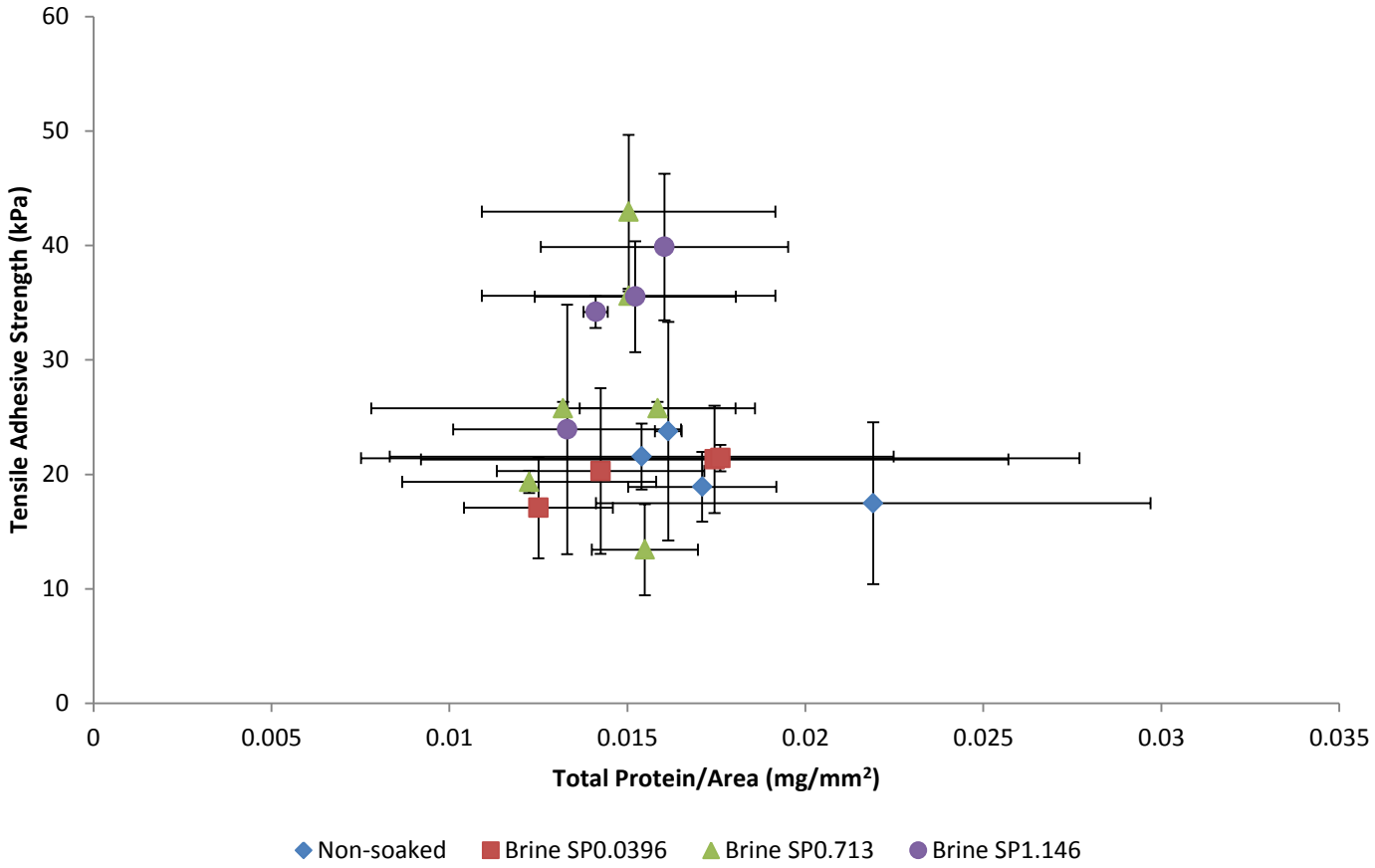

Figure 6.8. Tensile Adhesive Strength (TAS) (kPa) of meat cubes plotted against the total protein (mg/mm^2) measured on the meat cube surface. Means (±SD) from Table 6.5. Control (Non-soaked), Brine SP0.396=0.396 mol/L, Brine SP0.713=0.713 mol/L and Brine SP1.146=1.146 mol/L.

TAS was also plotted against myosin density relative to protein based on pairs of values matched for total work done as shown in Table 6.6. TAS increased significantly in a linear pattern with the increased myosin density on the surface of the meat ($p=0.001$) (refer Table 6.8 for the equation). Several studies have shown that increases in the amount of protein in the exudate result in increasing binding strength. Such a study was that of Pietrasik & Shand (2003), in which the binding strength of beef rolls was correlated to the amount of protein in the exudate, with more protein resulting in stronger binding. Similar results were reported for the production of restructured cured beef where more protein in the exudate resulted in higher binding strength (Ghavimi et al., 1987). However, these studies did not further analyse for specific proteins in the protein exudate. It could be suggested that myosin was the main protein influencing the binding of meat pieces compared to other proteins based on studies by Macfarlane et al. (1977) and Siegel & Schmidt (1979). This suggestion is supported by the results presented in Figure 6.9, which show that with more myosin in the exudate there was a stronger TAS.

Table 6.7. Matched pairs of values for TAS and exudate myosin concentration that have been paired because they were associated with similar or near applied averaged total work (J) ± SD values (4 values for TAS).

Brine Ionic Strength (mol/L)	Similar or near value of total work (J) done for TAS and myosin analysis		Value of the TAS and myosin	
	Work (J) from TAS analysis	Work (J) from myosin analysis	TAS (kPa)	Myosin (AU/mg)
0	0.00	0.00	17.48	0.00
	2.28±0.23	1.63	18.92	0.00
	2.28±0.23	2.93	18.92	187.05
	6.28±0.04	6.78	21.57	0.00
	6.28±0.04	5.74	21.57	228.55
0.369	0.00	0.00	17.09	366.30
	1.82±0.52	1.00	21.31	966.61
	1.82±0.52	2.83	21.31	529.73
	4.58±0.49	4.29	21.43	751.34
	4.58±0.49	4.32	21.43	0.00
0.713	0.00	0.00	13.43	401.13
	1.57±0.54	1.28	19.35	599.22
	3.51±2.88	4.34	25.77	464.36
	3.51±2.88	4.40	25.77	569.46
	3.51±2.88	4.21	25.77	1820.70
	7.92±1.39	8.42	35.60	2267.42
1.146	0.00	0.00	23.94	2702.69
	2.59±0.76	2.10	39.88	2582.86
	7.79±0.18	6.38	35.52	2199.00
	11.26±2.61	11.86	35.05	3314.78
	12.41±1.69	11.86	45.57	3314.78

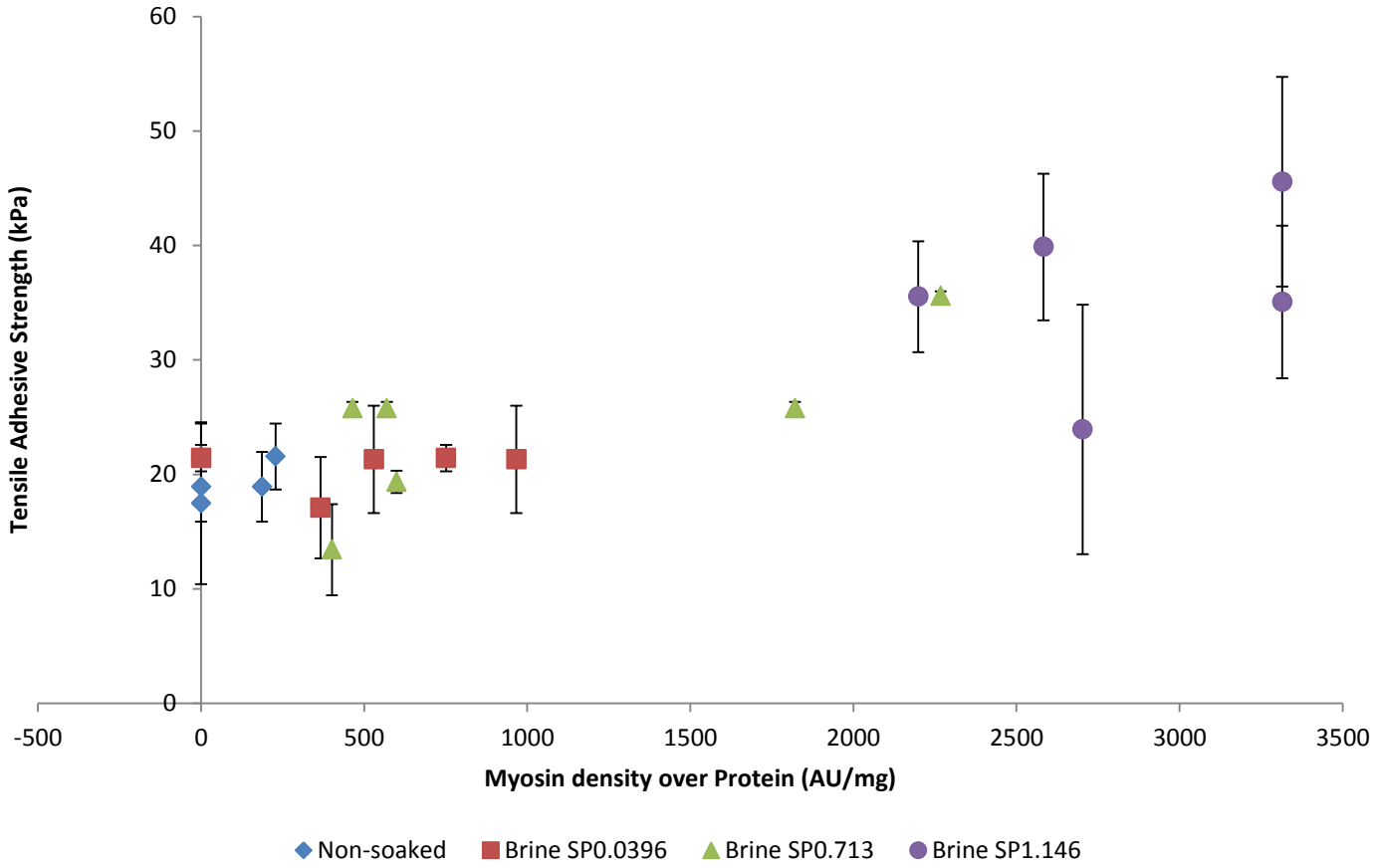

Figure 6.9. Tensile Adhesive Strength (TAS) (kPa) of meat cubes plotted against the myosin density over total protein (AU/mg) collected from the meat cube surface. Means (±SD) from Table 6.6. Control (Non-soaked), Brine SP0.396=0.396 mol/L, Brine SP0.713=0.713 mol/L and Brine SP1.146=1.146 mol/L.

Table 6.8. The linear equations produced with R^2 and Relative Standard Deviation (RSD). The factors were fitted in the sequence shown in the equations. The significance levels of the items involved are given at the relevant places in the text.

Figure	Equation	R^2	Significant value		RSD (%)
			Total protein	Ionic strength	
6.8	TAS = 59.9051 + 73053.1 Total Protein + 141.19 Ionic Strength	37.37%	p=0.421	p=0.01	71.7703 (kPa)
6.10	TAS = 18.494 - 1.09039 Ionic Strength + 0.00650581 Myosin	71.76%	p=0.001		4.6978 (kPa)

6.4. Conclusions

Trials were done to investigate the effect of ionic strength of brine used for pre-soaking and the work done on individual 20-mm cubes of beef semitendinosus muscle on protein extraction and strength of joined cooked meat:

1) Increasing the brine concentration increased the weight gain of the meat, probably because creating bigger spaces within the myofibrils, which allow more water to be stored.
2) Brine concentrations significantly influenced hitting loss, cooking loss and meat firmness. Total work done to the meat only affected firmness of the meat.
3) Total protein in the exudate scraped from the raw meat was not influenced by brine concentration or by total work applied to the meat.
4) Increased brine concentration or total work applied had increased the amount of myosin extracted.
5) The TAS of meat pieces was greater for the higher brine concentration and greater work done.
6) TAS was not affected by total protein in the exudate, but did increase when the ionic strength of the brine increased.
7) TAS increased significantly when the amount of myosin in the exudate increased, and when the ionic strength of the brine increased.

CHAPTER 7

Conclusions and recommendation for future research

7.1. Conclusions

The Impact and Friction Mechanical Robot (IFMR) developed could apply an impact force to the meat cubes and simultaneously measure the force and compression allowing the work to be calculated. The number of hits along with the speed for hitting and the gap between each hit could be pre-set. The method for applying the hitting force could be either 1) a specific target force or 2) the depth of meat compression. The specific force allows the meat to be subjected to a similar amount of force for every hit. The depth meat is compressed by the hitting process depends on the rigidity of the meat texture. The work done for every hit on the meat cube can be calculated from the information collected on force applied and depth or distance the meat is being compressed. The fixed distance method works with the meat cube being hit to a pre-set distance; consequently the force applied to the meat during every hit varies depending on compression of the meat. In this study, only the first method with a target force was used for all meat samples tests (see Chapter 3).

The protocols to collect exudate from the treated meat surface and to attach two meat cubes were successfully developed. The scraping method was chosen to collect exudate from the meat surface. The exudates was analysed for total protein and myosin content using the Bradford method and electrophoresis method, respectively (Chapter 4). The cooking protocol involved placing two meat cubes next to each other adjacent with fibre direction perpendicular to the joint. The cubes were then wrapped in plastic food wrap and cooked at 70 °C in water bath for one hour with applied weight of 250 g to press the two meat cubes to allow myosin gelation and thus bind the meat cubes together. The attached meat cubes then were tested for the tensile adhesive strength (TAS) using newly developed clamps attached to a texture analyser TA-XT2 (Chapter 5).

The total protein in the exudate scraped from the meat surface was not affected by the amount of total work done on the meat or by the different brine concentrations used for soaking the meat. However, extraction of myosin was increased by increasing total work applied to the meat and higher concentrations of the brine used for soaking. The salt and phosphates in the brine solubilised the myosin, and the work done on the meat helped to extract the myosin into the exudate on the meat surface. The brine also created more spaces within the myofibrils allowing more water to be absorbed, consequently increasing the swelling of the muscle and thus increasing the weight gain after soaking. The meat cubes with higher weight gain exhibited smaller cooking loss. In addition to the brine

concentration, the work done showed its impact on the meat texture by reducing the firmness when more work was applied to the meat. The work done altered the meat structure and contributed to softer and more pliable meat. This led to TAS being higher when more was work applied to the meat pieces. The TAS depended on the amount of myosin that had been extracted. It was concluded that:

1) Brine is needed to solubilise myosin and increase the quality characteristics of the meat.
2) Work is required to alter the meat structure and soften the texture. More importantly, sufficient work is needed to extract myosin into the exudate on the meat surface.
3) The brine used and work done act synergistically to extract myosin and increase meat pliability, which then increase binding strength between the attached meat pieces (Chapter 6).

7.2. Future recommendations

The advantage of the IFMR is that the total work done on the meat can be easily and quickly calculated. The same amount of total work can be applied to meat pieces using different speeds, gap between hits and total number of hits. Changes in the way the work is applied may affect myosin extraction and TAS of cooked meat cubes. Increasing the work can extract more myosin to the surface. The meat texture could also be more pliable after being treated with more mechanical work and along with more myosin, the TAS could be increased.

The effect of higher salt and phosphate concentrations than what had been used in this study should be investigated as they could still increase the TAS. This is because the failure of the TAS was measured at the binding junction while it did not reach the TAS of the original meat that failed at the clamping area. Different muscle types could be tested. The level of protein differs between different muscle type and among different species. It would be interesting to see what effect similar total work applied to meat from different muscles and species had on the various physico-chemical parameters measured in this study. The variation of myosin extracted and the TAS could also be observed.

This study had set up a good basis in terms of the technology to perform the experiment of imparting the impact force and calculating the total work done to the meat,

analysing the total protein and myosin extracted, and attaching the meat pieces together. It has also produced a set of data that showed the effectiveness of the mechanical work done on the meat towards the extraction of protein and myosin, and the TAS of the meat pieces with added various concentrations of salt and phosphates.

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Appendices

Appendix 1. Results on TAS against the cooking temperature and weight applied.

	Temperature: 60 °C					Temperature: 70 °C					Temperature: 80 °C				
Weight	0	250	500	750	1000	0	250	500	750	1000	0	250	500	750	1000
Treatment	60-0	60-250	60-500	60-750	60-1000	70-0	70-250	70-500	70-750	70-1000	80-0	80-250	80-500	80-750	80-1000
1	1.021	14.449	11.152	8.47	8.858	6.83	28.587	28.043	24.485	13.387	25.249	23.293	24.846	43.8982	35.5916
2	1.705	8.611	9.654	10.068	8.711	12.821	13.355	14.473	24.752	26.693	16.242	21.553	25.98	32.5932	43.1572
3	0.57	11.034	7.845	12.493	5.909	10.732	30.602	17.671	22.675	22.136	11.315	37.592	24.856	52.3347	33.6395
4	2.595	12.22	6.703	6.778	12.092	11.756	30.158	23.102	27.211	24.778	18.918	41.252	29.805	47.7837	37.6659
5	2.303	14.274	8.983	11.998	11.983	12.563	22.793	18.228	15.888	16.343	24.513	25.462	24.306	39.8636	36.0807
6	1.313	8.623	10.974	7.272	9.843	10.989	13.955	12.355	13.167	24.232	13.665	36.998	38.69	43.5677	37.2992
7	1.59	9.368	9.735	9.655	8.976	7.655	16.057	15.515	22.002	14.881	16.567	35.617	24.484	47.239	33.878
8	0.685	14.049	5.662	8.883	4.768	10.931	13.995	10.085	19.472	11.9	16.978	32.706	27.322	46.8673	37.7155
Average	1.473	11.579	8.839	9.452	8.893	10.535	21.188	17.434	21.207	19.294	17.931	31.809	27.536	44.268	36.878
SD	0.725	2.528	1.965	2.047	2.581	2.180	7.729	5.826	4.752	5.794	4.862	7.395	4.873	5.968	2.993
SE	0.256	0.894	0.695	0.724	0.913	0.771	2.733	2.060	1.680	2.049	1.719	2.615	1.723	2.110	1.058

Appendix 2. Publication

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Initial work on developing a cooking protocol for producing re-structured meat under controlled conditions

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Abstract

Restructured meat is made by binding individual pieces of meat together. To study the effect of mechanical work on the meat binding process, a standard cooking protocol must be established. This paper details the establishment of a standard cooking protocol for the cooking process using untreated beef *semitendinosus* muscle. The effect of different cooking temperatures and applied loads during cooking were investigated. Meat samples were cut in 20 mm cubes size from beef *semitendinosus* muscle and two pieces were held together with the muscle fibre parallel to each other by wrapping them with a plastic food wrap. Then the samples were placed inside square steel tubes, that act as a mold for cooking, and different weights (0, 250, 500, 750 and 1000 g) were placed on top of the meat cubes during cooking. The temperatures used for cooking were 60, 70 and 80 °C. There was a significant temperature effect, with increases noted between 60°C and 70°C and between 70°C and 80 °C. At 60 °C neither myosin nor collagen has gelatinized, leading to low binding strengths. At 70 °C the myosin component will have gelatinized. At 80 °C the collagen component will be contributing to the bond. To keep the collagen effect to a minimum the meat should be cooked at 70 °C. The effect of applied cooking load was significant at all cooking temperatures once sufficient load had been applied against no load to ensure good contact at the joint. There was a significant effect of applied load noted at 80 °C with increases up to 750 g and a drop occurring between the 750 and 1000 g loadings. The drop has been attributed to collagen being squeezed out of the joint as a bead of white material was noted around the joint.

Keywords: restructured meat; reformed meat; tensile strength; cooking; beef

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1. Introduction

In production of reformed or restructured meat, pieces of meat undergo physical treatment via a tumbler or massager, and with the aid of salt and phosphate [1], the myofibrillar proteins, mainly myosin, are extracted [2]. Further processing, which includes applying an appropriate pressure to the meat pieces and cooking [3, 4], coagulate the myosin thus binding the meat pieces into reformed meat.

The tumbling and massaging processes impart impact and friction forces to the meat. Products made from diverse type of meat require different level of forces. These physical treatments variations have been qualitatively determined previously through many trial and error procedures of tumbling and massaging to produce the most acceptable product. The parameters for tumbling or massaging processing that have been studied extensively are the time [5, 6], speed [7, 8] and intermittent or continuous method [9, 10].

There is no quantitative information on the effect of the physical forces on the meat pieces. In order to initiate such a study, a protocol for the cooking temperatures and applied load during cooking to the meat pieces needs to be established. This paper presents the result of the effect of different applied load on the meat pieces while cooking at different temperatures against the Tensile Adhesive Strength (TAS) of the bound meat pieces.

2. Materials & Methods

The meat samples were from beef *semitendinosus* (eye of round) bought from Taylor Preston Limited (Palmerston North, New Zealand). The meat was cut into 20 mm cubes with the fibre direction parallel to the cutting point. One meat cube was placed on top of another meat cube with the fibre direction of both meat cubes perpendicular to the joining junction. The combined meat pieces were wrapped with plastic food wrap (Glad) and stored at 4 °C overnight.

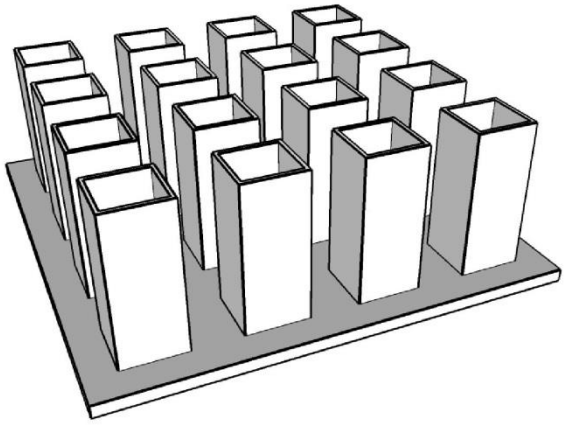

Fig. 1. The mold made of steel to put meat cubes and cook

Figure 1 shows a mold consisting of 16 steel square tubes with an opening of 22x22 mm and height of 60 mm attached to a steel plate (185x185x2 mm). The mold was used to retain the shape of the meat

during cooking and at the same time guided the weights applied to the meat. The weights were made from steel and consisted of two parts, Part A and Part B (Figure 2). Part A is the base with a total weight of 250 g. It was a steel rectangle with 62.5 mm height, had a bottom surface area of 21.8x21.8 mm and a steel rod at the top (110 mm height and 5 mm diameter), which functioned to hold additional weights. Part B consisted of steel cylinders with holes 5 mm diameter in the middle and weighing 250 g.

For cooking procedure, the combined meat pieces were placed inside the square tubes of the mold and weights (0, 250, 500, 750 or 1000 g) were added. The water bath was preheated to 60 °C before the mold containing the combined meat pieces was added and cooked for 1 hour. Then the meat samples were extracted and placed under cold running water for 10 minutes as a cooling process. The procedure was repeated with the temperatures of 70 and 80 °C for new meat samples. The wrap then was taken off and all the meat samples were stored at 4 °C overnight before the TAS analysis.

The TAS analysis was carried out by using the texture analyzer TA-XT2 (Stable Micro Systems, Surrey, England). Two identical clamps (Figure 3) were built to hold the combined meat pieces for the pulling action. One clamp was screwed to the floor of the texture analyzer and another clamp was screwed to the moveable arm of the texture analyzer using bolts. Each clamp consists of two plates; the static plate and the moveable plate connected by a hinge. A square plate was attached at 90° to the static plate to hold the meat. Three 9 mm length pins with 4.9 mm between adjacent pins were attached through the sides to spike the meat sample at both ends to secure the meat so tensile forces could be applied when pulling the meat apart. The movement of the pulling was at 0.1 mm/s and the highest peak was recorded.

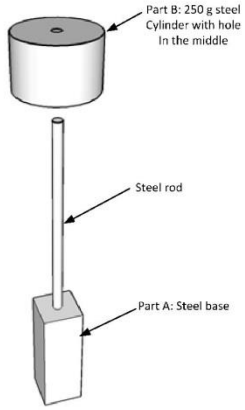

Fig. 2. The weights for the meat with Part A as the base and Part B is the additional weight

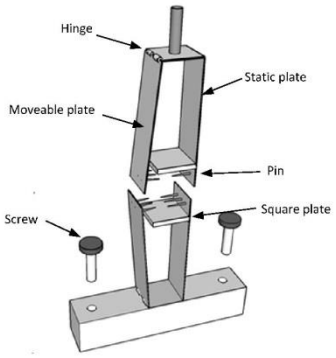

Fig. 3. The clamp used to clamp end of the meat for pulling action

The statistical analysis carried out was using a 3x5 factorial design with orthogonal contrasts to determine the differences between weight and temperature of cooking. As well, 2-way comparisons were used to analyze individual group of different cooking temperature and weight between the 15 treatment groups. The statistical software used was SAS 9.2 (SAS Institute, Carey, NC, USA).

3. Results & Discussion

The results of Tensile Adhesive Strength (TAS) (kPa), expressed as a pressure by dividing the force by the measured cross sectioned area, have been plotted against the applied weights (g) with cooking

temperature as a parameter are shown in Figure 4. There was a highly significant temperature effect, with increases noted between 60 °C and 70 °C ($p<0.0001$) and between 70 °C and 80 °C ($p<0.0001$). At 60 °C, myosin is the main protein for binding the meat pieces but it is still not fully aggregated and has not formed a complete network. At 70 °C the myosin reaches its strongest gelation [11]. At 80 °C the collagen component will be undergoing gelation and contributing to the bond [12]. When cooled, the solubilized collagen formed gels and at the same time adding more binding strength to the existing myosin gelation. To keep the collagen effect to a minimum the meat should be cooked at 70 °C.

The effect of applied cooking load was significant (p ranged from <0.0001 to 0.0036 for individual loads when compared against the no load condition) at all cooking temperatures once sufficient load had been applied against no load to ensure good contact at the joint. The suspected reason was that the meat cubes without applied loads had air pockets between the two meat surfaces, which prevented the protein-protein interactions [13]. Applying a weight has removed the air and the two meat cubes have full contact at the joining surfaces.

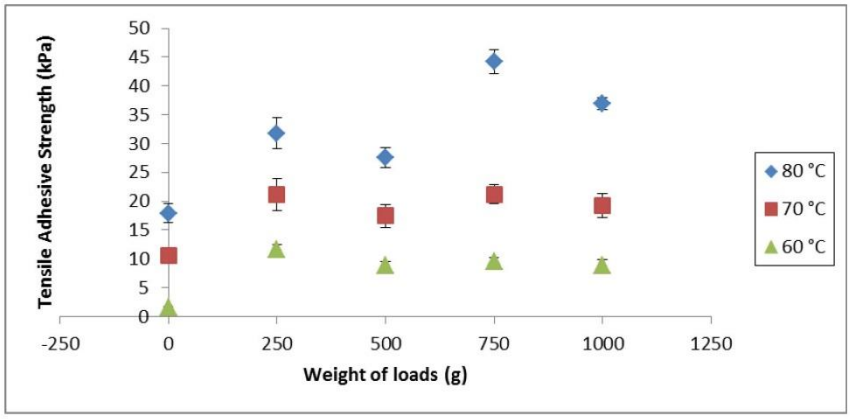

Fig. 4. Tensile Adhesive Strength of combined meat cubes applied with different weight of presser and cooked at different temperature. Eight replicates were carried out at each condition. Error bars are the standard error

Cooking at 80 °C showed a significant different ($p=0.068$) between applied weights of 250 and 500 g and when the weight was increased to 750 g there was a further significant increase ($p<0.0001$). This shows that pressure can influence the binding. However, when the load was increased to 1000 g it resulted in a significantly lower ($p=0.0019$) TAS value than the 750 g result (44.3 c.f. 36.9 kPa). Increasing the applied weight from 750 to 1000 g at a cooking temperature of 80 °C has resulted in the gelatinized collagen being squeezed out of the joint. This collagen was observed as a visible bead around the joint before tensile testing. Despite the increased bonding strength available at cooking temperatures of 80 °C, cooking of reformed or restructured meat at this temperature is not practical in industry due to higher cooking losses and the added effect of collagen gelatinization, hence 70 °C has been chosen as the cooking temperature for the protocol. Since the effect of applied load is not statistically significant at 70 °C, a loading of 250 g has been chosen to press the meat cubes together while cooking in order to minimize leakage effects on the solubilised myosin that may occur due to the applied weight. The 250 g weight is seen as being sufficient to produce a good bond where observation on the samples showed firm contact was formed with the gelatinized protein covering the edge of the binding junction.

4. Conclusion

Cooking at 70 and 80 °C gave good binding strength to the meat cubes. Cooking at 80 °C with heavier applied weights resulted in significantly higher TAS compared to 70 °C. The result was due to the gelation of solubilized collagen. Therefore, where myosin is being considered as the glue factor cooking at 70 °C is preferable and applying 250 g of weight is seen to be the best choice of loads.

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