Optimisation of the Thermal Processing of Mussels

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ABSTRACT

Perna canaliculus, more commonly known as the green-lipped mussel, is unique to New Zealand and is the foundation of the mussel farming industry in this country. This project aimed to identify practical ways to improve the thermal processing of mussels to maximise yield.

Initial work was carried out to characterise the composition of the mussel tissue. Following this, a method to quantify the cooking losses was developed. This methodology was used to examine the rate and extent of cooking losses in mussel tissue at various temperatures. Further to this it was possible, using differential scanning calorimetry, to examine the kinetics of protein denaturation associated with cooking losses.

The cook loss trials over various temperatures showed a definite increase in water loss once a temperature of approximately 65°C was reached. A relationship was developed between the water loss exhibited over a range of temperatures and the rate of protein denaturation. It was found that low temperature, long time cooking results in increased yields. These conditions will reduce the impact of temperature gradients through the mussel. The exact time temperature regime used commercially will be a compromise between moisture losses, microbial destruction, inactivation of rancidity causing enzymes and production restraints.

This regime was tested for whole and half shell mussels resulting in up to 4.5% increases in yield.

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CHAPTER 1

PROJECT OVERVIEW

1.1   Introduction

Sealord Group Ltd, Nelson processes on average 18,000 to 25,000 tonnes of green weight (raw) mussels per year. These mussels are processed in a variety of ways, which include the production of mussel meat and mussels in their half shell. The type of mussel farmed in the Marlborough Sounds, *Perna canaliculus*, is known more commonly as the green-lipped mussel. This species is unique to New Zealand and is the foundation of the mussel farming industry in this country.

During the pre-cooking and steam cooking of mussels prior to shucking and further processing, numerous functional and structural changes occur. The effect these changes have on the product yield and texture is important. Previous research has shown that the precooking of mussels can cause up to 6.5% moisture loss compared to freshly shucked mussels (Caseles *et al.*, 1988). It is anticipated that during steam cooking the water losses could be much higher, resulting in even lower product yield.

It is known that within the mussel processing plant, the average yield of cooked mussel meat is approximately 25% of the green mussel weight. Through minimising the cooking losses, there is the potential to greatly increase the profitability of the mussels. If the average yield of the mussels can be increased by 1% for both cooked meat and half shell products by alteration of the blanching and steam cooking processes, then Gains of up to $845,000 per annum are plausible, based on current prices and yearly production volumes.

1.2 The operation in nelson

At present Sealord Group Ltd., Nelson has two major mussel product lines. The first is for the production of a half-shell product, which is mainly exported, in various serving sizes. The second process produces mussel meat, where the mussel is fully unattached from the shell. Mussel meat is often sold as portions and then further processed into other products such as marinated mussels.

The current practice, for half-shell production, is to firstly subject the mussels to a hot water bath at a temperature of around 92 - 95°C for up to 5 minutes. The waterbath temperature is monitored in three positions over the cooker using probes connected to an automatic monitor. Variability in the mussel's themselves causes changes to the cooking operation, so that any one batch of mussels processed may be treated differently to another. The processing conditions are decided upon by the cook on the shift, and most of the decisions made are based on the cooks experience, and cooking yields performed on the mussels earlier. Every batch, after arrival in the bale-store, is sampled and a yield measurement is performed. Payments made to the various growers are based upon these yields as well as the amount of rejects produced throughout the process.

The purpose of the water bath step is to induce opening of the shells. However this method of opening does not always affect all the mussels, leaving many unopened at the beginning of the next processing step. Mussels are examined at various times throughout a processing day, with centre temperature readings taken hourly unless a size grade change is implemented. The mussel centre temperatures are checked using a temperature probe connected to a Shimaden SD15 digital indicator.

The reason for the variability between those mussels that open and those that do not, is unknown. It is hypothesised that of major influence is the mechanism causing the release of the adductor muscle from the shell. Whether the "glue" which attaches the muscle, the shell thickness or one of any number of other

possibilities, is responsible for the variability of the opening is yet to be discovered. It has been shown that the water cooking effects 'fat' muscles (those containing more actual meat) less than smaller muscles. In this instance it is thought that a possible reason for the un-opening is the strength of the membrane which holds the two shells together. Problems arise in the processing of these larger mussels to half-shell, as although they have high yield, difficulty in opening of the shells tends to generate more second grade meat due to cuts and nicks.

The second major product processed in the Nelson plant, the meat line, employs the use of the water bath, although at a lower temperature, around $50 - 60°C$. This initial step is used as a preconditioning step in order to loosen the byssal threads, making their removal in the debysser easier. Following the debysser, the mussels enter a pressure cooker, which, through direct steam injection, cooks the mussels to a minimum internal temperature of $68°C$. The pressure cooker is kept at a pressure of around 42 psig, and the mussels are held in the chamber. This step functions as the listericidal step in the process. Following pressure cooking, the mussels are conveyed to a shaker, which serves to agitate the mussels. This results in the release of the cooked meat from the shell. The loose mussels drop into a cool water bath, and are then fed through a quality control sector, where inferior mussels are removed by hand.

Both processes are similar from this point forward. Checked mussels are transferred to a spiral freezer, where they are chilled to a temperature of $-18°C$. Upon exiting the freezer, mussels are wet with a water glaze, and then portioned off into various packaging sizes. The process flow chart for the operation is given in Figure 1.1 below.

Figure 1.1: Mussel processing flowchart for Sealords operation, April 1999

MUSSELS ARE HARVESTED

MUSSELS TRANSPORTED TO SEALORD SHELLFISH

MUSSELS HELD IN CHILLED BALE STORE READY FOR PROCESSING

MUSSELS EMPTIED FROM BALES ONTO CONVEYOR BELT, INSPECTED FOR QUALITY AND GRADED FOR SIZE.

MUSSELS CONVEYED TO COOKER

MUSSELS COOKED TO ENABLE OPENING (LEAVE COOKER AT 73°C AFTER 3-5 MINUTES

WATER SPRAYS USED TO COOL PRODUCT
Material Input

PRODUCT CONVEYED TO OPENING ROOM

MANUAL OPENING OF MUSSELS
Offal And Shells To Waste

MUSSELS WASHED
Material Input

INSPECTION FOR DEFECTS
Seconds Processed Separately

MUSSELS COOLED
Material Input

SPIRAL BLAST TO -18°C

WATER GLAZE
Material Input

TRANSFER TO PACKING ROOM

SIZE GRADING AND INSPECTION FOR DEFECTS

BAGGED AND WEIGHED AND HEAT SEALED
Material Input

PACKED INTO INNERS AND OUTERS
Material Input

TRANSFER TO COLD STORE

COLD STORAGE

1.2.1
Process constraints
for food safety

Heat shock processes employed during shellfish processing are required in order to eliminate the possible effects of listeria poisoning from the seafood. Research carried out by Crop & Food Research (Bremner and Osbourne, 1997) determined that the thermal death time (D value) for *L. monocytogenes* in raw mussels is 16 seconds at 68°C and 24 seconds at 67°C. By assuming that fewer than 1 in 100

mussels are contaminated with *L. monocytogenes* then a 4 decimal retention process meets the required standard of fewer than 1 in a million mussels exhibiting contamination following thermal processing. For the case of cooking at 68°C, the time required would be 64 seconds, and for processing at 67°C it would be 96 seconds.

It was found by Bremner and Osbourne (1997) that at the Sealord shellfish plant in Nelson, cooking profiles showed that in the half shell process, all mussels were above 68°C for approximately 2 minutes, and in the meat line, mussels were held above 68°C for approximately 4 minutes. Therefore both processing regimes were well above the 4D range. These processing constraints must be considered when any optimisation of the process is made in an attempt to improve yield and product functionality.

1.3 Project aim

The aim of the project is to identify practical ways to improve yield during processing of mussels. The process that Sealord Group Ltd. has direct control over, that affects cooking losses, is heat treatment so this was the focus of the project.

Initial research was undertaken to understand the mussels in terms of composition, functionality and how they are influenced by the processing conditions to which they are subjected. Further work was aimed at characterising the extent of cooking losses that occur during heat treatment. The effect of cooking temperature, time and heating rate in the mussel meat on cooking losses was characterised. The factors influencing the heating rate in the whole mussel, (including size, shell thickness, time of opening etc.), were then investigated. By considering this information with the death kinetics for spoilage micro-organisms and proteolytic enzymes, optimal processing conditions and/or alternative grading regimes could be identified. This information was then used to recommend process changes that result in increased product yield.

CHAPTER 2

LITERATURE REVIEW

2.1   Introduction

The purpose of carrying out this literature review was to answer some of the underlying questions related to the current research. It was important to understand the characteristics of the actual mussel itself and how these properties are likely to be affected by outside stimuli. Secondly, the amount of literature on mussels, and in particular the species *Perna Canaliculus*, is limited, especially in terms of the influence of heat on the functional and structural properties of the mussel. Research into the composition of various other meat types was taken to provide a basis on which a correlation between well studied products and mussel meat could be made.

The affects of heat transfer and thermal denaturation of the proteins within the mussel were then investigated. By bringing together all the available literature on these topics possible reasons for both the extent and variability in cooking losses could then be identified.

2.2   Perna canaliculus

The *Perna Canaliculus* is a bivalve mollusc which has soft body tissue enclosed in a pair of hard shells or "valves" (Jenkins, 1979). Taxonomically, mussels belong to the *Mytilidae* family, the genus *Perna* being one of the most important in terms of commercial applicability (Marques and Pereira, 1988). More commonly known as the green-lipped mussel, *Perna canaliculus* is a species unique to New Zealand,

and in the Marlborough Sounds forms the foundation of the mussel farming industry. Green-lipped mussels are worth close to $NZ100 million annually in export earnings (Garden, 1998).

2.2.1.1 Soft body tissues

The majority of the tissue in a mussel is the mantle lobe. Seasonally it has been shown, through proximate composition analysis that the carbohydrate levels fluctuate significantly (Tavares *et al.*, 1998). This has been attributed to the fact that during the spawning season, the glycogen storage levels within the mussel reduce and are replaced by reproductive tissue (Jenkins, 1979). During spring the mussels tend to flourish and are in their optimum condition, becoming large and more swollen.
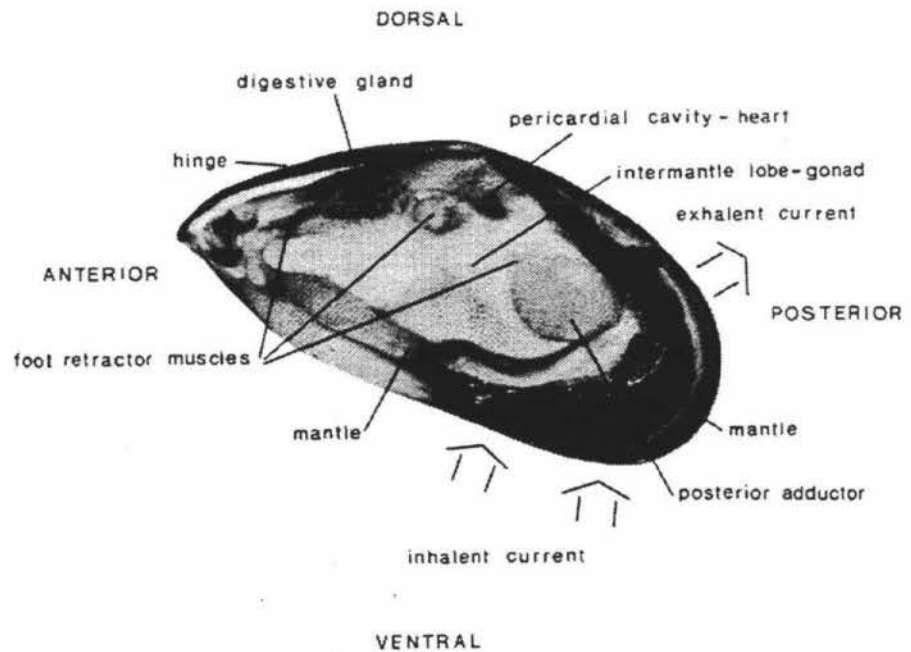
Figure 2.1: Mussel anatomy

The digestive gland, which surrounds the stomach, (shown in Figure 2.1) is a large mass situated near to the hinge. Adjacent to this is the pericardial cavity, which houses the heart of the mussel. A mussel heart is made up of a single ventricle and two auricles and can sometimes beat up to 25 times per minute (Jenkins, 1979).

The mussel tissue is attached to the valves by the adductor muscle. The adductor muscle is located at the posterior end of the mussel and is also responsible for shell closure. Additionally, the *Perna canaliculus* has three foot retractor muscles, one adjacent to the adductor muscle, one near the digestive gland and the third near the pointed or anterior end of the mussel. These muscles are paired and are attached to both the left and right valves, and join together at the base of the foot.

The foot, or tongue, of the mussel is a semi-permanent mechanism, which mussels have developed to enable them to attach to, and move along marine surfaces (Benedict and Waite, 1986). Usually the foot functions as juveniles seek out suitable positions for permanent attachment. Movement using the foot involves a snail-like gliding brought about by muscular lifting and shifting. Once the mussel has found an appropriate location, it ejects fluid down the length of the foot, which solidifies on contact with seawater, forming the characteristic bysuss thread. Further threads are laid down, the quantity of which is determined by how exposed the mussel is and how necessary it is for it to maintain its position.

Perna canaliculus has two paired gills, running the length of the mussel, which function as a respiratory device for the mussel as well as a means of providing food. The gills have tiny cilia attached to them, which beat regularly to circulate water through the gill filaments. The pattern of water circulation within the mussel is shown on Figure 2.1. The water carries food particles, which are trapped in mucus and transferred to the labial palps, which then sort out, through a size exclusion process, edible food particles. The food discarded by the labial palps, the pseudofaeces, is excreted through the mantle. Actual digested food is expelled through the anus, which is located near the exhalant aperture, allowing faecal matter to flow out with the exhalant current.

2.2.1.2 Mussel shell

The green-lipped mussel has two paired valves, or shells, which are joined at the hinge. Each shell has a common shape, with joint shells being very similarly curved. The hinge is situated at the pointed end of the mussel, the anterior, and on the dorsal, or angled edge of the shell. The shell is made up of a calcium carbonate layer, the inner layer, which is made up of two different crystalline structures, calcite and argonite. There is also an outer layer, which is known as the periostracal layer.
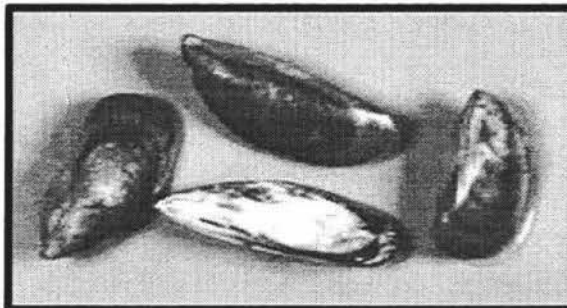
Figure 2:2:Shells

The rings that are apparent on the outside of the shell surface are indicative only of the environment to which the particular mussel has been exposed. In the case of some shellfish, rings on the shell can represent age, however for the *Perna canliculus* species this is not true. Physical interruptions in the mollusc's life such as exposure to air, and transferral to different living sites, can cause changes in the number of rings. Additionally mussels that are exposed to sunlight often exhibit a darker shell colour as the outer periostracal later darkens. This phenomenon has been observed in intertidal mussels located at the top of the culture ropes, where the shells are almost black (Jenkins, 1979).

2.2.2 Composition

Traditionally, protein, oil, moisture and ash contents (proximate composition), are indicators of the nutritional value of fish. In terms of applicability to the current work, the chemical makeup of the mussel, *Perna canaliculus*, is essential to provide a basis with which to compare the composition of various other, better-studied meats. There has been research carried out on several species similar to the *Perna canaliculus*, however it is more appropriate to use data focused expressly on the species farmed in Marlborough.

The Department of Scientific and Industrial Research produced a comprehensive book, "Proximate Composition of New Zealand Marine Finfish and Shellfish" in 1988. It details the proximate composition of some 62 New Zealand marine finfish species and 16 shellfish, which include *Perna Canaliculus*. The green-lipped mussels were caught in the Marlborough Sounds area, at a rate of 12 specimens per sample, in June of 1984.

Table 2.1: Proximate composition of shellfish (g/100g wet weight) (Vlieg, 1988 and George et al., 1988)

Species	Protein	Oil	Moisture	Ash	Soluble Carbohydrate
Perna canaliculus	11.9	2.1	80.9	1.7	3.4
Perna viridis	12.8	2.24	78.27	2.72	8.53 (Glycogen)

The study also calculated the gross energy of the edible part of the shellfish (per 100g wet weight) as 79 kcal or 332 kJ. It should be noted that the study conducted concentrated on calculating the composition of the mussels as a whole, whereas for the current work a more comprehensive breakdown-type analysis of the mussel is required. The breakdown analysis would seek to include a compositional analysis of the mussel as a whole as well as different sections, for example, the adductor mussel, the lip, the ends of the adductor mussel and various other portions.

A similar study was carried out on the species *Perna viridis*, a mussel species common in areas surrounding Calicut, India (George *et al.*, 1988). It was found that the majority of mussel meat solids are protein and glycogen, and that the meat can be considered to be a medium protein and medium fat food. A proximate composition analysis of the meat is shown in Table 2.1. Although these results are in a similar ballpark to the figures generated by the Marlborough Sounds experiments, the differences illustrate the importance of utilising data collected specifically in terms of the mussel species *Perna canaliculus*.

Mussels processed at Sealord Group Ltd., Nelson, are graded into three different sizes, based on the width of the individual shells. The majority of mussels

processed at the shellfish factory are medium or between 90 and 110mm in shell length.

Condition or "fatness" of a mussel is the term used to relate the body growth to shell growth (Jenkins, 1979). One method to measure this is by comparing the meat volume to the shell cavity volume. However, it has been found that a more straightforward approach of contrasting the wet weight to the total weight is equally applicable. Therefore:

$$Condition\ Index\ (CI)\% = \frac{wet\ weight\ of\ mussel\ meat\ (g) \times 100}{Total\ wet\ weight\ whole\ mussels\ (g)} \qquad \text{(Eqn 2.1)}$$

Naturally the accuracy of this measurement is dependent on the consistency of the procedure, and an ability to provide a representative sample of mussels. It has been found that with cultivated mussels the shells are relatively consistent in density and shape and are therefore not a significant source of error. Additionally, condition can be calculated on a steamed meat recovery basis as opposed to the total weight. In order to determine this mussels are cooked in a steam cooker at 100°C for five minutes, quickly cooled in cold water and weighed. The steamed meat recovery is calculated:

$$\%\ recovery = \frac{steamed\ meat\ weight\ (g) \times 100}{Total\ wet\ weight\ whole\ mussels\ (g)} \qquad \text{(Eqn.2.2)}$$

The wet weight should ideally be taken at the farm site, however processors often prefer to weigh the mussels on arrival at their processing site. It should therefore be noted that shell cavity water drains rapidly from mussels, and is responsible for a 10% weight loss over a five hours time-span. It is very difficult to determine the extent of water loss from the shell cavity (Fox, 1999) which adds to the variation in the mussels entering certain processing conditions. This is another aspect of interest in the current work.

2.3 General meat chemistry

There is a general lack of information concerning the cooking of mussel meat in terms of water losses, structural changes and alterations in functionality. This review will therefore begin overviewing muscle chemistry and then by comparison with different well-studied meats including beef and poultry, it may be possible to draw conclusions about mussel meat chemistry.

2.3.1.1 Muscle structure and functionality

2.3.1.1.1 Structure of skeletal muscle

Muscle contains water, protein, lipid, carbohydrate, minerals, organic extractives, and nucleic acids. Typically the chemical composition of an adult mammalian muscle is as shown in Table 2.2.

Table 2.2: Chemical composition of typical adult mammalian muscle (Lawrie, 1998)

Component	Amount (% by wet weight)
Water	75.0
Protein	19.0
Sacroplasmic	5.5 (28.9% of total protein)
Myofibrillar	11.5 (60.5% of total protein)
Myosin	50% of myofibrillar proteins
Actin	20% "
Regulatory proteins (e.g. tropomyosin)	8.9% "
Cytoskeletal proteins (e.g. Titin)	14.0% "
Connective Tissue	20 (10.5% of total protein)
Lipid	2.5
Carbohydrate	1.2
Soluble non-proteinaceous substances	2.3

There are three major types of protein found in meat; structural proteins, sacroplasmic proteins and myofibrillar proteins. Structural proteins (elastin and collagen) can vary between 2 and 8% in mature beef muscles, and are what is known as the connective tissue of the muscle. In fish, the amount of these

structural proteins can range between 3 and 10% (Ledward *et al.*, 1992). Sacroplasmic proteins are a very complex mixture of proteins, which are responsible for the metabolism within the muscle cell. Myofibrillar proteins, which make up about 60% of the muscle proteins, are fibrous in nature and their structural configuration is very important in relation to the properties of the muscle as a food.

Myofibrillar proteins are all soluble in a strong salt solution (typically about 0.6M) which gives rise to their importance in the emulsion stabilisation of salted or cured meats. They generally contain molecules of myosin, actin, tropomyosin and troponin, which all act to provide the overall functional properties associated with meat products. The sarcoplasmic protein is soluble in water and also in dilute salt solutions (0.1 M). They consist chiefly of albumins, myoglobin, haemoglobin as well as various enzymes. The structural proteins are for the most part collagen, which is insoluble in both water and salt solutions, but can be affected adversely when contacted with acid, or influenced by heat. Collagen tends to swell at higher pH value, and can ultimately affect the tenderness of meat due to the cross-linking of molecular chains (Sims and Bailey, 1992).

2.3.1.1.2 *Muscle proteins*

The muscles of various animals, from beef to different fish species, differ with regard to their visual appearance and quality. Structurally, however, there are several comparisons that can be made. A common structure exists which relates to the contractile cells or muscle fibres (Gault, 1992). The contractile mechanism is made up of actin and myosin filaments. The thick filaments are composed of the protein myosin, which has a long tail, and two curved pear-shaped heads. When aggregated in the form of the thick filament, myosin has a role in the increasing energy utilisation through regulation of ATP-ase activity and binding with actin in the contractile process. The thin filaments are made up of three different proteins, namely actin, tropomyosin and troponin, with actin present in the largest amounts. Actin molecules are small dumbbell-shaped particles, which on combining with 1 mol of ATP form a strand, known as fibrous actin. While a muscle is resting the thick and thin filaments are able to slide over one another inertly, however during contraction, the actin molecules are pulled over the myosin filaments, and form the complex actomyosin.
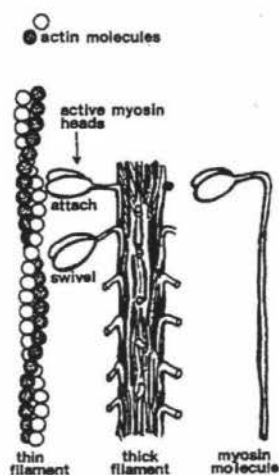
Figure 2.3: Diagrammatic representation of the binding of myosin heads to the thin filament. Source: Wilkinson, 1997

Two myofibrillar proteins, tropomyosin and troponin, combined in a complex regulate the interaction between myosin and the thin filament. Tropomyosin is a filamentous protein, which consists of two polypeptide alpha-helices which are coiled in a rope-like structure. This structure runs along the helical grooves of the actin strand. The structure of troponin is complex in that its globular form consists of three sub-units, (T) which bind strongly to tropomyosin (I), which in turn bind to actin but inhibits actin/myosin interaction, and (C) which binds calcium (Foegeding *et al.*, 1996).

2.3.1.1.3 *Connective tissue*

Connective tissue consists mainly of collagen. Collagen is inextensible due to intermolecular cross-links, which increase with animal age. Collagen affects the tenderness of meat, due to the toughening caused by these cross-linking molecular chains. It is the endomysial sheath, surrounding the muscle fibres (made up primarily of collagen), which is important when considering the thermal denaturation of all the muscle components.

The purpose of connective tissue is to surround muscle cells, so that the myofilaments are aligned and kept in a structural framework. The connective tissue exerts a force on the cellular proteins, and hence allows the contraction and

relaxation of the muscles. In terms of its concentration in meat, collagen is only a very small component, approximately 2%, however it is due to its high tensile strength it has been linked with meat texture (Foegeding *et al.*, 1996).

Table 2.4 shows the composition of several meat types. The data presented in this chart has been compiled from a larger number of resources, showing in this case the most representative research from each animal group. Overall mussel compositions vary quite significantly to those of other meats.

Table 2.3: Comparison of different meat types

	MUSSEL	BEEF	CHICKEN	TURKEY	OSTRICH	LAMB
	P.Canaliculus		Ducklin/Broil			Rib-Loin/Leg
	%(wetwt.)	%	%	%	%	%
	Vlieg (1988)	Paleari et al. (1998)	Smith et al. (1993)	Paleari et al. (1998)	Paleari et al. (1998)	Badiani et al. (1998)
Protein	11.9	20.1	21.39	20.4	22.2	20.2
Oil/Fat	2.1	4.5	1.99	3.8	1.6	2.6
Moisture	80.9	74.2	74.43	74.8	75.1	76.69
Ash	1.7	1.2	1.12	1	1.1	1.12
Soluble Carbohydrate	3.4					
pH		5.84		6.31	5.86	
Collagen		0.18		0.14	0.16	
Cholesterol (mg/100g)		50.1		36.6	33.8	73 (mg)
Fat/Protein		0.22		0.18	0.07	
Collagen/Pro x 100		0.89		0.71	0.71	
Gross Energy	79 kcal/100g		130 kcal/100g			104 kcal/100g

From the information it can be seen that the levels of protein found in most other meat types is greater than that found in the green-lipped mussel. Additionally, the mussels have approximately 6% more water than other common meats. Generally, most of the lipid levels are comparable between the different meats, including the mussel. The available information pertaining to the energy component of the animals indicates that the green-lipped mussel provides less energy than other meat products. In general it is suggested that any information regarding these meats could be used as a guide to the changes occurring within mussel meat, specifically during thermal processing.

Although compositional similarities are present between typical meat systems and mussel meat, differences do exist. In general, when most meat systems are cooked the meat is in a post rigor state whereby the actin and myosin complexes have formed. Mussels in comparison are in a pre-rigor state on processing and as such still have ATP present in the tissue. Additionally the bonds between the filaments will not have formed to the point where they are not naturally broken. Due to this difference it is important to ascertain as much information about the composition of the various mussel segments. The actual breakdown of the composition of the mussel, and in particular, *Perna canaliculus*, should help explain any trend variations exhibited in mussels compared to those trends normally shown in other meat systems. Additionally any attempt to model the changes occurring in mussels during cooking such as tenderness and texture will require initial knowledge of protein composition.

2.4   Structural and Functional Changes Induced by Heating

2.4.1 Overall effects of heat on meat systems

The retention of water in meat systems is important, both for economic reasons as well as in terms of consumer acceptance for the product. The amount of water retained in meat provide the producer with more product to sell in weight terms, and affects the quality of the product through influencing texture, flavour and juiciness (Offer *et al.*, 1984).

Upon heating meat there are several obvious structural changes that occur. There is a reduction in meat volume, combined with a loss of liquid, and a definite change in texture. It is generally believed that these changes are all related to the water holding capacity of the meat and the breakdown of several structural entities within the meat. These affect the ability of the muscle to retain water. The most marked changes in the heated meat system occur with the muscle proteins. It has been found that during heating, myofibrillar proteins breakdown resulting in the shrinkage of tissue and subsequent release of water (Hamm, 1986). The influence of heat on the connective tissue of the endomysium and perimysium has also been shown to effect the changes in cooked meat (Sims and Bailey, 1992).

Research has shown that several trends are exhibited in cooking meat, and that several relationships exist between different parameters involved in the cooking process such as shrinkage, extensibility, strength and shear force. Locker and Crase (1976) determined that the relationship between the degree of cooking and tenderness is closely related to the progressive denaturation of fibrillar and connective tissue proteins. Locker and Crase (1976) cooked strips of sternomandibularis muscles from prime ox. The strips were sealed in polythene pouches and heated in a water bath at various temperatures over different time periods.

At higher temperatures, the percentage cooking loss increased considerably. Additionally, the length of cook time influenced the amount of cook loss, where increases of 100% were encountered with a cooking time increase from 10 to 40 minutes (Locker and Crase, 1976) with the most distinct changes brought about at 70°C. The results of this work can be seen in Figure 2.3.
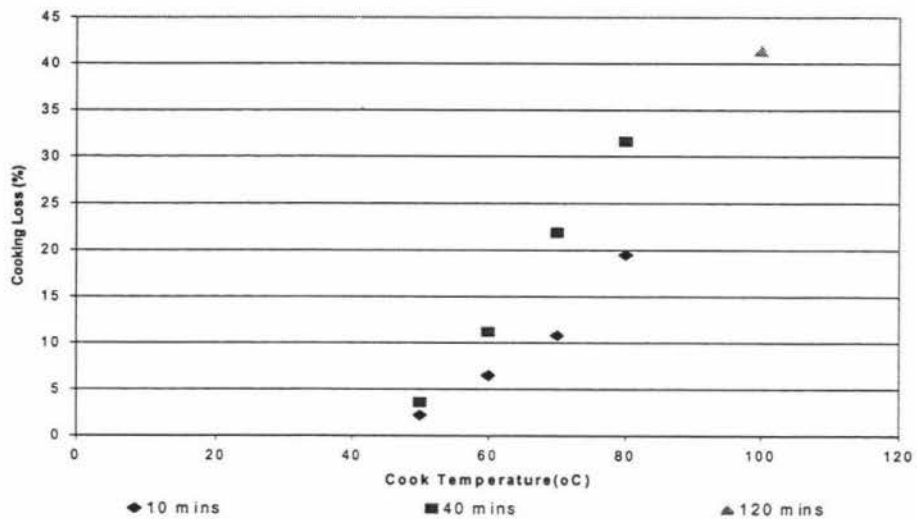
Figure 2:4: Changes in the cooking loss of strips of beef sternomandibularis muscle cooked to varying degrees. Adapted from data in Locker and Carse (1976)

Cook loss in turkey meat was measured by Northcutt et al. (1998). Muscles were weighed, and a cooking thermometer was inserted into the centre of each muscle. Using a water bath heated to 95°C, the muscles were heated to an endpoint internal temperature of 72°C. Each muscle was placed in a vacuum-sealed bag, and tempered to 20°C prior to immersion. Once the muscles reached the endpoint temperature, they were removed from the water bath and immersed in an ice water bath. Samples were then stored at 4°C overnight before being removed from the bags and weighed to determine cooking loss. The cook loss was reported as the percentage weight lost during cooking (Northcutt et al., 1998).

Sales et al. (1996) carried out a study to determine the fat content, cholesterol content, caloric value and fatty acid composition of both raw and cooked ostrich meat. The cooking loss methods utilised in this research involved the removal of external fat and epimysial connective tissue from steaks. Steak was ground, homogenised and vacuum-packed in plastic bags. The steaks were then stored at –20°C until analysis was performed. The steaks involved in the cooking trials were cooked to an internal temperature (monitored using thermocouples placed in the approximate centre of each steak) of 70°C in a preheated oven at 177°C. After

cooking, the steaks were then weighed, ground and homogenised. The cooking loss was determined from weights before and after cooking. It should be noted that in this case significant evaporative losses would have occurred.

In general, previous research studying the effects of heating on meat, have tended to measure the cooking losses on an overall percentage basis. The initial and final masses are often weighted against each other to give a percentage loss over the entire cook time. This approach is inappropriate for the current research, as it does not allow for an investigation into the changes occurring over time within the meat. In these studies, the temperature is changing in each sample as a function of both time and position in the sample. For this reason the results cannot be used to describe the kinetics of the cooking losses.

2.4.2 Mechanism of water loss

The mechanism for the changes due to heating described above are needed in order to best understand how to characterise cooking losses in mussels. Several studies have been carried out to generate a clearer understanding of the mechanism, which causes the loss of fluid from meat (Hamm, 1972, 1975; Blanchard and Derbyshire, 1975; Hansen, 1971). Water loss from meat products can be due to one of three things: evaporative losses, drip losses and cooking losses. The most prolific of these losses being those brought about through cooking (Offer et al., 1984). Muscle fibres are made up thousands of myofibrils, which in turn are comprised of both thick and thin filaments arranged in lattice of a hexagonal nature. Thick filaments are surrounded by six thin filaments. Myofibrils account for around 70% of the volume of lean muscle, and hence most of the tissue water is situated in the myofibrils (Foegeding et al., 1996). It has been proposed that it is the swelling and shrinking of the myofibril lattice that causes the uptake and loss of water from the muscle cell (Offer et al., 1984).

In raw muscle the fibres are surrounded by connective tissue, consisting of the endomysium and perimysium of the muscle cells. The fibres in living tissue are under tension with the collagen network, which holds everything together. Once the muscle cells go into rigor the fibres shrink, and although the collagen is still under

tension, the pressure it exerts does not act directly onto the fibres. Instead the pressure is applied to the aqueous solution held between the endomysial sheath and the fibres, causing it to be forced along this space to the cut ends of the meat ultimately producing what is known as drip.

During cooking, muscle undergoes a series of distinct changes that have been linked to the thermal denaturation of the various muscle proteins. From 45 – 60°C a shrinkage of the fibres transverse to the fibre axis is exhibited. Between the temperatures of 60 – 90°C a second shrinkage is apparent, this time in the longitudinal direction, parallel to the fibre axis (Offer *et al.*, 1984). The first change, at the lower temperatures, is due to the denaturation of the myofibrillar proteins, actin and myosin. It has been shown using electron microscopy that at this point the actin myosin complex shrinks within the still intact collagen network (Sims and Bailey, 1992). In this case, as with the "drip" situation, the force exerted by the endomysial sheath is now acting on the aqueous water separating the collagen and fibres. There is often a small loss of fluid at this stage, which could be due to the release of tension, on the denaturation of the actomyosin causing a flow of fluid down the annular channels (Offer *et al.*, 1984). Additionally, it has been found that an increase in the toughness of the muscle occurs at this stage, as indicated by increased shear force measurements (Sims and Bailey, 1992). This is probably due to the aggregation of the myofibrillar proteins.

The second loss of water occurs at a temperature of around 60 – 90°C, with an increased shear value at 65 – 70°C (Sims and Bailey, 1992). This is attributed to the shrinkage of the collagen (both the endomysium and perimysium) leading to an overall reduction in the size of the collagen network. The increased pressure on the aqueous solution causes it to be expelled more rapidly than from its original position (Offer *et al.*, 1984). The fluid released on the denaturation of the actomyosin is then forced out by the shrinking collagen network (Sims and Bailey, 1992). The degree of shrinkage as well as the amount of fluid lost is influenced by the nature and extent of the intermolecular cross-links stabilising the perimysial collagen fibres (Sims and Bailey, 1992). Therefore tissue which contains a large proportion of

highly cross linked collagen will demonstrate less shrinkage and hence less water loss attributable to the residual connective tissue strength not denatured by the heat (Sims and Bailey, 1992).

2.4.1.1 Factors affecting protein denaturation

The effect of heating pre and post rigor meat has been studied by Shin *et al.* (1993). It was found that increased shrinkage occurred at higher cooking temperatures and longer cooking times for both pre and post rigor meats. At set cooking temperatures it was shown that, apart from one exception, meats allowed to go into rigor lost significantly more weight than prerigor meat. Further it was determined that prerigor muscles were less tender than postrigor muscles. This effect was postulated to be due to the rate of heating at the various temperatures falling below the heating rate of 1°C/min required to achieve greater than 40% myofibrillar shortening (Abugroun *et al.*, 1985) which in turn produces tenderisation. This critical heating rate has been reported to bring about tenderisation of prerigor muscle as a result of heat-induced active contraction. It should be noted that mussels processed through Sealord Group Ltd., Nelson, are cooked in a pre-rigor state.

Many studies have been carried out to determine the influence of different salts on the water holding ability of meat (Hamm, 1986; Trout and Schmit, 1987). It has been found that myofibrils tend to swell in high concentrations of sodium chloride. The degree of swelling is more than enough to explain the water uptake in meat processing. By measuring the change in volume of the myofibrils when the salt concentration of buffer is changed it is possible to notice an increase in diameter of the myofibrils combined with an increase in sarcomere length. Increasing the concentration of NaCl buffers treating the myofibrils causes little change until 0.6 M is reached. At this point the myofibrils show a dramatic swelling of the myofibrils, with concentrations up to 1 M further increasing the swelling. Additionally it has been shown that the presence of pyrophosphate in salt solutions enhances this "swelling" property of salt solutions. Myofibrils have shown remarkable changes at

a concentration of 0.4 M NaCl. This trend is indicative of the synergistic effect of pyrophosphate, as the presence of pyrophosphate halves the required NaCl concentration of achieve maximum swelling. This may prove to be of importance due to the fact that often during processing mussels retain seawater within their shells. This would mean that during the cooking process, those with seawater would be subjected to water at higher salt concentrations than the standard cook water itself.

Research by Davey and Niederer (1977) showed the other factors, apart from the denaturation of the myofibrillar proteins and collagen may influence the textural changes in meat. The effect of the proteolytic activity of cathepsins and other tenderising enzymes may increase with temperatures around 65 – 75°C. Conversely, Bouton and Harris (1981) proved that compared to the denaturation of collagen, the role of proteases on texture at these temperatures is very low.

Differential scanning calorimetry (DSC) can be used to measure the changes occurring within a sample on heating. In this method a sample is maintained at the same temperature as a reference sample. The rate of heat input required to maintain the temperature is recorded and in the case of examining the thermal denaturation of myofibrillar proteins during heating it is possible to detect peaks at various temperatures. Peaks occur since the process of denaturation is endothermic, and the additional energy required by the heated sample during this dentuartion period shows as a deviation from the reference sample (Hastings *et al.*, 1985).

2.5 Characterisation of water in muscle

Several different ways have been developed to characterise the amount and degree of binding of water in muscle systems. This is termed the water holding capacity of the meat, but exactly what it is depends on the method of measurement.

Water holding capacity is a measure of the muscle tissue to retain its own water or added water during the application of a force. The location of the water in muscle tissue plays an important part in the losses of water from tissue on heating. Myofibrils constitute approximately 70% of the muscle tissue and hence most of the tissue water is located in them. They are therefore primarily responsible for the binding of water, which can exists in a variety of forms (Hamm, 1986).

- Constitutional water is located within protein molecules and comprises less than 0.1% of the total water in the muscle.

- Interfacial water is defined as water which is located at the surface of proteins, and makes up between 5 and 15% of the total water.

- Intracellular Water, which is the remainder of the intracellular water, not associated with the proteins. It is thought that this water behaves differently to those waters already mentioned, however the evidence to support this notion is inconclusive.

- Extracellular water is generally about 10% of the total water and is located in the dimension of extracellular space. This space alters in size depending on the degree of swelling or shrinkage of the myofibrils. Additionally it is possible that movement of water between the intra and extracellular spaces can occur.

Water that is not held in muscle as interfacial or hydration water can be expressed through the application of a force (Hamm, 1986). There can be significant changes in the WHC of stored or processed tissue. These changes are essentially dictated by the extent of immobilisation of the bulk cellular water within the microstructure of intact or comminuted tissue. It should be noted that changes in the WHC which are of most importance in practical processing sense, do not concern the fractions of either constitutional or interfacial water. Additionally there seems to exist a continuous transition between water, which is strongly immobilised within the muscle tissue which is difficult to get out, and loose water which can easily be squeezed out (Hamm, 1986).

There are several available methods for determining the water holding capacity of meat. They include the filter-paper press method, centrifugation methods, the suction method, as well as through measuring cooking loss. It is important to get an understanding of what it is that is actually being measured in these techniques. Hamm (1986) suggested the following terminology to describe the different phenomena associated with WHC.

Drip Loss (DL)   Formation of exudate from meat or meat systems (except thawing loss) without the application of external forces other than gravity.

Thawing Loss (TL)   Formation of exudate from meat or meat systems after freezing and thawing without the application of external forces. This is due to extra cellular water being frozen which freeze concentrates solutes. In order to reduce the concentration of these solutes, intra cellular water diffuses through the cell wall. Upon thawing, this water drips out rather than diffusing back into the cell.

Cooking Loss (CL)   Release of juice after heating of meat or meat systems either without or with the application of external forces. (e.g., centrifugation or pressing).

Expressible Juice (EJ)   Release of juice from unheated meat or meat systems (also after freezing or thawing) during the application of external forces such as pressing, centrifugation, or suction methods.

In order to measure these losses, two relatively simple methods to determine the water holding capacity are the filter-paper press method, and the centrifugation method.

The filter-paper press method (FPM) is commonly used due to its simplicity combined with the fact that past research has shown the method to be highly reliable. The WHC is measured by placing a sample between two Plexiglas plates on filter paper. A force is applied to the sample and the moisture that is released from the tissue is absorbed on the filter paper. The FPM is an excellent way to determine the WHC of a sample as it is also possible to use either ground or unground meat, it requires only small samples, and the sample can also be heat denatured. Additionally the technique is not time consuming and provides a result fixed on paper (Offer et al., 1984).

Methods of centrifugation have been proven to be relatively reliable provided both mincing and heating are avoided (Bouton et al., 1971, 1972). Additionally it has been noted that juice released from centrifuged meat needs to be decanted off very quickly to avoid readsorption. Following the extraction of tissue from the centrifuge tube it can be oven dried at a temperature of 105°C to determine the total water content. With the methods utilising non-minced samples, a very high gravitational force needs to be exerted by the centrifuge, however it has also been shown that at low speed, or indeed with minced meat, there is very little loss of juice on centrifugation (Wierbicki and Deatherage, 1959). In order to reduce the likelihood of readsorption, several variations to the centrifugation method have used fritted bottom to separate the juice from the meat sample.

The measurement of cooking loss has also been used to give an indication of the water holding capacity of a sample. Samples are heated in a pre-weighed centrifuge tube and differences between initial and final (on cooling) weights indicate the cooking loss on a percentage basis. The use of centrifugation methods combined with cooking loss, does enable cook juice to be separated however studies have shown that by simply blotting the sample after cooking often yields similar results to centrifugation.

2.6   Conclusions

From the literature review it is possible to draw the following conclusions.

1. The measurement of the condition of the mussels is required to account for the possible variability in the raw sample especially over time.

2. The measurement of composition is required, in terms of water and protein, in the mussel parts to identify similarities with more studied meat systems.

3. The measurement of cooking losses is required to be carried out in such a way that allows for the separation of the kinetics of cooking losses and the kinetics of heating (i.e. isothermal cooking experiments).

4. The determination of a method to characterise the water content in mussels is required that distinguishes the free extra cellular water from the intra cellular water that is primarily effected by the thermal treatment.   Centrifugation methods are most suitable for this.

5. It should be possible to relate the amount of water losses to the rates and extent of protein denaturation.

6. Protein denaturation can be most easily measured using differential scanning calorimetry (DSC).

CHAPTER 3

MUSSEL CHARACTERISATION

3.1 Introduction

Mussels destined for processing can exhibit extreme variability in physical size, meat to shell weight ratio, degree of stress, age, and also time out of the water prior to processing. As a consequence, mussels subjected to a particular processing operation will exhibit variability in finished product quality. A grading system is used whereby processing conditions are modified to account for shell size in an effort to reduce this variability.

To identify other ways to reduce variability and maximise product quality, it is important to understand the reason for the variation shown in the samples. Seasonal changes occur in mussels and these are primarily related to the sexual cycle (Jenkins, 1979). Glycogen accumulates in the gonad tissue of the mussel during the winter and spring months, and is subsequently utilised in the production of eggs and sperm. If a mussel has an insufficient food supply, the glycogen store is used up and mussel 'fatness' is lost.

Past research has measured the change in "fatness" by relating the proportion of the shell cavity filled by mussel meat. It was determined that a peak occurs in the spring with a drop during the spawning period of summer. In an environment with variable conditions such as in the Marlborough Sounds a more complex cycle is likely to exist (Jenkins, 1979).

3.2   Mussel condition

The variability of the raw mussels caused by the environmental conditions has the potential to be a significant factor in effecting the cooking yield of the mussel. For this reason, a measure of the condition of the mussels prior to experimental work was required. As stated in section 2.2.3, the condition or "fatness" of a mussel is the term used to relate the body growth to shell growth (Jenkins, 1979). For the duration of this work, the condition index was determined using the equation 3.1.

$$Condition\ Index\ (CI)\% = \frac{Wet\ weight\ of\ mussel\ meat\ (g)}{Total\ wet\ weight\ whole\ mussels\ (g)} \times 100 \qquad (Eqn\ 3.1)$$

It is preferable if the wet weight is recorded at the farm site, however in this case all measurements were carried out in Palmerston North, after transportation by overnight courier from Nelson. To give a more consistent basis for the calculation the influence of shell cavity water on the condition was not included. Throughout the course of the study it was found that "fat" mussels tended to be bulkier in the mantle tissue area and typically yielded a Condition Index in the high 30's percent range. Conversely, "skinny" mussels, with a Condition Index around 30 percent were also common. These "skinny" mussels were often provided by Sealords after periods when harvest was prohibited due to weather.

3.2.1 Methods and materials

3.2.1.1 Sample

Mussel samples were made available from Sealords Ltd. Nelson on a twice weekly basis, and arrived at Massey University at approximately 8am Tuesday and Thursday, after transportation from the Nelson site. Mussels were packed in a polystyrene-bin, and kept chilled with ice packing. On arrival at Massey University the sample were kept chilled in a 4°C room. Generally the samples which arrived had been harvested two days prior to arrival at Massey University and were used within two days of arrival.

Each mussel lot used in subsequent experiments was tested for Condition Index. The condition index was performed on a random sample of six mussels. The individual mussel shells were thoroughly cleaned to remove mud, crust and clinging byssus thread, and weighed to give the total wet weight of the whole mussels. Each mussels was then shucked raw by slicing the adductor mussel and freeing the meat from the shell by hand. The meat mass was weighed to give the wet weight of the mussel meat. From these measurements the condition index as given in equation 3.1 was calculated.

3.2.2 Variation over the sampling period	Over the sampling period the condition of the mussels altered quite substantially, with the condition index ranging from 31% to as high as 46%. In comparison to data published by Jenkins (1979), shown in figure 3.1, the condition of the mussels are very high. This could in part be due to variation in the experimental methodology used in this work. Six mussels were used to calculate the condition index for each batch. This was due to the limited amount of mussels supplied and the time required to characterise larger samples.
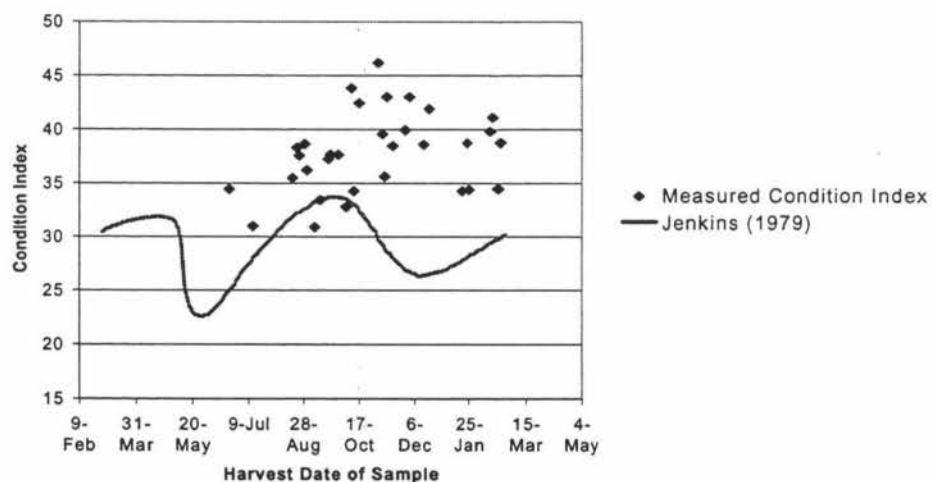
Figure 3:1: Variation in condition index over experimental period

The trend illustrated in Figure 3.1 is of increasingly better condition towards the end of the year through the spring months. The mussels sampled into the summer months did not show the characteristic drop in condition concurrent with spawning

season. Uncommonly low temperatures in the Marlborough Sounds during the 1999-2000 season may have contributed to this trend by limiting the spawning. The reason for the release of eggs and sperm is unknown, however explanations have been postulated ranging from the effect of moon phases, temperature changes and chemical stimuli on spawning (Jenkins, 1979). The samples contained both male and female mussels in varying ratios throughout the sampling period, which may have also contributed to the atypical trend. Additionally, since the Jenkins data was prepared in the late 1970's, improved farming practises may have contributed to a better quality product.

3.3 Natural pH variation

There is a natural variation in pH between mussels. When considering the volumes of mussels sampled, this variation could produce problems where a uniform initial sample is required. For subsequent cooking yield experiments, the pH of the sample could impact on the potential of the sample to bind water, since the pH of meat directly affects the water-holding capacity of muscle protein systems (Hamm, 1975).

The pH of individual mussels was determined by inserting a PROBE, attached to an Orion model 250A pH meter, into the mussel tissue. Three separate readings of pH were taken in order to determine the variability in the readings, with the probe positioned within the mantle of each mussel. The experiment was carried out different times of the year in order to assess the variability in pH over the changing season. Additionally the sex of each individual mussel was recorded to examine its influence on the pH.

Figure 3.2 below shows the variation exhibited in the natural pH of live, freshly harvested mussels. It was found that the average pH was around 6.8, with no excessive variation throughout the sample or between the different sampling times.

Interestingly, there was a noticeable difference between the pH of the mantle tissue between male and female mussels, however this was found to be insignificant.
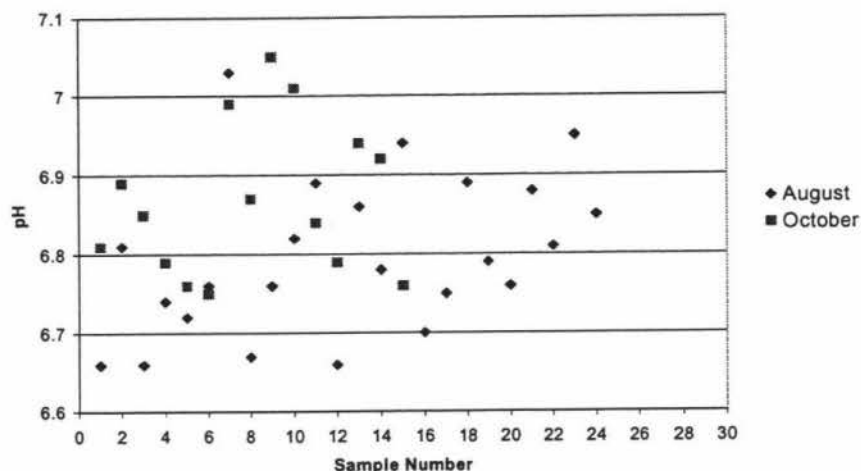
Figure 3:2: pH variation in raw mussels

3.4 Natural Salt variation

Salt is also known to significantly influence the water holding capacity of meat systems. For this reason the salt concentration of raw, freshly shucked mussels was determined in order to assess the potential of the salt to increase the water holding capacity of the mussel meat tissue. One gram of frozen minced mussel was diluted 100 fold with water. The sample was injected into a Dionex ion chromatograph (DX100 fitted with a Dionex AS98C cloumn) in a 20μL injection loop. The machine was calibrated to detect phosphate, nitrite, nitrate, chloride and sulphate ions.

It was found that in the natural state the average chloride concentration was 14 mg/L on a wet whole mussel basis. Since mussels are grown in salt-water conditions this value seems feasible as it is in the same order of magnitude as sea

water which in terms of chloride concentration is typically around 25 mg/L. Water constitutes up to 80% of the entire mussel weight therefore the water in the mussel would have a salt concentration of approximately 17.5mg of salt/L. This information is important when considering the effects of water cooking on mussel meat, where it is likely that the salt concentration will be diluted, due to leaching of the salt out of the mussel flesh.

3.5   Mussel composition

The actual physical makeup of the mussel has been discussed at length in section 2.2.2, where the proximate composition was given as:

Table 3.1: Proximate composition of shellfish (g/100g wet weight) (Vlieg, 1988)

Species	Protein	Oil	Moisture	Ash	Soluble Carbohydrate
Perna Canaliculus	11.9	2.1	80.9	1.7	3.4

The exact composition of the various mussel fractions is important when considering the heat transfer properties of the fractions as well as the ability of these fractions to retain water. The water holding capacity of muscle tissue is greatly dependent on the water-imbibing properties of myosin (a myofibrillar protein) (Hamm, 1986). Therefore, the quantities of these proteins in the mussel provide information as to the likelihood of the magnitude of water losses from the various mussel regions and the temperature range at which these losses occur.

In order to establish how much each proposed segment of the mussel constitutes to the overall mussel, a weight comparison of several segments of a sample of mussels was conducted.   Six mussels were dissected into four fractions; the adductor, the foot, the lip and the body. Each sample was then weighed to determine the percentage weight contribution. Additionally samples were minced and then duplicate samples of approximately 5g were held overnight at 105°C to

determine the moisture contents of the various samples. Figure 3.3 shows the typical makeup of a *Perna caniliculus* mussel.
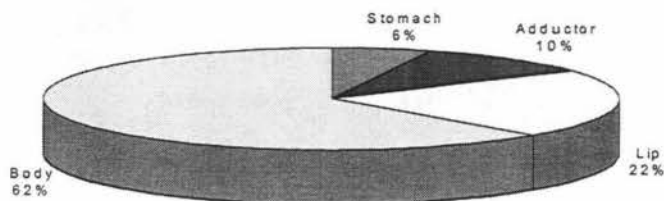
Figure 3:3: Make up of a typical green-lipped mussel by raw weight

From Figure 3.3 it can be seen that the major contributor to the overall mass of the mussels is the body. Furthermore it is interesting to note that the lip is also substantially heavier than the other fractions studied.

Work carried out by Williams (2000) sought to determine the composition of the segments outlined above. The method of Howell *et al.* (1991) was followed to extract the various proteins, sarcoplasmic, myofibrillar as well as the connective tissue. There were several problems encountered throughout this work, mainly in terms of quantifying the connective tissue content of the mussels. As a consequence the method was modified to include freeze-drying the samples to enable a course powder to be obtained following dissection, thus increasing surface area for contact during the various extractions.

Table 3.2: Protein content of freeze-dried mussel segments (Williams, 2000)

Mussel Segment	Myofibrillar Proteins (%)	Sarcoplasmic Proteins (%)
Body	68.0	25.1
Lip	67.0	29.0
Adductor	64.4	30.4
Foot	74.9	20.5
Whole Mussel	68.0	25.9

From Table 3.2 it is possible to see that the myofibrillar protein content ranges between 64.4 and 68.0%, the sarcoplasmic content between 20.5 and 30.4%.

In order to characterise the level of connective tissue in mussel meat the amount of hydroxyproline in the mussel meat was measured. Samples were sent to the Institute of Food, Nutrition and Human Health at Massey University for an amino acid screening. This method provided the most reliable measure of hydroxyproline, however the cost associated with this type of analytical analysis is very large and therefore only one adductor muscle sample was analysed. Table 3.3 shows the results from these analyses.

Table 3.3: Amino acid results for mussel adductor sample

Amino Acids	Mussel Adductor FD Basis
Hydroxyproline	1.59
Aspartic Acid	72.32
Threonine	29.14
Serine	29.38
Glutamic Acid	111.79
Proline	18.82
Glycine	53.89
Alanine	40.65
Valine	27.86
Met	17.05
Isoleucine	29.61
Leucine	55.75
Tyrosine	22.96
Phenylalanine	20.03
Histidine	11.17
Hydroxylysine	0.56
Lysine	49.91
Arginine	66.77
Units	mg/g

Table 3.3 shows that in any attempt to determine the amount of hydroxyproline in the mussel it would have difficult to quantify the very small amounts very accurately. In meat derived collagen hydroxyproline comprises 14% of the amino acids (Sims and Bailey, 1981). This results in an approximate collagen concentration of 11.4 mg/g, which is 1.14% of the dry adductor muscle weight.

To evaluate these results the work of Chinnamma et. al, (1988) can be used as a comparison. They found Perna viridis, a close relative of the Perna caniliculus, comprised of 2.033g% of protein nitrogen and 2.291g% of total nitrogen. Of protein nitrogen, approximately 53% was myofibrillar, with a further 22% of sacroplasmic proteins. Overall it was determined that the green mussel is a low fat, medium protein food, with a large amount of carbohydrate reserve. In comparison, the results obtained by Williams (2000) determined a myofibrillar content of 68% for a whole mussel sample. The sarcoplasmic content was more comparable at 26%, indicating that the collagen content of the mussel tissue is likely to be low. The low collagen content would explain the tenderness of shellfish compared to red meat. Collagen in red meat muscle is 2-10% of dry weight (Ledward et. al, 1992) explaining the increased toughness of red meat systems.

3.6   Conclusions

From the work described in this chapter regarding the determination of mussel characteristics it is possible to draw the following conclusions:

1. Mussels are highly variable in condition which can contribute difficulties in terms of carrying out reliable, consistent cooking experiments.

2. Mussels examined from the Marlborough Sounds exhibited a range of condition indexes, mainly in the 30 – 40 percent region. Differences are apparent between visually "skinny" and "fat" mussels as well as different times of the

season, with mussels of better condition found through the spring months. On the whole, condition was better than previously reported by Jenkins (1939).

3. There was no evidence of variation in pH throughout the sample of mussels tested, with an average pH of 6.8 common for both male and female mussels. This suggests the mussels were in good condition upon arrival at Massey University.

4. The average concentration of salt in mussels in their natural state is 14mg/L on a wet basis. Considering mussels are approximately 80% water, there is 17.5mg/L of salt within the water fraction of the mussel.

5. The major source of moisture in mussels and hence the source of moisture loss of is the body.

6. The myofibrillar content of mussels ranges from between 64 and 68%, the sarcoplasmic content between 20 and 30% and the collagen content was 1.14% on a dry weight basis.

The preliminary characterisation of the mussel condition and composition outline in this chapter highlighted the need to establish a consistent basis for subsequent cooking loss experimentation. The condition changes throughout the season and composition can vary with mussel part. The literature review highlighted how water can be categorised according to binding strength. For these reasons experimental methods for characterising changes in moisture binding during cooking must be developed before work on determining kinetics of cook losses could be carried out. This is the focus of the next chapter.

CHAPTER 4

CHARACTERISATION OF THE MOISTURE CONTENT OF MUSSEL MEAT

4.1 Preliminary variability

There are numerous problems associated with the determination of water within a meat sample, and these are compounded when studying a system such as mussel meat. Preliminary trials have indicated the vast variability present between different mussels in terms of meat to shell ratios, pH. In order to work towards the development of a method to establish consistent cooking loss results it is first important to understand the different types of water in the mussel tissue, and the variability associated with this water. Much research has been carried out in the field of meat research on these properties, which affect the loss of water from meat systems. There are several mechanisms by which the loss of water from muscle tissue can be explained.

4.1.1 Nature of water associated with muscle

There are three major types of water losses, which can be used to describe meat systems. These include drip losses, expressible juice, and cooking losses. Additional losses can occur during the thawing of meat products, however these losses are not relevant to the current work. Drip losses occur in meat without the exertion of any external force, whereas expressible juice is the release of juice from unheated meat under the application of some external force. Finally cooking losses are similar to the expressible juice however they are measured once the meat system has been subjected to heat (Hamm, 1986).

It is important to focus on the water which is located in the muscle tissue as bulk or free water. The immobilisation of this water in interfilamental spaces, or the filaments themselves, causes the major variations in muscle water holding capacity. Bulk water can also be found in extracellular space, which can be easily expressed through the application of an external force, such as pressure or cooking. Therefore it is important to quantify the cooking losses in a way that distinguishes them from the free water present in the mussels. Without doing this, it will be difficult to accurately quantify cooking losses. It is likely that the main cause of variability in the water content of mussels is due to differences in the amount of extracellular moisture present. For this reason some form of measurement of bound water is likely to reduce the variability between individual mussels. This will maximise the chance of quantifying the changes brought about by the cooking process.

The problems facing research into the water holding capacity of mussel meat are extensive mainly due to the lack of uniformity in the raw material. Provided a suitable method is established to determine the quantities of free and bound water in raw mussel samples, this could be used to examine the changes in water content in mussels caused by cooking.

4.2 Characterisation of moisture in mussel tissue

Initial work was carried out to establish a reliable method of determining how much water is contained within an individual mussel. Such a method was required to allow the measurement of the affect of cooking time and temperature on mussel meat cooking losses. Two methods commonly used to characterise water in meat systems were investigated for this purpose, the filter-paper press method and centrifugation both described in Hamm, 1986.

When considering each method it was important to consider that there are two forms of water present in meat tissue, free and bound water. It is the free water,

which is located between the filaments which is more easily removed. In comparison, bound water is more tightly bound to the actual meat structure and is often connected with the muscle proteins. As such it is likely that the cooking process releases bound water due to protein denaturation reactions resulting in the production of more free water.

4.2.1 Water holding capacity

In order to gain a better understanding of the water holding properties of mussel meat it is important to examine these properties at a fundamental level. Through utilising techniques usually applied in the area of meat research it will be possible to gain a closer insight into the functionality of the mussel meat.

Water holding capacity is a commonly used method to characterise the degree of water binding in meat products. These are methods which aim at distinguishing bound from free water. For this reason the methods available for measurement of water holding capacity were investigated for use in the characterisation of mussel tissue. The specific objectives of the water holding capacity trails were to:

1. Compare the water holding capacity of ground and unground meat, in terms of water losses from both the bulk water phase and the bound water. It was thought that the grinding process would not affect the bound water content.

2. Try to eliminate the variability between individual mussels by mincing together several mussels.

3. Avoid the problem of temperature gradients occurring during the heating of the mussel samples in subsequent cooking trials. A minced product could be squeezed into a flat layer which provides very fast heat transfer.

Overall the objective of this body of work was to determine a consistent method to measure the amount of water in mussel meat, both in terms of free and bound water. From this work it would be possible to determine a feasible method by which to quantify losses from minced mussels and whether it will be possible to carry out further experiments with communited meat. It was expected that there would be

differences in using minced meat compared with whole samples, however for ease of experimenting in the future minced meat was a more practical media. It was expected that for the mussels, although water losses in the bulk water may be affected, the bound water that tied up in proteins will be affected by heat as they would, were the mussel intact.

The water holding capacity is one of the most important factors in terms of the economic value and quality of meat. This is because it influences weight changes during transport and storage, drip losses, cooking weight losses, including shrinkage and weight loss, as well as sensory qualities (Irie *et al.*, 1996). The water holding capacity of the meat will be examined by first taking into account drip loss, then through subjecting the meat to an external force. This will provide information as to the quantities of water associated with the bulk water phase. Further oven drying of samples will indicate the amount of water held as bound water in the mussel tissue. The filter paper and centrifugation methods were investigated to achieve these purposes.

4.2.1.1 Filter paper press method

The filter-paper press method can be used to quantify the amount of water present in a sample. This method was developed by Grau and Hamm in the 1950's for both intact and ground tissue samples (Hamm, 1986). It involves the excision of a small sample, around $1cm^2$, which is placed on filter paper between two plexiglass plates. The plates are then subjected to an external pressure which in this case was 6kg, for a period of 15 minutes. Once the filter paper is removed from between the plates, there are two areas which need to be considered to determine the water holding capacity. The first is the area covered by the actual meat sample itself (meat area), and the second, the area of filter paper through which the water in the sample has travelled (liquid area).

There are several methods available to quantify the water holding capacity following essentially the same experimental technique. Kauffman *et al.* (1986) derive a ratio of meat area to sample weight (g), whereas by Hoffman *et al.* (1982) and Sakata et

al. (1991) used a meat to liquid ratio. Irie *et al.* (1996) took the process one step further and quantified the meat and liquid areas on the filter paper using video image analysis (VIA) and a planimeter. A planimeter measures the area of the sample by measuring the circumference of the water spread. Irie *et al.* (1996) presented a correlation between the VIA , the meat/liquid ratio and the meat area/sample weight (g) ratio. It was found that correlation coefficient of the meat/liquid area ratio by VIA to the conventional method was 0.92. The ratio comparing meat area to sample weight was less highly correlated therefore it is more appropriate to use the liquid/meat ratio despite not having the facilities to perform VIA. In this work a simplified approach was taken where it was assumed that the areas were directly proportional to the weights of photocopied samples. Using this assumption the areas were calculated using equation 4.1.

$$A = \frac{\text{Weight due to Meat}}{\text{Total Weight - Weight due to Meat}} \qquad \text{(Eqn. 4.1)}$$

Trials were performed using both whole mussel tissue and homogenised samples. The intact mussel tissue was taken from the mantle of the mussel and approximately 1cm^2 samples were used. These samples were of varying thicknesses due the variability within the raw sample. An effort was made to reduce the inconsistencies by sampling similar areas, however it is likely that a large experimental error will still exist with this practise.
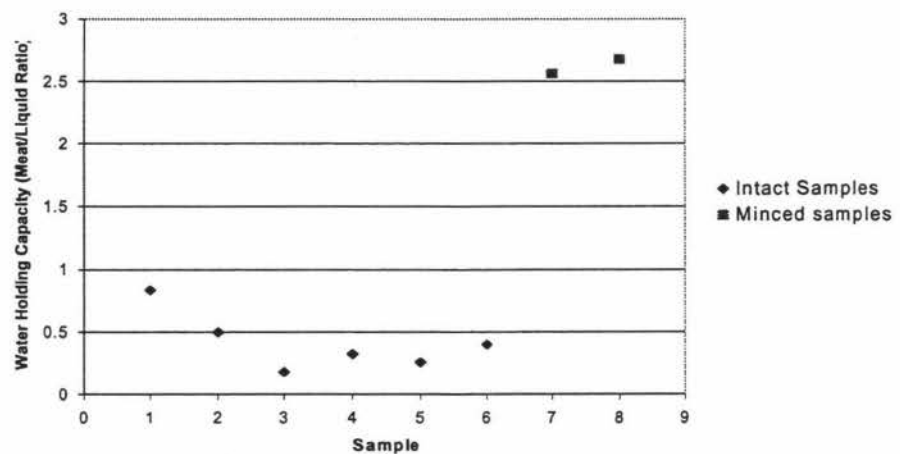
Figure 4:1: Water holding capacity using filter paper press method

From figure 4.1 it is possible to see the scatter present in the water holding capacity of the intact mussel tissue as well as the results of the homogenised samples.

It was interesting to note that the homogenised samples were highly mobile in that the sample moved to the outer boundary of the liquid area. The resulted in no difference between the meat and liquid areas, hence the odd results presented in Figure 4.1. It was found that although the method was relatively fast and required little in the way of experimental equipment, the results were very inconsistent.

Additionally the results from the actual sampling were varied, with some samples expressing large amounts of liquid and others very little. It became apparent that in order to sample the mussels for the filter-press method, the whole of the mussel could not be accounted for. The use of homogenised mussel to counter this was found to be inappropriate due to the spread of the tissue with the liquid.

From the filter paper press trials it was determined that although the method provides a simple and relatively fast approach to determining the water holding capacity, the benefits are overshadowed by not being able to obtain consistent results.

4.2.1.2   Centrifuge method

The centrifuge method can be used to determine the water holding capacity of meat samples. This method involves the centrifugation of a weighed sample. The water which is 'spun' out of the meat is then quickly decanted to avoid readsorption and the liquid loss can be quantified by weighing the meat sample. Further to this, the sample can then be dried to 105°C overnight to determine the bound water content. Literature suggests that for red meat systems the centrifugation method be carried out using extremely high rpm's and for extensive times. For the mussel meat, although the majority of the muscle proteins are myofibrillar, as in meat, the actual tissue is not as strong as ordinary meat (containing less collagen) and therefore a method of centrifugation at lower speeds could be possible. The following sections outline developmental work in applying the centrifugation method to mussel meat.

The initial method employed involved the spinning of mussel samples, both intact and ground, in a Hermle Z320 centrifuge set at 4000rpm for 30 minutes. The water removed by the application of this force was then decantered, and the remaining sample weighed to determine the liquid loss. The remaining meat sample was dried at 105°C in an oven overnight to determine the remaining water "bound" within the meat structure. This method presented many problems as it was trialed. It became evident that even if a centrifuge tube was decanted as soon as the centrifuge came to a halt, there was a fair amount of readsorption of water.

The possibility of utilising a false-bottomed centrifuge tube was mentioned in literature as a way to help to eliminate the readsorption problem (Hamm, 1986). The method was modified to include a false bottom made of wire mesh (size 60/60) suspended on glass beads upon which the mussel or mince was placed. This allowed for the separation of liquid from the meat since the mesh could then be removed and the water forced through the mesh accounted for separately. Trials proved that greater separation was achieved using the mesh and glass beads however concurrent with this there was also a loss of suspended solids through the mesh, which otherwise would have remained with the solid sample.

An additional trial was performed to test the need for running the centrifuge for extended periods of time. Duplicate samples of whole mussles were centrifuged for 5, 10, 15 and 30 minutes and it was shown that, in terms of the free water expressed, the longer centrifugation time provides for a better separation of water from the mussel.

From Figure 4.2, below, it is possible to see that after a centrifugation time of around 25 minutes the free water content spun off reaches a steady level. Therefore the spin time of 30 minutes, initially employed was maintained for future experiments. It was also found that if the water content of the mussels were expressed on a dry basis the variability in the samples was reduced.
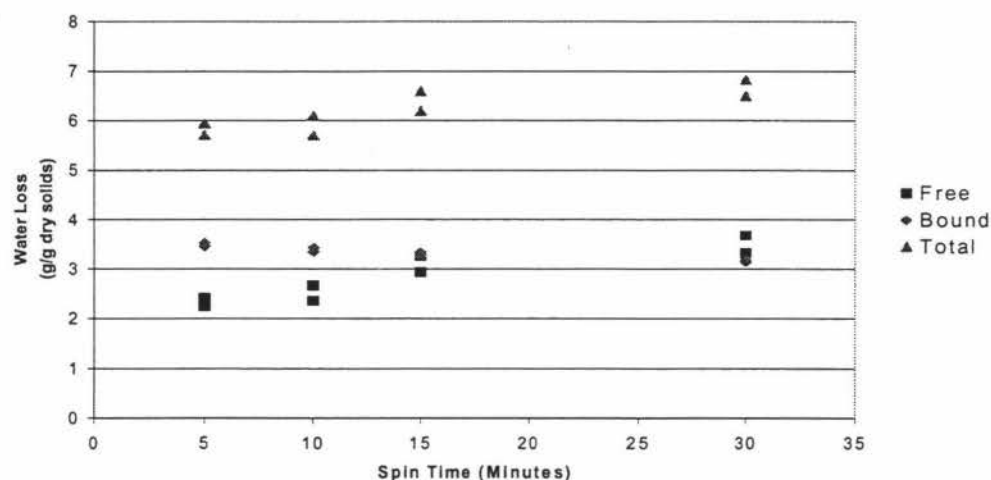
Figure 4:2: Water losses as a function of centrifuge spin times

As a consequence of mincing the meat during these experiments significant solids were lost through the wire mesh. Due to the unexpected occurrence of suspended solid loss through the mesh trays, it was necessary to account for these losses. Some efforts were made in an attempt to lower the losses. However as Figure 4.4 shows after centrifuging for short times, the suspended solids lost through the mesh are as high as the losses exhibited at longer centrifuge times. Although some solids are lost through the mesh, this can be incorporated into the results by drying the extract, and the separation benefits of the mesh far out weigh the possible problems associated with the suspended solid loss.
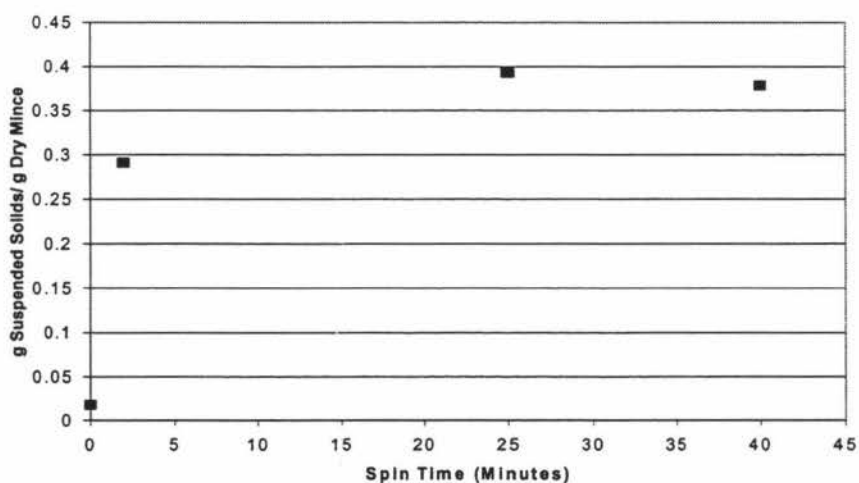
Figure 4:3: Loss of suspended solids at various centrifuge spin times

Throughout the trials the suspended solids lost through the mesh ranged between 10 and 30% of the dry weight of the sample. This illustrates the necessity of quantifying the suspended solid losses and incorporating them in the overall dry weight of the sample. Additionally the fact that the developed method was intended as a comparative technique meant that provided consistent results were obtained the method would be suitable (i.e. WHC of mussels cooked at 50°C compared to the WHC of mussels cooked at 90°C).

4.2.1.3   Comparison of moisture in whole and homogenised mussels

Initial trials utilising the centrifuge tube, without the glass beads or wire mesh, yielded interesting results in terms of the differences between sampling whole and minced mussels. Minced mussels were used to try and eliminate the variation in mussel characteristics from mussel to mussel. Preliminary cooking experiments were carried out to determine the consistency of results from individual mussels. It was found that there is extensive variability between the separate mussels. This was carried out by blending six mussels into a homogenous solution. Figure 4.5 shows that blending gives much more consistent results when comparing different samples. The moisture content (both free and bound) were much more uniform between blended samples than for whole mussels.
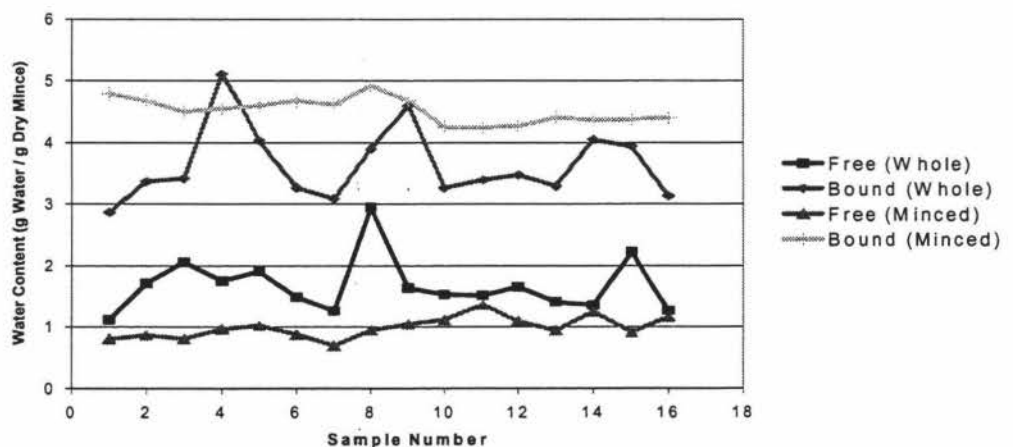
Figure 4:4: Comparison between uncooked whole and homogenised mussel water contents

Homogenising the mussel meat clearly provides more consistent separation of water through the centrifugation stage, and thus more consistent results from what is essentially a highly variable sample. Additionally the use of homogenised mussels could also prove useful in terms of the heat transfer during cooking. In order to study the changes occurring in mussel meat during cooking it is important to investigate protein denaturation and heating separately. If the mussel is too thick the rates of thermally induced change will vary depending on the position within the mussel. Since the size of mussels can vary a lot, cooking rates throughout the mussel are likely to affect the overall cooking losses.

The use of a minced mussel sample would allow for the sampling of one large homogeneous sample. A small thin sample of homogenised mussel could be used, reducing the temperature gradient in the mussel. On the other hand, the use of whole mussels is closer to the actual processing situation employed at Sealord Group Ltd, and only poses the problem of increased variability. For these reasons it must be clear that the results of a study on a homogenised sample are directly applicable to the individual processing of whole mussels.

Mussels are processed into the water-bath alive, and hence in a pre-rigor state. There is the possibility that accelerated rigor onset may be caused by mincing the mussels. In animal meat it is generally considered that before the onset of rigor mortis, muscle tends to be tender and the muscle proteins have a high water holding capacity (Laakkonen, 1973). When meat undergoes the transformation to the post rigor state, bonds between myofibrillar proteins result in a more rigid (meat) structure which has quite different functional properties than pre rigor muscle. In order to keep ATP levels high, anaerobic glycolysis occurs after death. This causes production of lactic acid in turn causing a pH drop. Mincing of pre rigor meat has shown a very fast pH drop.

To investigate the possibility that mussels experience accelerated rigor onset during mincing trails were carried out to examine the change in mussel pH after mincing. Figure 4.6 shows the trend in pH from the initial pH of a whole mussel after mincing.

The rapid pH fall shows the changes after mincing are significant and indicate the modification of the mussel meat to a post-rigor state.
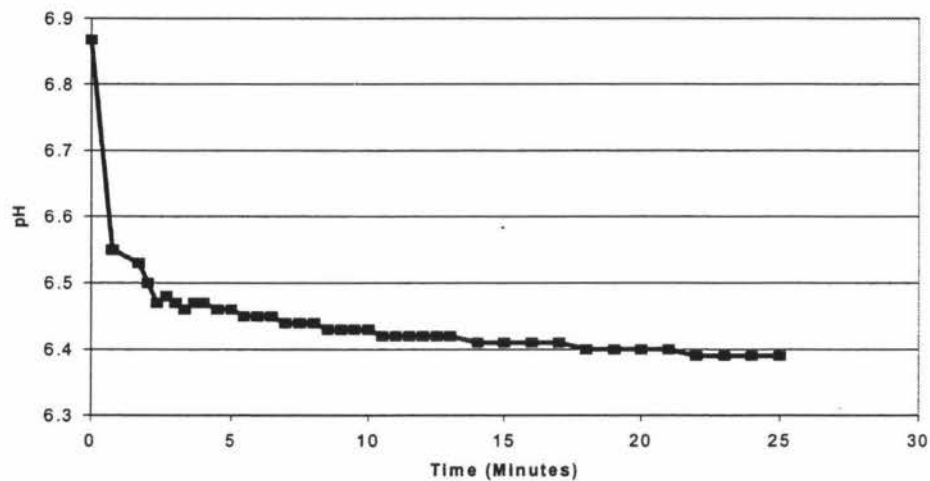
Figure 4:5: pH changes in mussels after mincing

The pH of various segments of the mussel were measured individually following the homogenisation of each fraction through a blender. This was done in order to determine the possible reasons behind the evident pH fall. It was thought that some parts of the mussel might be more acidic than others especially when minced, perhaps due to the digestion processes. The segments tested were the lip, foot, adductor, stomach and body, as detailed in section 3.7.
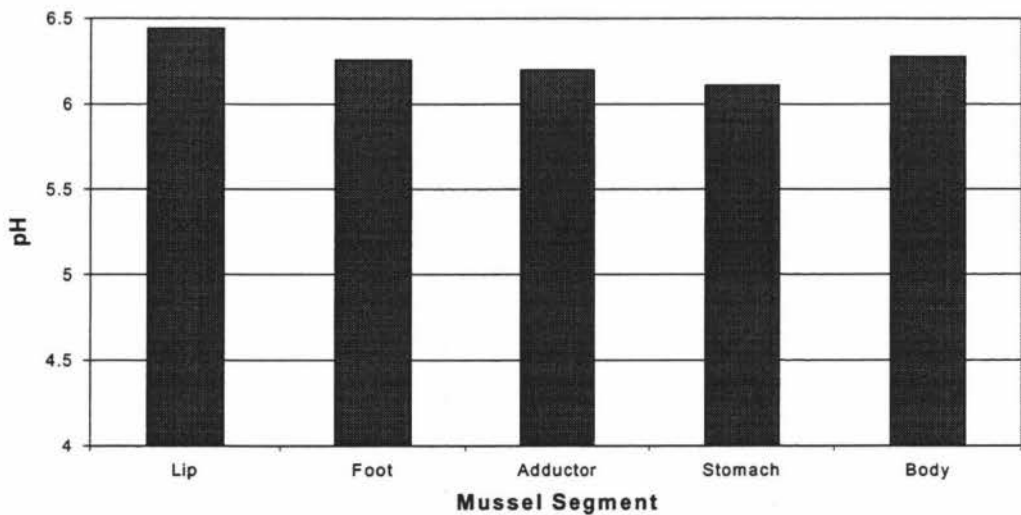
Figure 4:7: pH variation of segments of mussel tissue following homogenisation

Figure 4.7 shows the ultimate pH of each of these fractions after mincing. The stomach had slightly lower pH than the other fractions of the mussel. This is thought to be due to greater levels of smooth muscle which have more mitochondria and therefore result in a lower ultimate pH than striated muscle.

From this initial study it is possible to see that although mincing allows for more consistent water measurement and more uniform temperature during cooking, it also causes an increased rate of rigor development, and a consequent drop in pH. Due to this change in state, the blended mussels are not appropriate for use in cooking trials in this work. The variability between samples can be reduced by cooking whole mussels, and then mincing several cooked mussels together before centrifugation. In this way some of the advantages outlined for homogenised mussels are maintained without changing the nature of the mussel cooking process under investigation.

4.3   Developed methodology

In order to determine the water contents of whole and homogenised mussel samples the following methodology was followed in subsequent cooking trials:

1. Mussels were removed from their shells.
2. Mussels were either combined with other individual mussels and homogenised (after cooking) in a small food processor, or left intact.
3. Centrifuge tube, wire mesh and the centrifuge tube with glass beads weights were recorded and were arranged as shown in Figure 4.7.
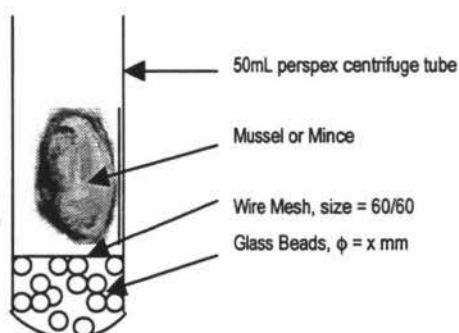
Figure 4:7: Diagram of the set-up of the centrifuge tube, glass beads and wire mesh

4. Weights were taken of the centrifuge with sample. Generally a 10g sample was used.

5. Samples were centrifuged for 30 minutes at 4000 rpm using a Hermle Z320 centrifuge.

6. The mesh (containing sample) was removed and weighed. The centrifuge tube containing the beads, free moisture and suspended solids were weighed to determine the free water content.

7. Both mesh and beads were placed in a 105°C-oven overnight and the dry weight recorded after cooling in a desicator.

The calculations to determine the free and bound water followed simple mass balance principles whereby the free water content is derived from water losses in the centrifuge (CT) and the bound water from changes at 105°C.

Whole mussels were used for subsequent cooking trials but homogenised for water content analysis post thermal treatment. Thus the problem of variability was reduced, as is the introduction of post rigor meat to the cooking process.

4.4 Conclusions

This chapter focuses on the work towards the establishment of a method, which enabled the consistent collection of information on both the free and bound water present in a sample, and was crucial to later experiments. Characterisation of the water content by this method allowed subsequent investigation of effects such as cooking time and temperature.

In order to successfully characterise the extent and rate of water losses in mussels during cooking it was necessary to develop methods that could adequately measure the water present in mussels. A modified centrifuge method was developed which

quantified two types of water present in the system; 'free' water, which could be removed by centrifugal force and 'bound' water, which could only be removed by subsequent drying. It was found that reproducible results could be obtained in 'bound' water measurements between mussels if results were expressed on a dry basis.

Experiments also showed that mincing raw mussels resulted in accelerated rigor onset. This changes the protein structure of the mussel meat and therefore mincing raw mussels is not suitable for subsequent cooking studies, even though this would have been a useful tool to reduce the variability between mussels. It was decided that mincing cooked mussels would be carried out to obtain averaged results in later experiments.

CHAPTER 5

FRESH WATER MUSSEL COOKING

5.1 Introduction

This chapter outlines the work aimed at quantifying the rate and extent of water losses during thermal processing of mussels. The method used was developed to simulate the cooking conditions used in commercial operations. That is it needed to employ the application of heat in excess water. Most published results for cooking losses in red meat systems are based on drip during cooking of samples contained in a plastic bag. Such methods were not suitable for this work. It was also important to eliminate temperature gradients through the mussel itself otherwise kinetic information on water losses is difficult to distinguish from the dynamics of heating. By understanding the kinetics of cooking losses and how they are affected by temperature it would be possible to identify optimal commercial cooking processes. This was the aim of work outlined in this chapter.

5.2 Initial cooking trials

Preliminary trials were carried out by cooking shucked mussels and measuring the weight losses associated with the individual mussels. Cooking trials using individual mussels immersed in water were carried out at a set temperature of 85°C. This approach was taken since literature suggests that it is useful to examine cook losses in terms of weight loss over the cooking period (Boleman *et al.*, 1995;

Jeremiah et al., 1997; Sales et al., 1996). Each individual mussel was weighed following raw shucking and then heated in the water bath prior to reweighing. Each sample lot used for experimental purposes was tested for condition index so that samples from different harvest lots and dates could be compared.

Varying results were found (shown in Figure 5.1) suggesting that a different approach to measuring the cooking losses was required other than those normally applied in meat research. The results exemplified the variation between individual mussels, where several mussels sampled on varying days and even after the same cooking length, showed no sign of converging at a particular weight loss point.
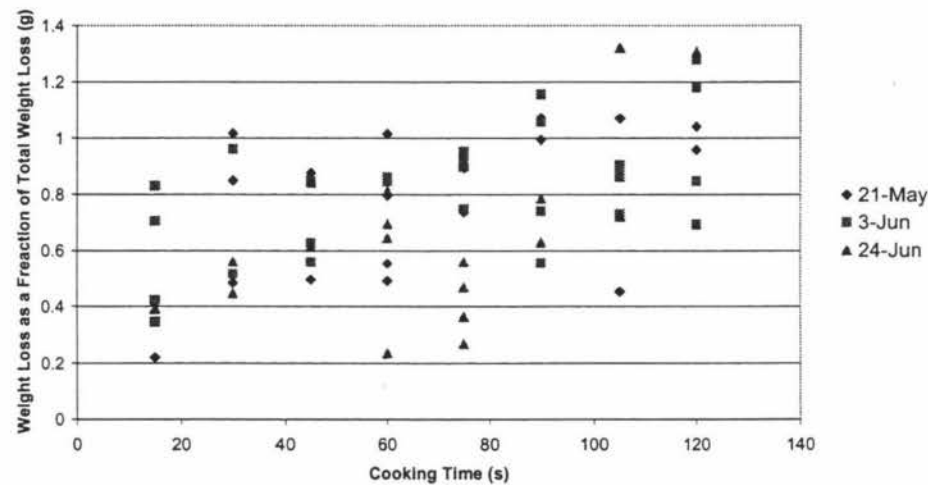
Figure 5:1: Individual weight losses over various cook times and sampling days

Figure 5.1 shows the extensive variability in the different samples. There was no discernible difference or trend shown between the different dates illustrated in figure 5.1, highlighting the need for an improved method to investigate cooking losses in mussels. However, in spite of the scatter the data shows a trend of increasing cook loss as cook time increases. Trends such as this have been found in past research for other meat systems (Laakkonen, 1973; Locker and Carse, 1976; Trout and Schmidt, 1987).

Following the initial trials, the cooking losses as a function of cook time were examined utilising the centrifuge method detailed in section 4.2.1.2. Free water content was determined during the centrifugation separation, where homogenised samples were suspended over glass beads on wire mesh. The samples were centrifuged for 30 minutes at 4000 rpm. In order to determine the bound water content of the mussels, the centrifuged samples were placed in a 105°C oven for 24 hours to completely dry the mussel tissue.
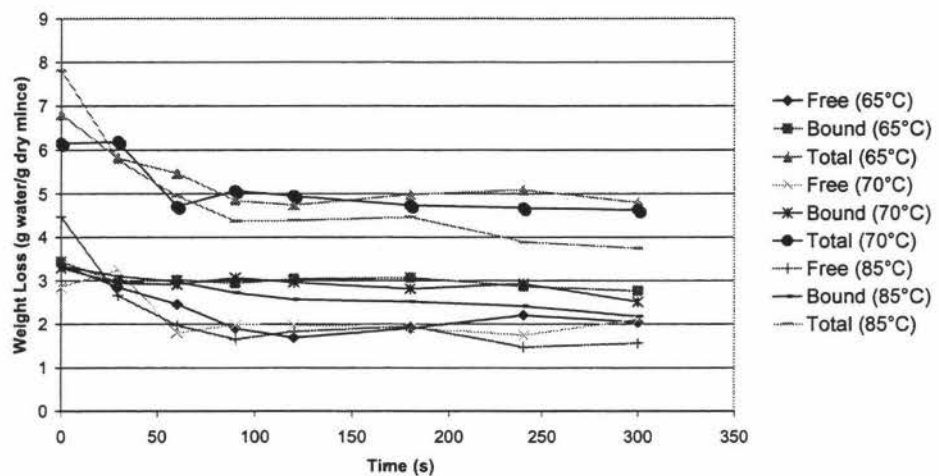
Figure 5:2: Water losses over time in mussel meat at varying temperatures

Figure 5.2 shows that the total amount of water remaining after cooking decreases for longer cooking periods. The level of bound water did not appear to change significantly over the heating period. This trend was unexpected as protein denaturation affects the ability of the myofibrillar proteins to retain water, so as the proteins denature it was expected to see losses in bound water. Figure 5.2 also shows clearly that as the temperature increases, the total water losses increase. There was very little difference in bound water vontent after 200s for mussels cooked at 65 and 70°C but lower bound water contents were recorded at 85°C. This suggests that more protein denaturation occurred at 85°C. The change in free water content appeared relatively similar at all temperatures. Although the data reveals relatively clear trends it is possible that the time dependency of cook losses

was due to the dynamics of the heat transfer rate through the mussel, rather than the rate of protein denaturation and subsequent water loss.

As a result it was decided to carry out experiments where the rate of heat transfer through the mussel was not limiting (i.e. a step change in temperature is experienced by the mussel, rather than promoting temperature gradients through the mussel). With such a method it would be possible to determine the true kinetics of the water loss from the mussels. If the rate of water losses were measured at different temperatures, Arrhenius kinetics could then be used to characterise the effect of temperature on reaction rate. Such a model could be then used to optimise the thermal processing of mussels by also considering the rate of listeria destruction as a function of time and temperature.

5.3   Experimental method development

An experimental cooking method was needed where step change increases in temperature could be achieved throughout the mussel mass. It was shown, in Section 4.2.1.3, that minced mussel samples lower the amount of variability between individual mussels. However, as a consequence of mincing the mussel meat is that chemical and structural changes in the actual meat occur. This is due to the meat changing from a pre to a post-rigor state. Therefore an approach was required which would minimise the effects of mussel thickness, without employing the homogenisation of sample.

Mussels cooked in the preliminary trials were cooked in a water bath, freshly shucked. The two sides of the mussel mantle often opened and therefore cooking was only required through thinner pieces of tissue. To investigate the rates of heating in the water bath, temperature profiles of mussels cooked at various temperatures were monitored using fine thermocouples connected to a Grant

Squirrel 1000 data logger. It was found that in whole mussels, the time taken for the centre of the mussel to reach the water temperature was around three minutes.
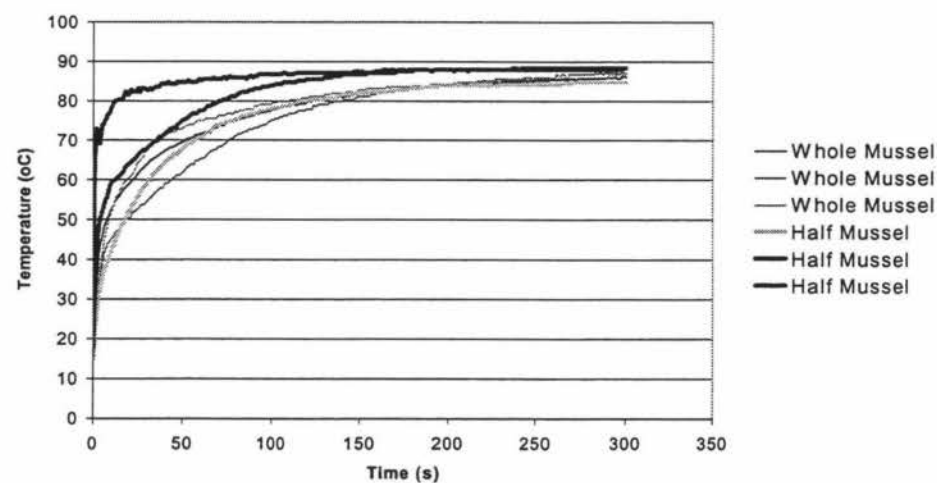
Figure 5:1: Temperature profile for whole and half mussels heated in an 85°C water bath

It is of interest that this was approximately the time period before cook losses reached steady state in initial trials (see Figure 5.2). Further temperature profiles on mussel meat halved at the hinge area were carried out to ascertain the influence of meat thickness. It can be seen in Figure 5.3 that halved mussels heated up considerably faster than whole mussels.

During the cooking of shucked mussels, variations occurred in how open the two sides of the mantel of the mussel were, as well as with position in the cooking water, especially in relation to other mussels. For these reasons it was decided to build to build an apparatus which regulated the thickness of the mussel and held them in fixed positions in the water bath. Such an apparatus would provide uniform, repeatable cooking experiments which could be carried out at near isothermal conditions

The necessity for uniform heat transfer is important when considering the actual water loss kinetics of the mussel. When carrying out the experiments it is crucial to ensure that the rate at which the mussels heat up is faster than the rate at which the

actual cooking losses occur. Therefore a mathematical model, describing the heating of a mussel in the water bath, was used to determine analytically the most feasible width for experiments. This would allow the design of an appropriate device to provide rapid and uniform heat transfer through the mussels.

The affect of mussel thickness and water agitation on the heating of the mussel meat was investigated using a mathematical model. The heating of a thin piece of mussel can be approximated as an infinite slab of thickness 2R, with either a fixed surface temperature or convective heat transfer occurring on the surfaces. It was assumed that:

- The water bath temperature was constant over the heating period
- The thickness of the mussel was uniform
- Heat transfer in the lengthwise and breadthwise directions was small compared to the depth. Heat from the water bath reaches the centre of the mussel faster through the shortest route. (Path of least resistance).
- The mussel will maintain constant thermal properties.

The physical model can be seen in Figure 5.4.
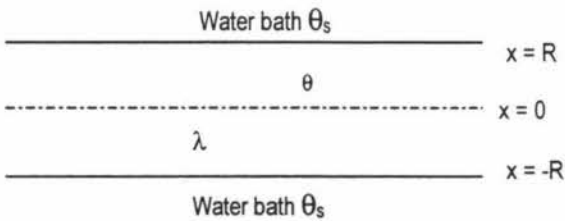
Figure 5:4: Physical model of heat transfer through the mussel.

Equation 5.1 below describes the rate of heat transfer through the mussels.

$$\rho c \frac{d\theta}{dt} = \lambda \frac{d^2\theta}{dx^2} \qquad \text{for t>0 and 0<x<+R} \qquad \text{(Eqn. 5.1)}$$

with the boundary conditions given below.

$$\frac{d\theta}{dx} = 0 \qquad \text{for t>0 and x=o} \qquad \text{(Eqn. 5.2)}$$

$$\theta = \theta_s \qquad \text{for t>0 and x=R} \qquad \text{(Eqn. 5.3)}$$

$$\text{or,} \quad -\lambda \frac{d\theta}{dx} = h_A(\theta_S - \theta) \qquad \text{for t>0 and x=R} \qquad \text{(Eqn. 5.4)}$$

And initial conditions,

$$\theta = \theta i \qquad \text{for t=0 and 0<x<R} \qquad \text{(Eqn. 5.5)}$$

Equation 5.3 describes the situation where there is no resistance to heat transfer on the outer surface of the mussel. This means the surface temperature is the same as the water temperature. Alternatively Equation 5.4 can be used if there is external heat transfer resistance (i.e. a significant difference between the water and surface temperature). An analytical solution for this model exists if the heat capacity and thermal conductivity of the mussel are constant over the temperature range of interest. These are given below as Equation 5.6 and 5.7 for the situation where there is a fixed surface temperature, and where external heat transfer resistance is present respectively.

$$Y = \frac{4}{\pi} \sum_{m=0}^{\infty} \frac{(-1)^m}{2m+1} \cos\left[(2m+1)\frac{\pi}{2}\frac{x}{R}\right] \exp\left[-(2m+1)^2 \frac{\pi^2}{4} Fo\right] \qquad \text{(Eqn. 5.6a)}$$

$$Yav = \frac{8}{\pi^2} \sum_{m=0}^{\infty} \frac{1}{(2m+1)^2} \exp\left[-(2m+1)^2 \frac{\pi^2}{4} Fo\right] \qquad \text{(Eqn. 5.6b)}$$

$$Y = \sum_{m=1}^{\infty} \frac{2Bi \cos(\beta_m \frac{x}{R})\sec(\beta_m)}{Bi(Bi+1) + \beta_m^2} \exp\left[-\beta_m^2 Fo\right] \qquad \text{(Eqn 5.7a)}$$

$$Yav = \sum_{m=1}^{\infty} \frac{2Bi^2}{\beta_m^2 [Bi(Bi+1) + \beta_m^2]} \exp[-\beta_m^2 Fo]$$ (Eqn. 5.7b)

where,

$$Fo = \frac{\lambda t}{\rho c R^2} = \frac{\alpha t}{R^2}$$ (Eqn 5.8)

$$Bi = \frac{hcR}{\lambda}$$ (Eqn 5.9)

$$Y = \frac{\theta - \theta_a}{\theta_i - \theta_a}$$ (Eqn. 5.10)

$$Yav = \frac{\theta_{av} - \theta_a}{\theta_i - \theta_a}$$ (Eqn. 5.11)

and β_m are the roots (m, 1-4) of equation 5.12,

$$\beta \tan \beta = Bi$$ (Eqn 5.12)

5.3.1.1 Thermal properties

The thermal properties for mussels (specific heat capacity, c, and thermal conductivity, λ) are unavailable in literature. Therefore an empirical equation based on the composition of the mussels was used to estimate these values. The composition given by Vlieg (1988) was used to achieve this. This was summarised previously in Table 2.1.

5.3.1.1.1 Specific heat capacity

The specific heat capacity is defined as the amount of heat necessary to raise the temperature of a unit mass of the material by unit degree and is therefore measured in J/kgK (Rahman, 1995). Choi and Okos (1986) developed an expression to determine the specific heat capacity (c_i) of the component phase based on the mass fraction (X_i) of the various components (Equation 5.13).

$$c_f = \sum_{i=1}^{N} c_i X_i \qquad\qquad \text{(Eqn.5.13)}$$

Using this correlation and the various mussel components (including water, protein, fat, carbohydrate and ash) the specific heat for mussels was determined. The model predictions often yield results, which differ from experimentally derived data (Rahman, 1995). This is due to:

1. The specific heat capacity of the component phases varying with the source or origin,
2. Bound water or unfrozen water having a different specific heat capacity than bulk water,
3. Excess specific heat capacity due to the interaction of the component phases.

Rahman (1993) combined all the effects together to give an estimate of the excess specific heat capacity c_{ex}, which is:

$$c_f = \left[\sum_{i=1}^{N} X_i c_i \right] - c_{ex} \qquad\qquad \text{(Eqn. 5.14)}$$

where for fresh seafood the correlated excess specific heat capacity is (Rahman, 1993):

$$c_{ex} = -33.77 + 85.58 X_w - 53.7 X_w^2 \qquad\qquad \text{(Eqn. 5.15)}$$

Using these correlations it was possible to determine that for mussel meat the specific heat is 3.4963 kJ/kgK. Using the density of mussel meat (1086 kg/m³), the volumetric specific heat was calculated to be 3.797×10^6 J/m³K. In comparison, the specific heat capacity for a typical beef meat product is 3.56 kJ/kgK at 20°C (Chen, 1985). Additionally Calamari mantle, with a water content of 0.8002, has a specific heat of 3.41 kJ/kgK (Rahman, 1993) which indicate that the correlation provides a reasonable answer.

5.3.1.1.2 *Thermal conductivity*

The thermal conductivity is a measure of the ability of a material to conduct heat. In order to determine the thermal conductivity the effective medium theory can be used. For a medium with N components the following expression can be applied (Davis *et. al*, 1975):

$$\sum_{i=1}^{N} X_i \frac{\lambda_e - \lambda_i}{\lambda_i + 2\lambda_e} = 0,$$

(Eqn. 5.16)

where λ is the effective thermal conductivity of the medium and X_i is the mass fraction of each component 'i' with thermal conductivity (λ_i). Using this equation it was found that the thermal conductivity for mussel meat was 0.5681 W/mK. By comparison a typical value for the thermal conductivity of the calamari mantle is 0.508 W/mK, (Rahman *et. al*, 1991) which illustrates that the predicted mussel thermal conductivity is a realistic value. This value was determined with heat transfer through the transverse (perpendicular to muscle fibre) direction and interestingly other products studied showed variation in thermal conductivity based on whether the heat was transversely or longitudinally applied.

5.3.1.2 Model solution

Using the analytical solutions given in Equations 5.6 and 5.7, the changes of temperature occurring within the mussel can be determined to provide information on optimum thickness during cooking to give as close as possible instantaneous heat transfer through the meat. It was found that for mussels flattened to a thickness of 3mm, the centre had reached the water temperature after 2 minutes. In comparison, the 1.5mm width provided a faster heating rate, where centre temperature reached the water temperature in 60 seconds. Figure 5.4 shows the predictions for temperature changes within the mussel using both analytical solutions for the situation where there is a fixed surface temperature (1st kind of boundary condition) and resistance to external heat transfer is a factor (3rd kind of boundary condition).

A heat transfer coefficient of 150 W/mK was used for these simulations. This value is typical for a water system with mild agitation. As can been seen from the chart, the heat transfer is markedly faster when there is no resistance to heat transfer and the surface temperature is equal to the water temperature. This is unlikely to be a true representation of the real system, in which is it reasonable to assume a certain extent of resistance to heat at the surface.
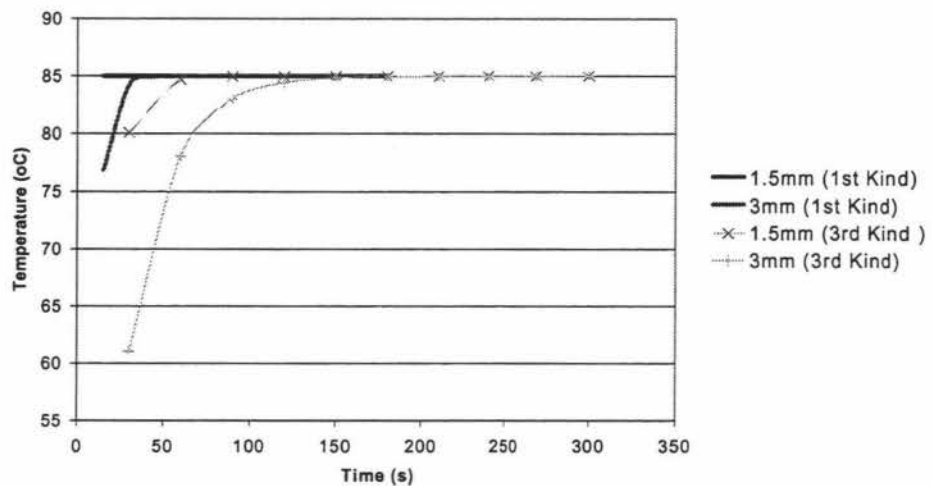
Figure 5:5: Heat transfer predictions determined using both a fixed surface temperature and resistance to external heat transfer

From figure 5.5 the assumption that the 3rd kind of boundary condition is the best model to use, leads to the question of what heat transfer coefficient is likely to be regulating the flow of heat over the surface of the mussel. Initially a conservative estimate of 150w/mK was used in the predictive equations, however this was later increased to 300w/mK. This could be achieved in the water bath by agitating the water. By altering the heat transfer coefficient to account for the increased heat transfer capacity of agitated water, it would be possible to accurately determine what thickness of mussel was required to heat the entire mussel to a given temperature within a minute. The differences in heating rates for these two heat transfer coefficients are shown in Figure 5.6 below.
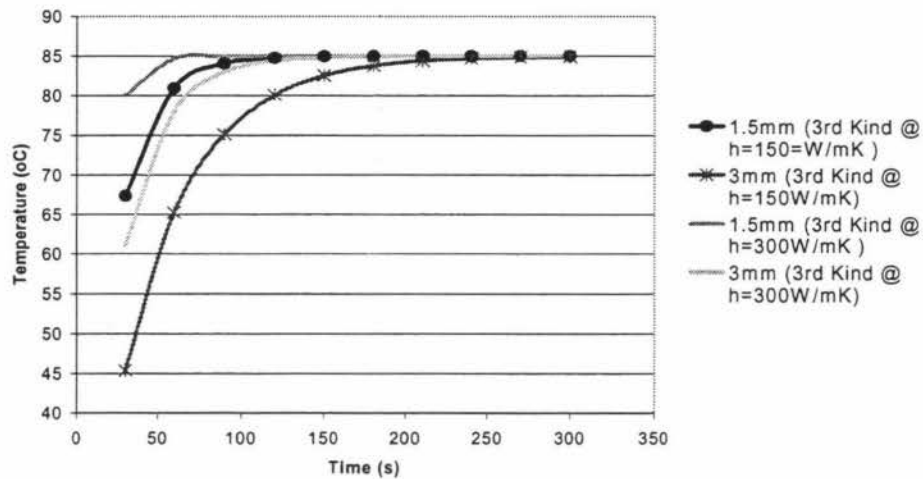
Figure 5:6: Affect of increased heat transfer coefficient on the heating rate within mussel meat

From these predictions it is possible to determine that a thickness of 3mm should heat the mussels at a sufficiently fast rate so as to ensure a near to instantaneous step change in mussel temperature. If mussels are squeezed further to 1.5mm this will improve the heat transfer rate. Limitations as to the smallest feasible thickness will depend on the actual size of the mussels, and how destructive the flattening method is.

5.3.2 Apparatus development and assessment

Initially a device was developed consisting of two stainless steel perforated plates, which were separated by 3mm spacers. The mussels were compressed between the plates to the desired thickness
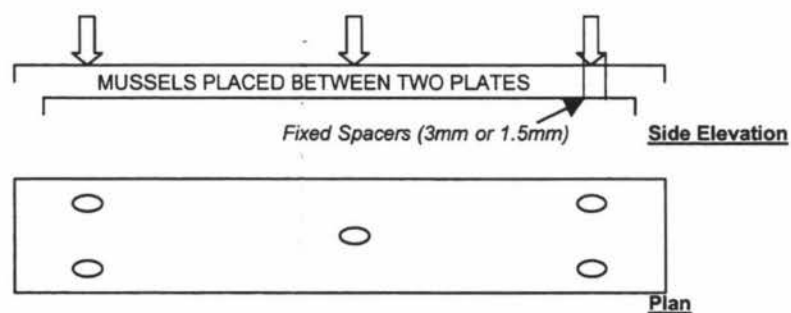
Figure 5:6: Apparatus used to control mussel thickness during heating (Arrows indicate the placement of screws)

By using perforated plates, it was possible to ensure the intimate contact between mussels and cook water that occurs during commercial cooking operations. The stainless steel has a high thermal conductivity (? = 14 W/mK) and would not limit heat transfer rates.

The prototype plate was constructed with four screw-down corners, however it became apparent that the mussels nearer the edge of the device heated up at a faster rate (see Figure 5.8). This indicated that they were slightly thicker in the centre of the apparatus, due to flexing in the stainless steel mesh. As a result the apparatus was modified to include a central screw which ensured uniform thickness across the whole apparatus as shown in Figure 5.7.
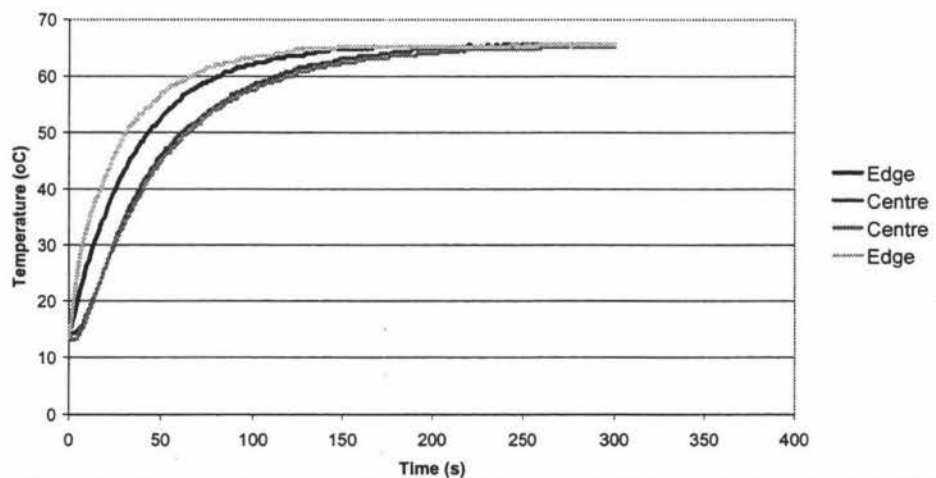
Figure 5:8: Heating rates of mussels in 65°C water bath, showing the dependence on position in the initial prototype

Initial trials were carried out in the new apparatus using 3mm and 1.5mm spacers based on the predictions from the mathematical model. The centre temperature of mussels contained in the apparatus were recorded using fine gauge thermocouples and the Grant datalogger previously described. This data is shown in figure 5.9 below.

It was found there was very little effect of mussel thickness on the heating rate. This was contrary to the initial model prediction suggesting the heating process was limited by external heat transfer resistance rather than through the mussel itself. Work on the predictive equations had included the possibility that the system would have significant external resistance to heat transfer. The variations in the heat transfer coefficient for the predictive model showed that where there was increased heat transfer to the mussel the heating rate would be substantially increased. Therefore to eliminate the effect of heat transfer resistance between the mussel and water a stirrer was added to agitate the water, increase the heat transfer coefficient of the water to mussel, and thus the heating rate within the thinner mussels.
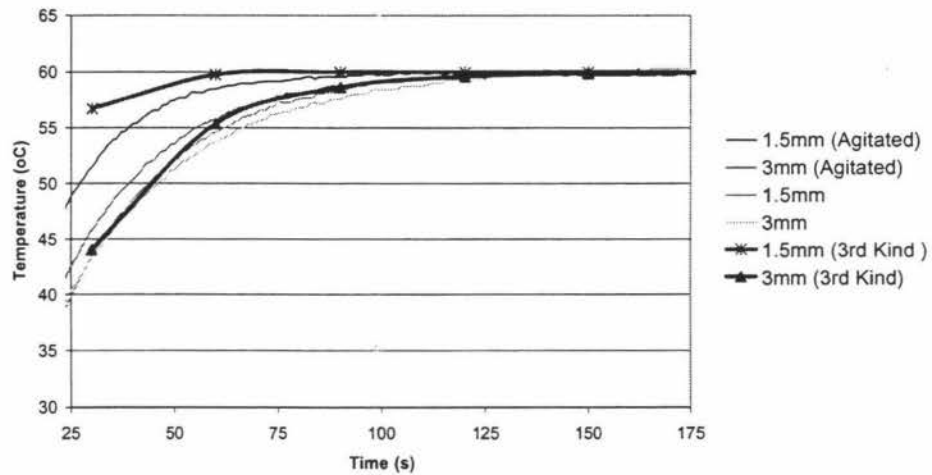
Figure 5:9: Comparison between mussel heating rates in an agitated and non-agitated water bath

It can be seen in Figure 5.9 that by agitating the water, the heating rate increased substantially, as did the difference in heating rate between the two thicknesses. This shows that without stirring of the water bath the system is convection limited. Figure 5.8 also shows the comparison between the mathematical model and the actual experimental heating rates. These results compare relatively well and differences could be attributed the estimation of the convective heat transfer coefficient.

From this analysis it can be seen that the approach taken here in the design of the experimental apparatus resulted in a clear well defined method. Rather than basing the design on trial and error, it was possible to predict the required thickness from the modelling work. This allowed the construction of an apparatus that would fit the requirements of the dynamic cooking experiments carried out in this work.

5.3.3 Conclusions

It is evident from the experimental heating results obtained, that it is possible to achieve fast heating rates (less than 1 minute) within the mussels and that the device constructed performs well. The chief elements of the design of the device include the ability to control the thickness of the mussels, and the agitation of heating water to increase the heat transfer coefficient. Overall by addressing these points, the designed apparatus is able to provide a fast and consistent method to heat mussel meat to given temperatures.

5.4 Dynamic cooking trials

Having established a suitable method of quickly and evenly affecting a temperature rise in mussel tissue, using the designed apparatus, it was possible to conduct experiments to examine the effect of cooking time in a controlled environment. The rate of cooking loss at varying time and temperature profiles was determined for both whole mussels and mussels cooked utilising the designed equipment in order to identify any possible outstanding effects caused by employing the flattening device. The overall aim of these experiments was to determine whether the water loss in mussels cooked at various temperatures exhibited significant cooking time dependence.

Studies to characterise the rate of cooking losses were carried out over a range of temperatures. During processing, the internal centre temperature of the mussels can range from 20 to 90°C depending on the size grade, and length of time in the cooker. From the cooking loss trials it was expected that a clearer understanding

as to the mechanism of water loss from the mussels would be achieved. It was hypothesised that at higher temperatures that the water losses originate from the breakdown of protein structures, thereby releasing water.

The first dynamic cooking trials were performed using freshly shucked mussels immersed in the water bath. At different time intervals (zero to five minutes) samples were removed and the water content (both free and bound water) were measured as outlined in section 4.4. In addition the centre temperature of a typical mussel was also recorded using fine thermocouples connected to a Grant datalogger.

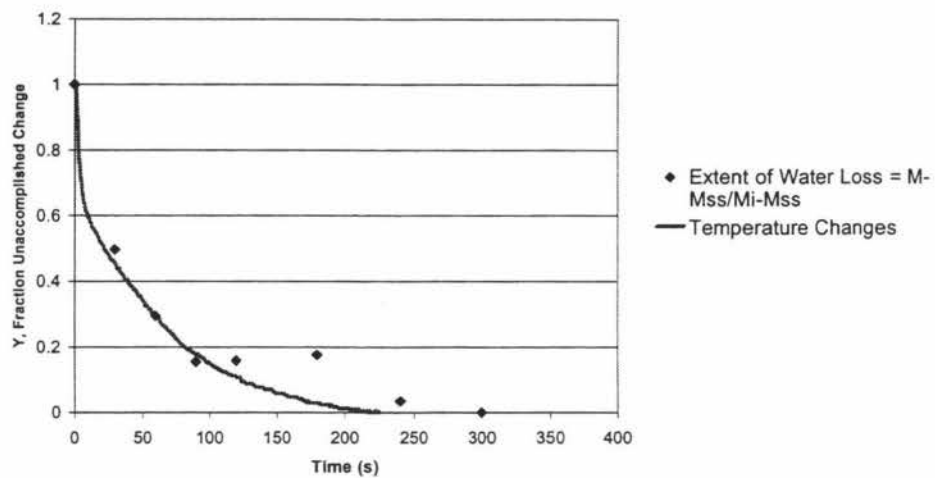Figure 5:10: Relationship between temperature change and rate of water loss in whole mussels

Figure 5.10 shows the relationship between the temperature changes occurring at the centre of the mussel and the water lost as a fraction of the initial water content. The data has been expressed in terms of unaccomplished change 'Y' for both water loss and temperature.

$$Y_T = \frac{T - T_\infty}{T_i - T_\infty}$$

(Eqn 5.17)

$$Y_{WL} = \frac{m - m_\infty}{m_i - m_\infty}$$

(Eqn 5.18)

It is interesting to note that in this case, where whole unflattened mussels were used, the rate at which the mussels heated up to maximum temperature was slow. Hence, although there was a trend of time dependent water losses during the heating phase, the mussel temperature was not constant during this phase. To derive kinetics for the cooking losses it is imperative to know that the changes occurring are happening at a set temperature throughout the entire mussel. The results do, however, illustrate that the temperature increase and water loss appear to follow the same trend. Therefore it is likely that the water loss is more dependent on temperature than cooking time.

This further illustrates the necessity for a device to reduce the effect of limited heat transfer through the mussel meat. Using the apparatus described in section 5.2.2, mussels were compressed to the 1.5mm-spacer width. Larger mussels exhibited signs of tissue damage on compression and so the 3mm spacer was used in these cases. In order to measure the cooking changes dynamically as opposed to the standard method or cooking for a length of time, several of the compression apparatus were built. At each temperature several cook time periods were examined; 0, 1, 2, 3, 4, and 5 minutes. All five mesh plates were placed simultaneously in the water bath at t=0, upright and adjacent to the agitator to provide even mixing throughout. At each time interval one plate was removed and placed into a plastic bag and then into an ice water bath to rapidly cool the mussels and stop reactions from continuing.

The method required several modifications throughout its implementation. Initially, as stated above, the heated mussels were placed in ice water. Problems arose when the steel mesh tore through the bags, allowing water to come in contact with the mussels and possibly becoming reabsorbed by the mussel meat. Eventually, the mussels were placed in the plastic bags between layers of ice, which despite having poorer heat transfer qualities avoided the reabsorption difficulties. After

cooling, the five mussels present on each plate were homogenised together and dried at 105°C for 24 hours to obtain the moisture content. The relationship between the temperature changes that occur within the mussels and the observed water content changes can be seen in Figure 5.11.
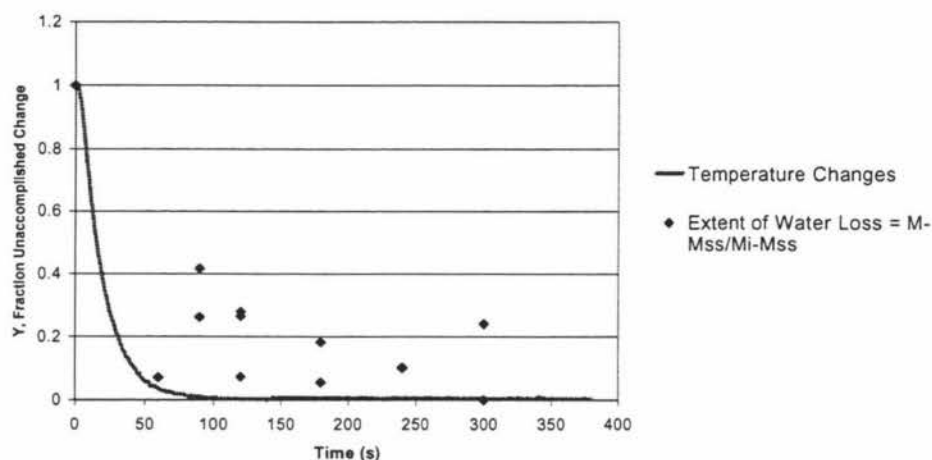
Figure 5:11: Relationship between temperature changes and rate of water loss for flattened mussels

From Figure 5.11 it is possible to see that the rate at which the temperature increases toward the endpoint is significantly faster with the apparatus. This overcomes the difficulties that arose when attempting to analyse the data presented in figure 5.10. It can be seen in figure 5.11 that some scatter was present in terms of the extent of water loss. This scatter was due to the fact that the results are plotted as n extent of water loss. The initial water content was taken at a time zero, where the mussels had not been affected by any outside stimuli, however as mentioned previously, the variability in the water content of raw samples of mussels is considerable. Additionally the calculation of the extent of water loss also incorporated the steady state value for the water loss at essentially an infinite time. This value was taken at five minutes and could have introduced error since it mussels could conceivably have lost more water on further cooking.

The scatter in Figure 5.11 also illustrates that cooking at a faster heating rate with flattened mussels, there is no real trend exhibited in terms of cooking losses

increasing with increasing time. The data suggest that there is a rapid reaction rate and subsequent rate of cook losses in the mussels on heating.

The initial experiment showed that by utilising the apparatus, rapid heating rates could be achieved within the mussel meat. It is interesting to note that, even though the use of the flattening apparatus afforded a method of overcoming heat transfer considerations, problems arose with respect to the reproducibility of water loss results. The principal cause of these problems was the expression of water caused by compressing the mussels to a defined thickness. The degree of this phenomena was dependent on the initial mussel size.

Damage to the mussel tissue prior to cooking, caused by flattening mussels of varying thicknesses to a similar width, caused excess leakage in the form of drip losses. Water, which under normal circumstances would have been held within the mussel as free water, was released under the pressure exerted by the screwed down plates. Some of this water loss was evident during the trials where small amounts of water pooled underneath plates left on the bench for short times.

As a result of this the method was adjusted to quantify the changes in mosiure content during cooking using the 'bound' and 'free' measurement techniques outlined previously in section 4.3. In this way the effect of the expression of free water during sample preparation could be allowed for and useful data could be collected to describe the time dependency of cooking losses as a function of temperature.

5.5 Effect of temperature on rate of cooking losses

Studies concerning the denaturation of fish muscle are limited and for mussel tissue there is no reported literature. Connell (1961) determined that in comparison to

mammalian myosin, fish myosin is thermally less stable. This is evident particularly in terms of freezing and frozen storage (Hastings *et al.*, 1985). Additionally, Hastings *et al.* (1985) found that the temperature of the natural habitat also affects fish myosin stability, and considering fish can live in water ranging from between − 2° and 28°C there exists much room for variability. The instability of fish proteins is likely to be similar for mussel tissue and highly dependent on temperature.

Given the lack of literature on this subject, the aim of the work described in this section was to collect water loss data over time for mussel meat. The experiments were conducted at a range of temperatures between 60 and 90°C in order to examine the cooking losses at varying temperatures. Based on this information it would then be possible to develop a kinetic model to characterise the affect of temperature on the rate of water losses in cooked mussels.

Freshly shucked mussels were placed in the device developed earlier in this chapter. Within the device the mussels were flattened to a width of 3mm and placed in a heated water bath. The affect of water at temperatures of 60, 65, 70, 75, 80, 85 and 90°C were examined over the course of the experimentation. For each experiment five plates were used, each holding five mussels. The plates were held upright, perpendicular to the base of the water bath, by a converted dish rack, in order to enable uniform heating. A mechanical stirrer was placed within the water bath at the rear, centre of the water bath, providing the best possible agitation to all five plates. Since the plates were held in place by the 'dish-rack', they could all easily be placed in the water bath at time zero. Each minute thereafter one of the plates was removed. It was quickly placed within a plastic bag before being cooled on ice in order to prevent further heating reactions. Once all five plates had cooled sufficiently, the mussels were removed.

Each batch of mussels (5) were placed within a small blender and homogenised until the final consistency of the mussels was suitable for further testing. The homogenised samples were then weighed and centrifuged at 3000rpm in order to determine the free water content of the sample. Following this the samples were reweighed and placed in an oven at 105°C to determine the bound water content of the mussels. From this information it would then be possible to ascertain any

difference in the water content of mussels cooked at various temperatures to the level of free and bound water contents.

The results of the experiments are shown in figures 5.12 – 5.14 for total, free and bound moisture contents respectively
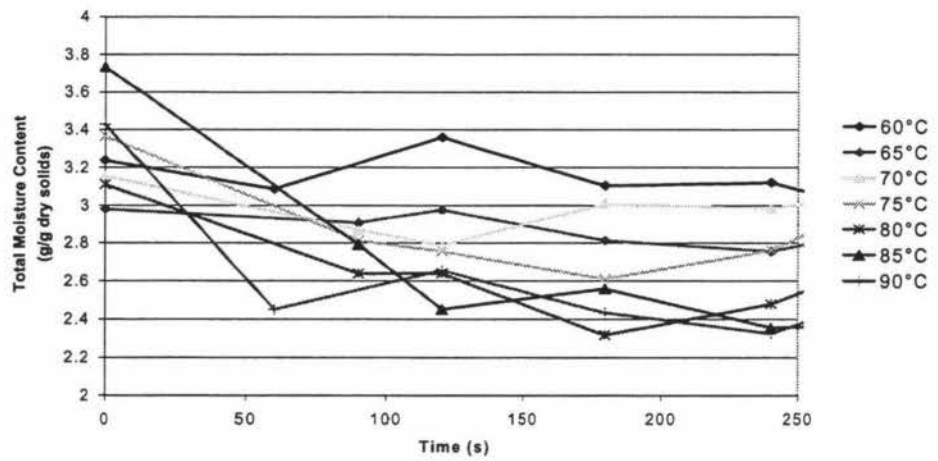
Figure 5:12: Effect of cooking on total moisture content at various temperatures
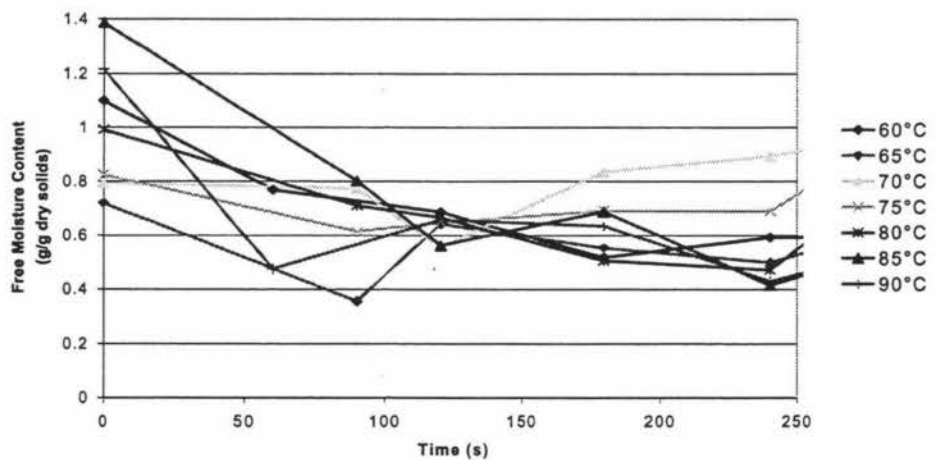
Figure 5:13: Effect of cooking on free moisture content at various temperatures
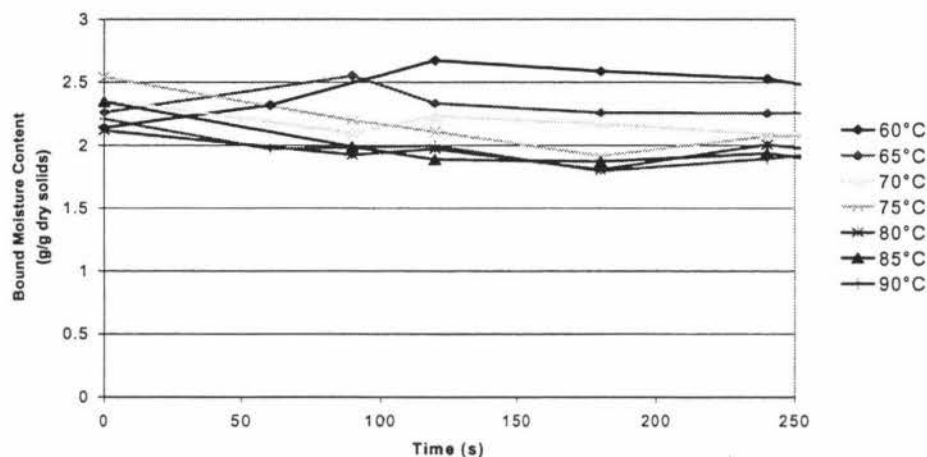
Figure 5:14: Effect of cooking on bound moisture content at various temperatures

From figure 5.12 it is possible to see that there is certainly an overall trend in the total moisture content, where at all temperatures between time 0 and subsequent points there is a loss of water. However, it is also important to note that the initial moisture content of the samples was quite variable. This would be due to the variable levels of free moisture in the raw material and due to the effect of squeezing the mussels to different extents during sample preparation depending on individual mussel size. This can also be seen in the free moisture content graph (figure 5.13) where the t=0 points are highly variable and follow no specific trend with respect to temperature. Interestingly, however, the initial water contents for the bound water contents were quite uniform for all temperature trials.

From figure 5.13, it is possible to see that the free moisture content drops rapidly to a relatively constant level within the first 100 seconds. This is the same order of magnitude as the time required to reach steady state temperature. There were no further changes in free moisture levels upon heating for longer times. This suggests that any change in free moisture will happen within two minutes of heating. The steady state free moisture content levels are essentially the same and irrespective of cooking temperature. Therefore it follows that whatever variation is present is random with respect to cooking temperature.

In terms of bound water, the trends are a little more difficult to see, however, at higher temperatures there is a trend of a drop in bound water content immediately after heating after which the levels remain relatively constant. This trend is clear for temperatures above 70°C. At 60 and 65°C there is more scatter in the data but the final bound water contents are significantly higher than for the mussels cooked at temperatures above 70°C. There is a clear trend at increased temperatures where a lower steady state bound water content results. This can be seen more clearly in figure 5.15.
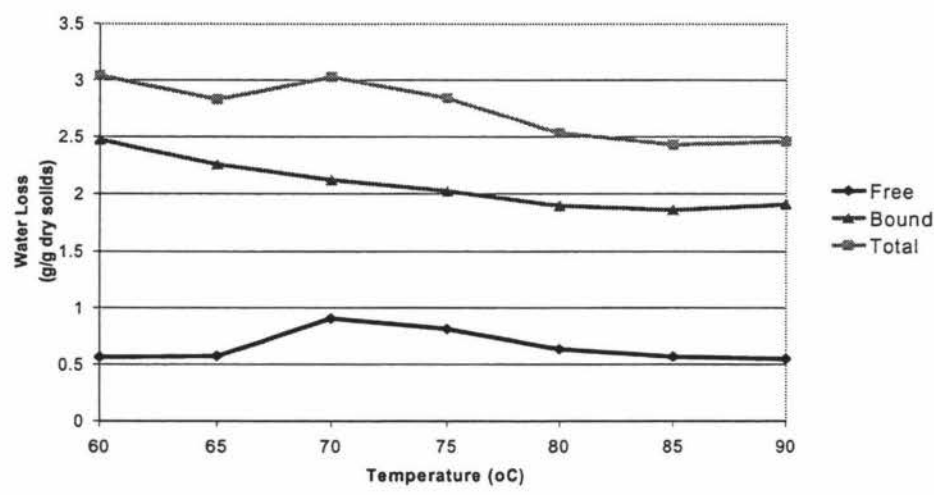
Figure 5:15: Steady state cooking losses

It can be seen that as the temperature increases the bound water content decreases.

Overall the data presented in this section suggests the following behaviour of mussel water losses during cooking.

- Immediately upon heating mussels loose the majority of their free water. The final level of free moisture appears to be relatively constant and independent of cooking temperature.

- There is also a rapid loss of bound water from mussels upon heating, but the final level is temperature dependent. This result is consistent with the mechanism proposed for cook losses being caused by protein denaturation.

- From a commercial operation perspective, this results in the conclusion that yield is affected by the temperature that the mussel reaches, rather than the length of time the mussel is kept at that temperature.

Since it is difficult to characterise the initial uncooked moisture content of mussels, it is best to report results based in terms of final free, bound or total moisture contents. This result means that it is more useful to quantify steady state moisture contents after cooking mussels at a range of temperatures. This investigation is outlined in the next section.

5.6   Effect of temperature on extent of cooking losses

The previous section clearly demonstrated that the time mussels are held at a particular temperature is not important (i.e. the rate of cooking losses is fast compared with heating time). In steady state the extent of cooking losses was shown to be affected by the cooking temperature.

To investigate this effect further a series of trials were carried out to characterise the relationship between temperature and the level of water losses observed. For these temperature trials the methodology was essentially the same as earlier trials, however the samples were held in the water bath for an interval of 5 minutes and then removed. The only variable being investigated was the temperature of the water bath. The trial, in essence, determined the steady state point provided in the previous trials after 5 minutes of heating. The results of this work are shown in Figure 5.16 below.
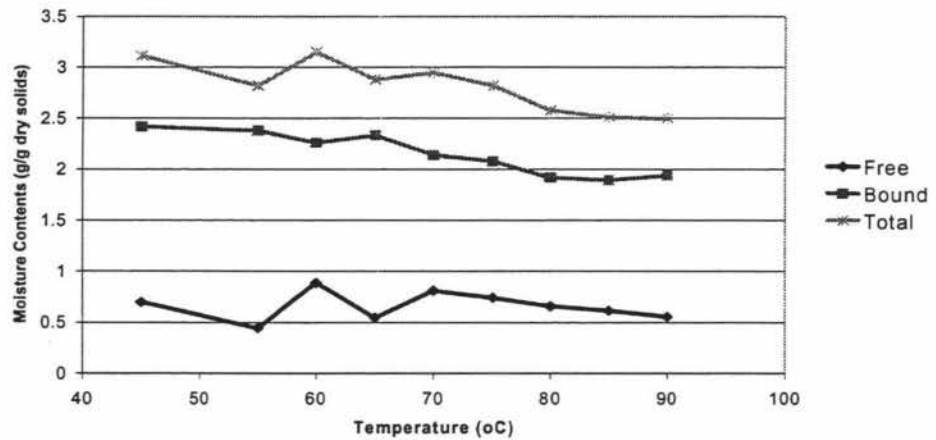
Figure 5:16: Average steady state moisture contents of mussels cooked for 5 minutes at various temperatures

From figure 5.16 it is possible to see that the after five minutes of cooking the free water content does not exhibit any clear trend. However the total water content, including the bound water, clearly shows that increasing temperatures reduce the overall water content of mussels. The results shown in figure 5.16 were further analysed and the total water contents were compared to the initial water content to give the results shown in figure 5.17.
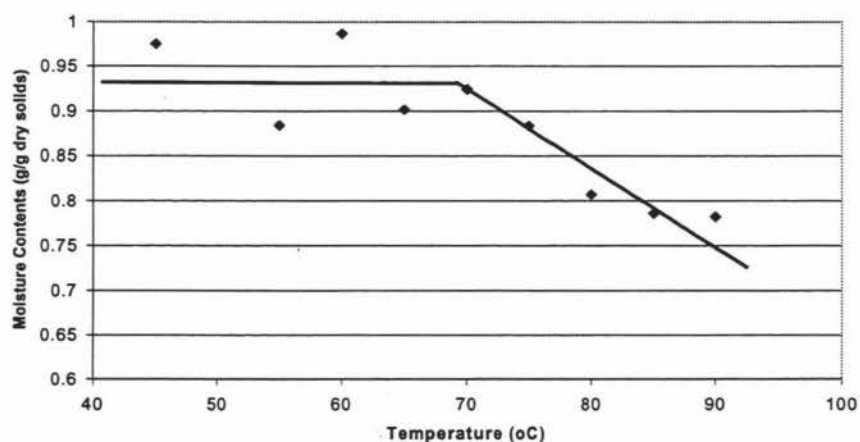
Figure 5:17: Summary of changes in water content as a function of temperature

From Figure 5.17 it is possible to see the changes brought about by increasing temperatures. The chart indicates that at temperatures around 60-65°C there appears to be a transition point where the water retained is relatively constant to where increasingly large changes occur. There is approximately 10 to 15% less water remaining at 90°C compared with the amount of water present at 50°C, based on the dry weight of the samples taken.

These findings are particularly apparent when we express them in terms of reduction in yield compared with not cooking at all. Raw mussel meat has a moisture content of approximately 80%. For every 1kg of mussel meat, there is initially 800g of water. If the mussels are cooked to 65°C from figure 5.17 it can be seen that 93% of this water is retained (7% lost). This results in (0.07 x 800g) 56 grams of water being per kg of mussel meat. At 80°C this loss increases to 14%. At 90°C the loss is up to 20% reduction in yield. This illustrates the importance of reducing the temperature the mussel meat reaches during cooking.

5.7   Conclusions

In conclusion the work described in this chapter has illustrated a number of points crucial in terms of optimising mussel processing.

The time a mussel spends at a particular temperature does not impact the water losses within the mussel. The temperature the mussel reaches is the factor that determines the extent of cooking losses.

The temperature at which changes in water losses become apparent is between 60°C and 65°C. There is little evidence of a change in water content with mussels cooked up to these temperatures. Above 65°C water losses increase as the temperature increases.

In order to increase the yield of mussels, the temperature of the mussels must be kept low.

The results obtained in this chapter suggest the water losses are directly linked to a protein denaturation reaction with high activation energy. This is investigated in Chapter 6.

CHAPTER 6

KINETICS OF COOKING

6.1 Introduction

This chapter will examine the different factors involved during the cooking of mussel meat, as well as the underlying reasons for the results found in the previous chapter. Dynamic cooking trials have shown that the water lost during the cooking of mussels is not very dependent on the length of cooking time. More significant effects are observed at changing temperature. It is obvious through trials performed at various temperatures that there is a distinct change occurring in the extent of cooking losses from the mussels at around 70°C. The likely cause of these changes is the denaturation of different proteins, which would contribute to the loss of water. It follows that in order to study the effect and occurrence of cook loss it is important to determine what factors affect the rate and extent of protein denaturation at a range of temperatures.

As mentioned in section 2.3.1 meat consists of several different proteins. Of these the largest group is the myofibrillar proteins. Mussels, also, have a higher percentage of myofibrillar proteins than other proteins. Myofibrils can be broken down further to myosin (50% of the myofibrils) and actin (20% of the myofibrils). Since the myofibrillar proteins play a large role in the process of water loss from meat products it follows that they will influence the losses experienced in mussels. Research has shown that in meat samples, it is possible to determine the denaturation of both myosin and actin through the use of differential scanning calorimetry which measures the endothermic reaction of protein denaturation.

The third type of protein that is important in cooking loss is the connective tissue, consisting mainly of collagen. It is the shrinkage of collagen during cooking which is associated with the water losses from meat. On heating, the collagen fibres shrink both transversely and longitundinally, forcing out the cellular water from within the muscle fibres. From the composition analysis of mussels discussed in section 3.5, it was shown that the amount of connective tissue is very low compared with the myofibrillar and sarcoplasmic proteins. As a result it is likely to be difficult to detect collagen denaturation through the use of DSC. Based on published literature it would be expected that collagen would denature at around 60 to 70°C (Davey and Gilbert, 1974; Judge and Mills, 1986), which would explain the changes in cooking losses at this temperature.

6.2 Differential scanning calorimetry

Differential scanning calorimetry (DSC) provides a way to directly observe the thermal transition temperatures of muscle proteins. It is also possible to use this technique to determine the kinetic parameters of the denaturation of the various proteins. It is simple enough to track the changes in temperature associated with certain processing situations, but to understand what is happening at a fundamental level is central to the optimisation of process conditions. Heating affects meat in terms of texture, appearance and flavour, however it also induces many structural changes which lead to water losses, shrinkage and toughening (Kijowski and Mast, 1988). DSC works by monitoring the heat induced endothermic protein unfolding which commonly occurs as temperature increases. The additional energy required by the protein sample to match the linear temperature increase of a reference sample is recorded (Hastings et al., 1985).

Previous research has been carried out making use of DSC for the measurement of protein denaturation in various meat and fish systems. Beas et al. (1991) examined the thermal denaturation of fish muscle proteins during gelling, concentrating on the myofibrillar proteins. Peaks were detected at 46°C and 75°C (commonly called the

Tmax of the protein), with a shoulder at 46°C. These results were similar to DSC patterns described for other fish species, however the Tmax details were slightly different. These differences were suggested to be due to differences in species habitat temperatures. Additionally the effect of salt on the fish muscle system was studied, and it was found that at a high ionic strength, there was a noticeable and important drop in the denaturation enthalpy.

Differential scanning calorimetry was utilised by Findlay et al. (1986) to quantity temperature induced changes to the thermomechanical properties of beef muscle. An initial transition was noted at 57°C and was attributed to myosin denaturation. The second transition was observed at 70°C and was ascribed to the denaturation of sarcoplasmic proteins, myosin fragments and tropomyosin and the shrinkage of various types of collagen. A third transition was noted at 80°C which was explained as the denaturation of actin.

Research has shown that the thermostability of proteins is dependent on the environmental temperature of the species, particularly when considering marine species. Hastings et al. (1985) compared cod, herring and squid which are all North Atlantic species, and where the water temperature is between 0 and 8°C, to jumbo prawns, which originate from a more tropical climate where water temperatures can range between 25 and 30°C. In this case the first peak in the jumbo prawn was 10°C higher than the fish species from the colder Atlantic region (Hastings et al., 1985). Similarly, Howell et al. (1991) found that the onset temperature of protein denaturation in tropical fish was 11°C higher than cold water species. Interestingly, they also observed the effect of ionic strength and pH on the onset temperature. It was found an increase in both parameters with tropical fish, but not for cold water species which actually exhibited a decrease in denaturation. Previous work by Connell (1961) showed that the myosin of fish muscle are less thermally stable than those originating from mammalian tissue. This has been related to the temperature in which the fish live (Howell et al., 1991).

Howell et al. (1991) compiled a table of the peak temperature of the first myosin transition temperature for various species as shown in Table 6.1.

Table 6.1: Peak temperature of myosin for various species

Species	Environmental Temperature (°C)	Tmax (°C)	Reference
Rabbit (hind-leg muscle)	Warm-blooded	60	Wright et al. (1977)
Bovine (M. semimenbranosus)	Warm-blooded	59	Park & Lanier (1989)
Titapia. Areochromis aureus		58	Park & Lanier (1989)
Tiger Prawn. Penaeus monodon	25-30	53	Hastings et al. (1985)
Herring. Clupea harengus	4-8	43	Hastings et al. (1985)
Catfish. Calrius gariepinus	c. 30	55.1	Howell et al. (1991)
Nile Perch. Lates niloticus	26-30	55.7	Howell et al. (1991)
Red Snapper. Lutianus sebae	23-27	53.6	Howell et al. (1991)
Sea-Bream. Gymnocranius rivulatus	23-27	51.7	Howell et al. (1991)
Trout. Salmo gairdneri	c. 10	45.7	Howell et al. (1991)
Cod. Gadus morhua	2-8	42	Howell et al. (1991)

From Table 6.1 it is possible to see that various species exhibit markedly different peak denaturation temperatures, therefore it is important to examine the mussel itself, and not base the findings directly on literature. Overall it is possible to study the individual proteins in mixed systems such as meat, on the condition that the proteins denature at easily distinguishable temperatures, thereby generating different and discernible peaks (Stabursvik and Martens, 1980).

6.2.1 Methods and materials	**6.2.1.1 Standard DSC trials**

DSC studies were carried out using a Perkin Elmer DSC7 attached to a Perkin Elmer Thermal Analysis Controller (TAC) 7/DX. The system was calibrated using both a water and indium thermogram. Samples (5-15mg) were taken from fresh mussels (except for those subjected to alternative conditioning) and placed in hermetically sealed aluminium DSC pans. Samples were subjected to a temperature range from 20°C to 100°C at a rate of 10°C/minute for most trials excluding the kinetic investigation. An Endocal, refrigerated circulating bath set at – 30°C was used as the system's cooling accessory. For a reference a sealed empty pan was used. Kijowski and Mast (1988) found that there was no statistical

difference in the onset temperature of meat proteins using water, denatured samples and empty pans as references. Following DSC analysis pans were punctured and stored at 105°C for 24 hours to determine the dry matter content in the sample.

6.2.1.2 Kinetic studies

In order to determine the kinetic parameters related for protein denaturation in mussel meat, the variable heating rate method of Ozawa (1970) was used. This method is based on the fact that Tmax ,"the temperature at which the maximum rate of heat input occurs" (Wright and Wilding, 1984) is sensitive to heating rate, but the conversion at the reaction peak is constant (Duswalt, 1974). DSC thermograms were run from 20°C to 100°C, with varying heating rates of 2, 5, 7, 10, and 15°C/minute. The kinetic constants were determined using equation 6.1. (Ozawa, 1970).

$$\ln\left(\frac{\beta}{T_{max}^2}\right) = \ln\left(\frac{ZR}{Ea}\right) - \frac{Ea}{RT_{max}} \qquad \text{(Eqn 6.1)}$$

where β corresponds to the heating rate (K/min); Tmax is the peak temperature (K); R is the gas constant (J/mol/K); Ea is the activation energy and Z is the pre-exponential factor of the Arrhenius equation. Using a plot of $\ln(\beta/T_{max}^2)$ vs $1/T$ it is possible to determine the value of Ea, Z and consequently the rate constant (Kd) for the protein transition peaks for myosin and actin (Beas *et al.*, 1991).

Work performed by Goodno and Swenson (1975) concerning the thermal transitions of myosin examined the influence of the heating rate on the Tmax value of myosin. In most types of melting experiments such as scanning microcalorimetry, a variation in Tmax with respect to the heating rate is observed (Goodno and Swenson, 1975). In terms of the myosin protein fragment it was determined that with a heating rate fall between 1.86 and 0.44°C/min, there was a concurrent drop in Tmax of around 1.5°C. It is this dependence on heating rate that the kinetic information can be derived from.

6.3   Thermal transitions in mussel fractions

Before performing any of the kinetic trials it was first important to understand how the various parts of the mussel itself differ with respect to thermal treatment. As mentioned earlier compositional analysis of the mussel showed that the majority of the weight of the mussel is made up in the lip and body area. It is therefore important to examine how the different segments outlined in section 3.5, behave when heated, and to determine which segments should be used for future DSC work. The thermal transitions for the various parts of mussel tissue were determined by scanning all samples at 10°C/min. The mussel was segmented as described in section 3.5. Figure 6.1 profiles the mussel fraction DSC curves. Enthalpy peaks can be seen at 50°C and 80°C, in each sample, that can be attributed to the denaturation of myosin and actin respectively (Wright et al. 1977, Martens and Void, 1976).
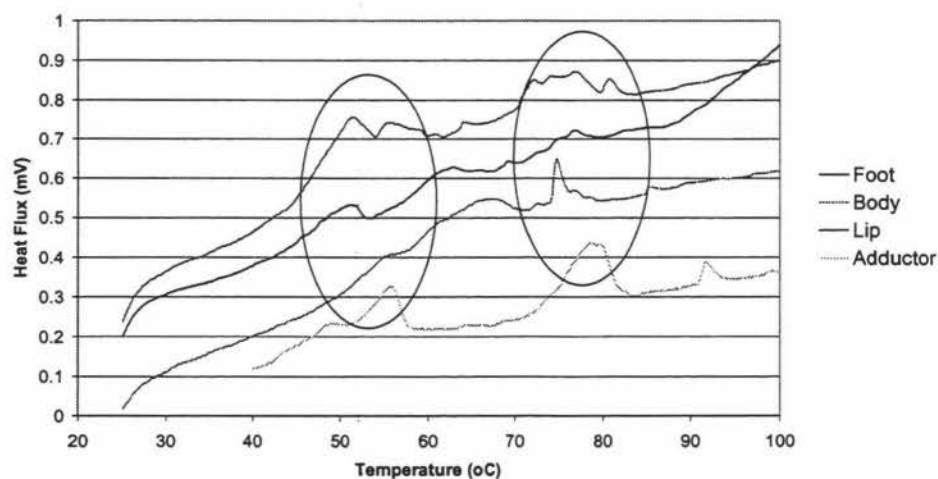
Figure 6:1: Comparison of the thermal transition temperatures of different sections of mussel anatomy

It can be seen in Figure 6.1 that the peaks for the denaturation of actin and myosin are much clearer in the adductor muscle compared to the other sections of the

mussel sampled.

During the initial DSC trials, work was also carried out to determine the extent of damage to the protein structure during heating. Past research has shown that myofibrillar proteins in particular are subject to irreversible protein damage (Quinn *et al.*, 1980). This was shown to be the case during the thermal denaturation of mussel proteins, whereby adductor samples were heated twice, with the second heating showing no endothermic changes, hence irreversible protein denaturation during the first heating treatment. This is important in terms of establishing the optimum processing conditions, as once the protein damage occurs there is no way to recover the water holding capacity afforded by the intact proteins.

Howell *et al.* (1991) stated that although it is possible to study the thermal denaturation of proteins in muscle systems complex problems can often arise. They suggested that the isolation and characterisation of individual proteins could serve to simplify the matter (Howell *et al.*, 1991). Howell *et al.* (1991) separated the myofibrillar from the sarcoplasmic proteins. From Figure 6.1 it can be seen that the denaturation of the sarcoplasmic fraction of a fish myofibril merges with the denaturation of the myofibril fraction. Using the method of Goodno and Swenson (1975) it is possible to isolate the different fractions, derive the DSC curves for the individual proteins and then be able to see them more clearly in the DSC scan of the whole muscle. In terms of viability for the current research, this method would be too time consuming, and judging from the difficulties associated with the more simple compositional analysis it is unlikely that this course of action would provide any fruitful results in a reasonable time frame. Stabursvik and Martens (1980) stated that it is possible to study individual proteins in meat on the condition that the various proteins denature in different temperature ranges, resulting in distinguishable peaks. As shown from Figure 6.1, the peaks in the mussel adductor tissue act in this manner.

As a result the adductor muscle was selected for subsequent analysis. Because of the clarity of the peaks the samples showed that all protein fractions were present in the sample.

6.4   Characterisation of the kinetics of protein denaturation

As mentioned in section 6.1.1.2 it is possible to use the DSC to characterise the kinetics of protein denaturation. By scanning samples of the same substance at varying scanning rates the value of Tmax, the peak temperature, will differ. This variation can be used to determine the underlying kinetics of the protein denaturation itself. The adductor muscles of various mussel samples were scanned at scanning rates ranging from 2 K/min to 15 K/min and the resultant scan profile was used to determine the thermal denaturation peaks of myosin and actin at the differing scan rates. Table 6.2 shows how the scanning rate affected the peak temperature of the mussel proteins, enabling the kinetics of the system to be determined.

Table 6.2: Effect of scanning rate on the thermal denaturation peaks in mussel meat

Scanning Rate (K/min)	Peak I – Myosin (°C)	Peak II – Actin (°C)
2	52.35	73.31
5	56.85	77.4
7	59.25	79.32
10	59.27	80.4
15	62.5	85.6

From the information in Table 6.2 it is possible to plot $\ln(\beta/T_{max}^2)$ vs $1/T_{max}$ as shown in Figure 6.2 to determine the kinetic parameters.
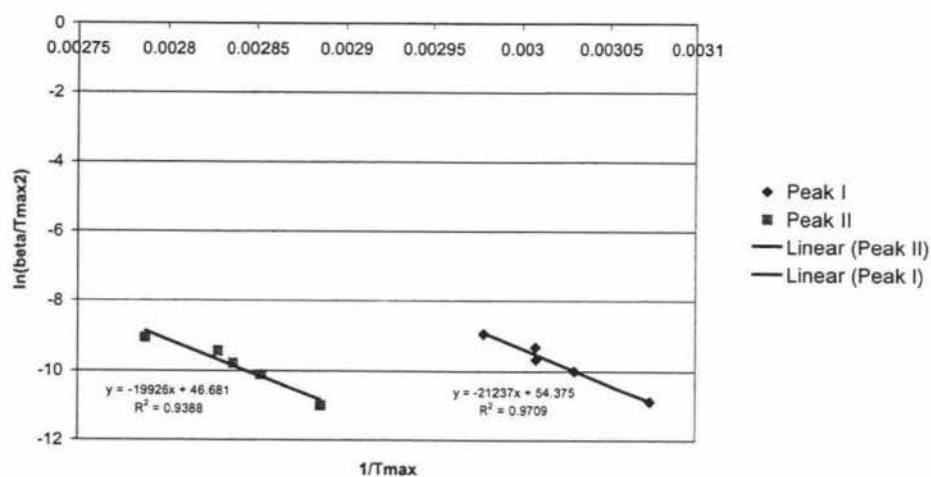
Figure 6:2: Determination of kinetic values for myosin (peak I) and actin (peak II)

From Figure 6.2 it is possible to determine the kinetic parameters of both peaks using the equation described in section 6.1.2.2. Table 6.3 below shows a summary of the data used to determine the activation energy, Ea and Z, the pre-exponential factor of the Arrhenius equation for myosin and action.

Table 6:3: Determination of the Ea and Z

Variables	Peak I - Myosin	Peak II - Actin
Slope = -Ea/R	-21237	-19926
R	8.314	8.314
Ea	176564.418 (177 kJ/mol)	165664.764 (166 kJ/mol)
Intercept = ln(ZR/Ea)	54.375	46.681
$e^{(\text{intercept})}$	4.11872×10^{23}	1.87629×10^{20}
$Z = ((e^{(\text{intercept})} \times Ea)/R)$	8.75×10^{27}	3.74×10^{24}

For Peak I, myosin, the activation energy, Ea, and Z, the pre-exponential factor of the Arrhenius equation were found to be 177kJ/mol and 8.75×10^{27} min^{-1}

respectively. For Peak II, attributed to actin, Ea was determined to be 166kJ/mol while Z was calculated to be 3.74 x 10^{24} min^{-1}. This information compares favourably with research studying the thermal denaturation of fish muscle proteins (Beas et al. 1991). Beas et al., (1991) found that for hake muscle the activation energy for myosin ranged between 52.2 and 67.6 Kcal/mol, depending on whether the fish were pre or post spawning, and Z ranged between 9.33 x 10^{35} and 4.94 x 10^{46} min^{-1} under the same conditions.

6.5 The effect of salt concentration on protein denaturation

Howell et al., (1991) carried out work detailing the thermal stability of fish myofibrils including the effects of ionic strength. The work concluded that as the ionic strength increased, the onset temperature of myofibrillar denaturation decreased. Increasing the ionic strength reduced the stability of fish myosin, except in the case of cod where there was no perceptible effect. The actin onset temperature was similarly effected in all species studied, with the actin destabilising at increasing ionic strengths (Howell et al., 1991). It was found that warm water species were thermally destabilised by up to 8°C when ionic strength was increased from I=0.05 to I=1.00.

Work was carried out in this study to determine whether this trend exists in mussel tissue, which can potentially have varying ionic concentrations depending on cooking conditions (i.e. cooking in seawater or freshwater). Experiments were carried out as described in section 6.2.1.1, with the adductor muscles subjected to salt solutions ranging from 0 to 2.5% v/v (NaCl), and scanned using DSC at 10°C/min. Figure 6.3 below shows the results of the trials.
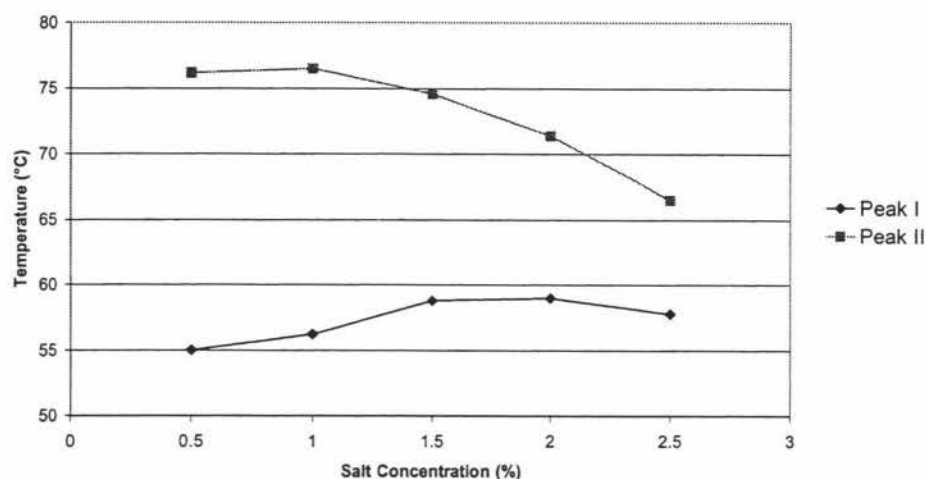
Figure 6:3: Influence of salt concentration on Tmax of myosin and actin in mussel tissue, where peak I and II are myosin and actin respectively

From Figure 6.3 it is possible to see that for mussel tissue the Tmax temperature of myosin increased, then declined slightly with increased salt concentration, whereas the actin peak decreased. This in not in complete agreement with the findings of Howell *et al.*, (1991) described above however, some useful conclusions can be drawn from the exercise. Howell *et al.*, (1991) attributed the destabilisation of the myosin molecules at an increasing ionic strength to the fact that at a low ionic strength myosin molecules combine to form filaments. These filaments have a greater stability than individual myosin molecules which are prevalent at a higher ionic strength. The filaments provide extra stability and hence account for the higher transition temperatures at low ionic strength.

The obvious decrease in transition temperature of actin with increasing salt concentration does have importance with respect to mussel processing. It is desirable to avoid protein denaturation during cooking if moisture loss is to be avoided. These results indicate that cooking in low ionic strength conditions would be preferable. As such cooking baths should not be allowed to increase significantly during cooking with the addition of excess seawater.

6.6   Effect of cooking at various temperatures

DSC can also be used to examine the effect of cooking on the various proteins within the mussels post processing.   Previous trials have concentrated on identifying protein denaturation within uncooked mussel meat in order to establish why water losses are prevalent as temperature increases. The aim of this section was to look at the effect of cooking on DSC thermograms. In this way it was possible to ascertain how cooking at different temperatures irreversibly denatures the constituent proteins in the mussel.

Mussel samples were cooked for five minutes at temperatures ranging from 20°C to 90°C. Samples of the cooked tissue were taken and DSC runs were performed on the samples at a scanning rate of 10°C/min. The effect of cooking at the various temperatures is shown in Figure 6.4 below.

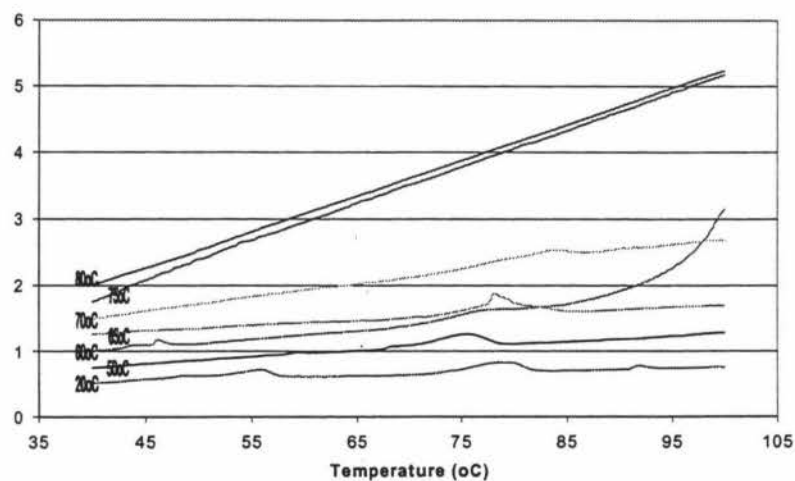**Figure 6:4: DSC profile of mussel samples cooked at various temperatures**

It is possible to see from the chart that as the temperature of the cook increased the myosin peak is the first to disappear from the differential scanning output. The second peak, actin, can be seen at the lower temperatures from 20°C up to approximately 65°C. This would indicate that in mussels cooked for five minutes at

the temperatures above 65°C actin has already denatured. This is important to know as previous DSC work has shown that a first peak exists at some point around 60°C to 65°C, with a second peak prominent at temperatures above 75°C attributed to actin (Findlay and Stanley, 1984; Howell et al., 1991). From figure 6.4 it is possible to see that for meat heated to 70°C there is no evidence of a second peak from the thermogram. This is interesting as it indicates the susceptibility of the mussel actin fraction to denaturation and further highlights the need to heat process mussel tissue at low temperatures.

6.7   Prediction of moisture loss from kinetic data

The effect of temperature on the denaturation of the proteins in mussel tissue has been shown to culminate at around 60 - 65°C. Concurrently, previous work has shown that there are definite moisture losses expressed at this same temperature range. Therefore, it follows that it should be possible to develop a relationship between the kinetics developed in this chapter and the moisture loss results measured in Chapter 5.

From the kinetic information shown in Table 6.3, it is possible to use first order kinetics to further investigate the changes occurring within mussel tissue at increasing temperatures, since:

$$\frac{dC}{dt} = -kC \qquad \text{(Eqn. 6.1)}$$

where:

$$k = Ze^{(-E/RT)} \qquad \text{(Eqn 6.2)}$$

It is possible to prove that at a constant temperature the kinetics result in:

$$\ln\frac{C}{C_o} = -kt \qquad \text{(Eqn. 6.3)}$$

$$\ln\frac{C_o(1-X)}{C_o} = -kt \qquad \text{(Eqn. 6.4)}$$

$$\ln(1-X) = -kt \qquad \text{(Eqn. 6.5)}$$

$$X = 1 - e^{(-kt)} \qquad \text{(Eqn. 6.6.)}$$

By calculating the value for X at individual temperatures for both myosin and actin it is possible to determine the rate of change in terms of the individual proteins. All data collected in Chapter 5, concerning the water losses associated with heating, were carried out after cooking for 5 minutes, however it took approximately 1 minute for the mussel to reach this cooking temperature. Therefore for the calculations shown in figure 6.5 below, a time of 4 minutes was used.

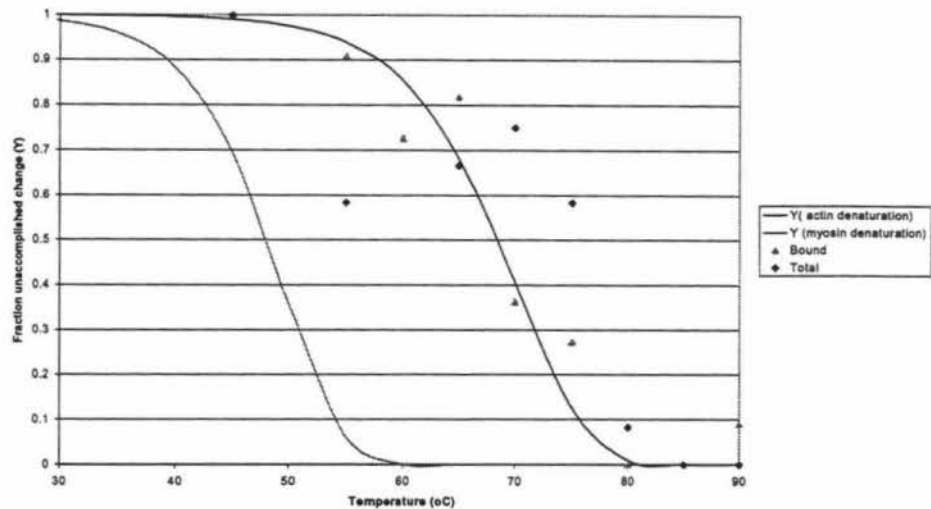**Figure 6:5: Actin and myosin denaturation as a function of temperature. Also showing the unaccomplished change in both bound and total water contents as a function of temperature.**

In addition to the actin and myosin denaturation curves shown on figure 5.6, the moisture losses identified after 5 minutes of cooking for both bound and total water content as a function of initial and steady state water contents are also shown. The

trends are similar for both bound and total water since the free water in mussels was relatively constant. In comparison, the bound water content shows much less variation and exhibits the same trends as those shown for actin denaturation. These results overall highlight the fact there are very many similarities in terms of actin denaturation and water losses in mussel tissue. Since it has been determined that actin denaturation in mussel tissue is evident at temperatures below 70°C (unlike other meat systems which tend to have a higher Tmax for actin), and that this coincides with apparent water losses, processing regimes for cooking mussels must be cognisant of this fact. Figure 6.6 below shows the relationship between water loss and actin denaturation and it can be seen from this that the correlation is strong.
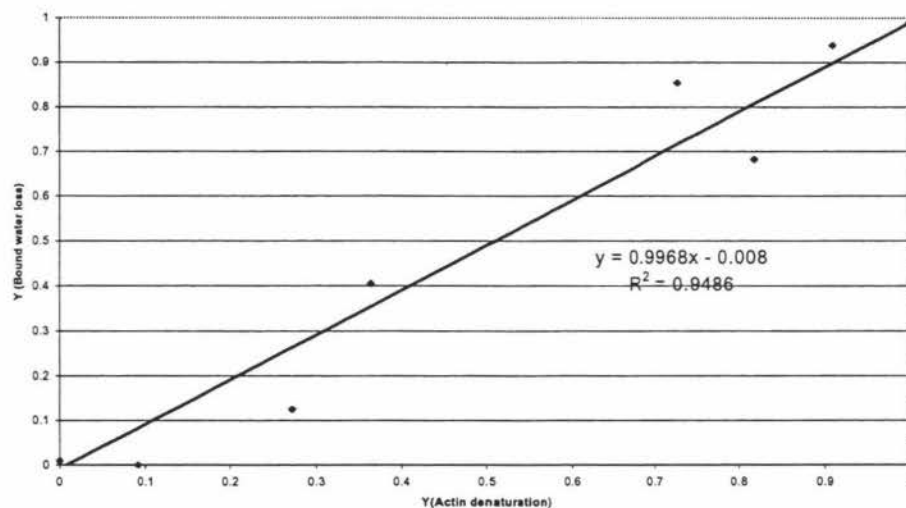
Figure 6:6: Relationship between Y(water loss) and Y(actin denaturation) where the R^2 value is 0.9486

Additionally there is the effect of collagen on the water losses experienced in heated mussels. Since collagen normally denatures in a similar temperature range to actin, the fibrillar shrinkage associated with collagen denaturation may also have influence the rate and extent of water losses within mussels. However, the compositional analysis of mussel tissue in Chapter 3 has shown that there is very little collagen present in mussel tissue compared to other meat systems. It is therefore more beneficial for the processor to concentrate on the water losses from the myofibrillar proteins through ensuring that temperature is controlled below the critical, protein denaturation levels.

6.8 Conclusions

Overall this chapter has provided a useful link between the phenomena of protein denaturation and the moisture losses experienced in a cooking mussel. Through the moisture loss trials detailed in Chapter 5 it was possible to determine a definite trend with respect to certain temperatures. At temperatures over 65°C the amount of moisture lost from a cooked mussel increased significantly. This chapter has closely examined the denaturation of the proteins using DSC. Using simple kinetics it is possible to illustrate a correlation between protein denaturation and water loss. The next step is to prove outright that the temperatures where the changes are occurring (65°C) is applicable in a more commercial situation – heating whole shell and half shell mussels. This work is detailed in Chapter 7.

CHAPTER 7

VALIDATION

7.1 Introduction

The work carried out in his thesis leads to the conclusion that to maximise yield, mussels should be cooked at low temperatures for as long as is necessary to provide microbial safety to the product (i.e. listeria death). Cooking at high temperatures for even a short time will result in protein denaturation and irreversible water loss. A final experiment was performed to validate these conclusions.

Conventional cooking treatment of mussels aims at cooking at high temperatures (e.g. 85°C) until the centre temperature reaches a level required for listeria death (68°C for 16s) (Bremner and Osbourne, 1997). By cooking this way, the bulk of the mussel is exposed to the high temperature. Cooking slowly, for longer periods of time will reduce the temperature gradients through the mussel and as a consequence reduce water losses. To test these theories experiments were carried out on whole and half shell mussels to confirm better cooked mussel yields could be achieved in this way.

7.2 Methodology

Two trials were performed in order to prove the validity of the proposed cooking methodology. The first involved heating whole mussels for a set length of time to

simulate heating in a commercial water bath. This was compared against a trial designed to simulate conventional processing.

The aim of these experiments was to derive the variation in yield associated with the different temperatures. This was determined from changes in cooked mussel water content following the different cooking temperatures.

It was important when attempting to simulate industrial cooking that the temperature within the mussels be monitored. However in the case of the whole mussel cooking a suitable method to record the temperature change within the unshucked mussel was not possible. Therefore, initial tests were performed which determined that mussels at 95°C and 80°C required 5 and 9 minutes of heating respectively to reach the those temperatures. Whole mussels were placed into a heated waterbath, and on removal the centre temperature was measured using a thermocouple. The water content of the samples was then determined by drying the samples at 105°C. The percentage of water remaining compared to the initial sample weight was determined and a boxplot of the data is shown in Figure 7.1 below.

Boxplots of Whole 80 - Whole 95
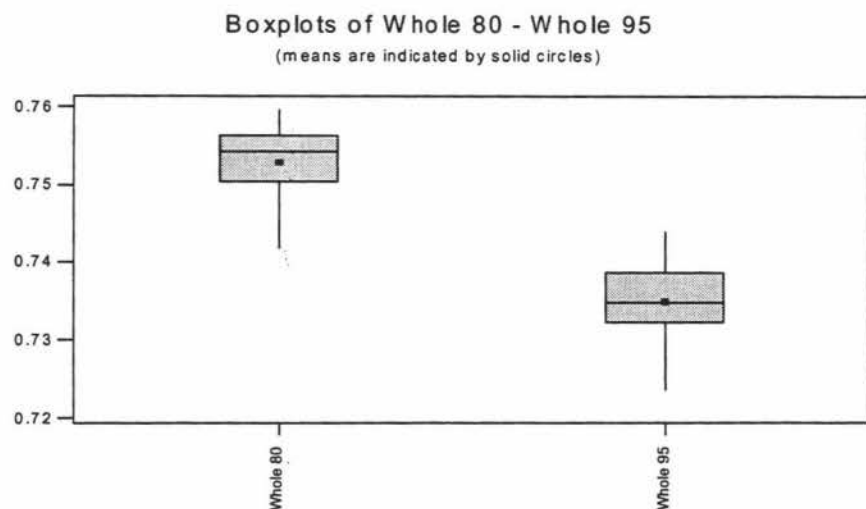(means are indicated by solid circles)

Figure 7:1: Boxplot of water content remaining at 85°C for 9 mins and 90°C for 5 mins for unshucked whole mussels, where the y-axis is the water remaining compared to the initial weight of the sample.

Figure 7.1 shows the difference between the two cooking trials. In order to illustrate that the differences were statistically different, a one-way analysis of variance was performed on the data. It was determined that at a 95% confidence interval, the two means for whole mussels cooked at 80°C and 95°C were significantly different. The values shown on the y-axis of the Figure 7.1 show approximately a 2% decrease in water retention in mussels cooked at the higher 95°C.

The second set of trials aimed to more closely simulate the current processing conditions at Sealords. The methodology for this experiment, therefore, differed slightly from that described above. In this case the mussels were initially preconditioned in a waterbath at 95°C for one minute. The mussels were then removed from the waterbath and moved to a conventional oven, where they were placed approximately 5-7cm below the grill. The mussels remained under the grill for a period of two minutes in an attempt to facilitate the opening of the shell. Through applying sufficient heat to the outside of the shell it was possible to encourage the release of the adductor muscle from one valve and hence the opening of the shell. Following the 'popping' of the mussel shells the unattached valves were removed and the mussels in their half shells were placed into the waterbath. For this experiment the water was again set at 80°C and 95°C for purposes of comparison.

Thermocouples, attached a data logger, were used to monitor the internal temperature of the mussels in real-time. The removal of one the valves from mussel resulted in much faster heating rates. Therefore mussels were only left in the 95°C water bath for just under two minutes, and in the 80°C waterbath for three minutes. Figure 7.2 below shows the variance in the sample means between 80° and 95°C in terms of the water content of the samples.
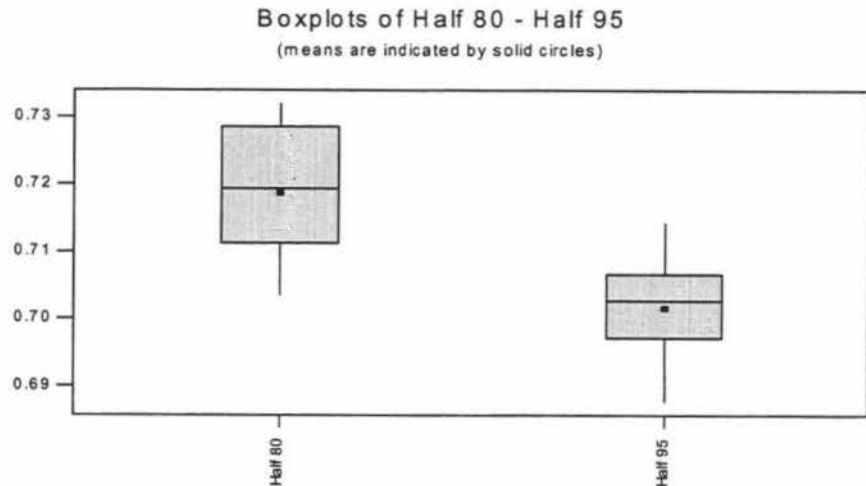
Figure 7:2: Boxplot of water content remaining at 85°C for 3 mins and 90°C for 2 mins for half shell mussels, where the y-axis is the water remaining compared to the initial weight of the sample.

It can be seen from Figure 7.2 that while there was a difference in water losses between the two temperatures treatments, the percentage of water remaining was less than for trials on whole mussels. This is explained by the fact that with the water achieving direct contact with the mussel meat, the rate of heat transfer was faster and more of the mussel reached the water bath temperature with the half shell mussels.

This result reinforces the discussion earlier that minimising temperature gradients (in the case of whole mussels by the presence of intra vavleular spaces) can result in lower moisture loss. This data suggest that even lower temperatures for longer times should be investigated for half shell cooking.

Interestingly, however it should be noted, that the heating rates achieved in commercial factories may infact be faster than those determined experimentally. It is likely that movement within the water baths used in processing, such as that created by moving belts, could increase the turbulence within the water and thus increase the heat transfer coefficient of the water.

Statistical analysis on the half shell mussels proved again a significant difference between the means of the percentage water loss at 80°C and 95°C. The actual variance in the percentage water content between the two temperatures is just under 2%. This is very similar to the findings for the whole mussels, and illustrates again the impact of removing the valve of the mussel.

7.3   Commercial implications

The most important detail to examine from these experiments is the potential yield recoveries afforded through heating at lower temperatures.   In terms of cost savings, the retention of water within the mussel meat will be most beneficial. The following calculations show the potential the cook loss savings described in this chapter have to save money.

WHOLE   COOKED MUSSELS   The dry weight of mussels has the same basis for the two moisture content measurements. From figure 7.1 above, it is possible to see that after cooking at 80°C, the water content, $X_{80°C}$, is 0.754 g water/g mussel cooked, whereas cooking at a temperature 95°c, decreases the water content, $X_{95°C}$, to 0.734 g water/g mussel cooked.

To determine the increase in dry weight the following calculation, based on a comparison of the dry mussel weight at each temperature can be performed, where:

$$\frac{\text{g dry mussel at 95°C}}{\text{g dry mussel at 80°C}} \quad = \quad \frac{(1-X_{95°C})}{(1-X_{80°C})} \quad = \quad \frac{(1-0.734)}{(1-0.754)} \quad = \quad 1.081$$

This corresponds to a increase in yield of 8.1%, which at an average value of $5/kg translates to an increase in revenue of $0.4/kg. Considering that the typical yield on a green weight basis is 0.25kg of cooked mussel meat per kg of green weight, this would increase to 0.27kg of cooked mussel meat per kg of green weight.

MUSSEL MEAT COOKED ON HALF SHELL

Using the same methodology as for the whole cooked mussels, it is possible to determine the water content of the half shell mussels after cooking from figure 7.2. This shows that for mussel meat cooked on half shells, $X_{80°C} = 0.72$ and $X_{95°C} = 0.704$. Performing the same calculation to determine the dry weights of the mussel meat and hence the yield increase in half shell mussels results in the following:

$$\frac{\text{g dry mussel at 95°C}}{\text{g dry mussel at 80°C}} = \frac{(1-X_{95°C})}{(1-X_{80°C})} = \frac{(1-0.704)}{(1-0.72)} = 1.057$$

This translates to an increase in yield of 5.7%, which at an average value of $5/kg corresponds to an increase in revenue of $0.285/kg. In terms of additional greenweight yield this equates to 0.264 kg cooked mussel per kg greenweight.

HALF SHELL BASIS

If mussels are **sold** on a half shell basis it is likely that there will be approximately 0.8kg shell per kg of cooked mussel at 95°C. Therefore yields on half shell basis will be:

Cooked whole: 8.1%/1.8 = 4.5% increase.

Cooked in half shell: 5.7%/1.8 = 3.17% increase

In terms of value, for mussels cooked whole, a 4.5% increase, where half shell product sells for approximately $4.25 per kg, equates to a dollar value of **$0.19 per**

kg of half shell product. In terms of greenweight yield, the typical yield is approximately 0.45 kg half shell product per kg greenweight. This would increase to 0.47 kg half shell product per kg greenweight.

Similarly for mussels cooked in the half shell, a 3.17% increase would be worth **$0.135 per kg of half shell product**. In terms of greenweight yield, this would increase to 0.464 kg half shell product per kg greenweight.

CHAPTER 8

CONCLUSIONS AND
RECOMMENDATIONS

Overall there are several rules of thumbs which have been revealed through the current work and which, if followed should enable considerable savings to be made in the commercial processing of mussels.

1. Processing temperatures should be kept as low as possible to avoid protein denaturation and temperature gradients through the mussels should be minimised.

2. The length of the cook time can be extended to ensure that listeria death requirements are fulfilled. Additionally the inactivation of lipases is required to avoid rancidity development during frozen storage of the cooked product.

3. Lower temperatures for longer times will mean either a reduced production rate or larger capital equipment requirements. This must be balanced against yield savings.

4. Lower temperatures will reduce the chances of the whole mussel opening, which could potentially mean an increased risk of damage to the mussels through knife opening. Additionally the opening rates will be much slower and as such productivity may be affected.

5. To avoid both problems, rapid high temperature opening could be employed (e.g. steam, infra red heating etc). This would allow cooking half shells which will be much faster than for whole mussels and should not slow production.

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NOMENCLATURE

Bi	Biot number	
C	Concentration	
c	Specific heat capacity	J/kgK
C_o	Initial Concentration	
Ea	Activation energy	J/mol
Fo	Fourier number	
h_A	Heat transfer coefficient	W/m^2K
k	Rate constant	min^{-1}
m	Moisture content	kg/kg
R	Gas constant	J/mol/K
R	Slab half thickness	m
T	Temperature	K
t	Time	s
T_{max}	Peak temperature	K
X	Extent of reaction	
x	Position	m
Y	Fraction unaccomplished change	
Yav	Mass average fraction unaccomplished change	
Z	Pre-exponential factor	min^{-1}
β	Heating rate	K/min
θ	Temperature	°C
θ_i	Initial temperature	°C
θ_s	Surface temperature	°C
λ	Thermal conductivity	W/mK
ρ	Density	kg/m^3