Development of a gelled form of lactic-fermented green shell mussel (*Perna canaliculus*)

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Abstract

New Zealand's isolation makes it a natural home to many unique species. One of them is the now cultivated New Zealand green shell mussel (*Perna canaliculus*) which is sought after by seafood lovers around the world. This mussel is by far the largest seafood export from New Zealand accounting for about 85% and exported to 78 countries in 2011. This research was conducted to create a variant of an existing value-added product: lactic-fermented comminuted mussel meat. This product has been named *Perna*. The existing knowledge relates to a spreadable form of *Perna*, which is currently undergoing commercial evaluation.

This work is mainly about developing a gelled form of *Perna* using hydrocolloids, specifically carrageenans and gelatine. The proposition was that a gelled, sliceable form of *Perna* would expand its culinary potential. The spreadable form would be used for crackers and breads, while a slice of *Perna* could be part of an entrée in domestic cuisine or food service.

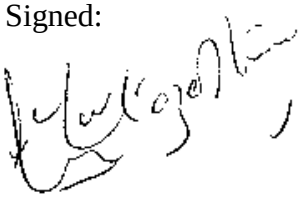
However, as the project progressed it became clear that unacceptable variations in pH of *Perna* were affected by the time the mussels were cooked in boiling water prior to comminution and fermentation. Because of the fundamental importance of this variation in terms of odour/flavour, the first part of the thesis explores the chemistry of how pH could be better controlled. It was found that the buffering capacity of mussel proteins changed with cooking. Although no explanations were obtained at a biochemical level, the empirical outcome was that cooking live mussels between 7 to 10 minutes would predictably result in a desired final pH of around 4.5 that yields superior organoleptic properties such as pleasant smell, taste and texture.

Moving to gelation as a goal, it was found that kappa and iota carrageenans were incapable of forming useful gels in *Perna*, the former producing excessive syneresis and latter a soft, spongy product. Experiments with gelatine were initially unsuccessful, inadvertently because of the low gelatine concentrations tested. After a more concentrated gelatine colloidal suspension of about 8% was incorporated into the mussel mince, a useful gel was finally created, although the texture was crumbly. However, this final success is considered an excellent starting point for sliceable product refinement.

Statement of Originality

I declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person, nor material which has been accepted for a qualification of any institution, except where acknowledgement is made.

Signed:



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10 September 2015

"Part of the secret to success in life is to eat what you like and let the food fight it out inside."

– Mark Twain

Chapter 1

Introduction

Aquaculture

Aquaculture is the farming of fish and other aquatic animals and plants based in both salt water and freshwater, and is practised in natural waterways and artificial enclosures. Marine aquaculture is practised in the sea, generally in calm coastal waters. Aquaculture is now the world's fastest growing primary industry, and demand for aquaculture products is expected to strengthen significantly as the world's population increases, and wild-catch levels remain relatively static. United Nations Food and Agricultural Organisation figures show aquaculture produces about 47% of seafood consumed globally by humans with production levels growing at a rate of approximately 6.3% annually since the past decade. Estimates suggest aquaculture will soon produce more seafood than wild fisheries (FAO, 2014).

The important marine aquaculture species of New Zealand are the green shell mussel (*Perna canaliculus*), the king or quinnat salmon (*Oncorhynchus tshawytscha*) and the Pacific oyster (*Crassostrea gigas*). These three are the major export items by value and volume (Table 1) (New Zealand seafood exports, 2012). Land-based aquaculture typically involves growing fish in hatchery tanks and outdoor ponds. Presently, the main marine and freshwater species farmed inland include salmon, paua, and rock lobster.

In 1969, a group towed the first mussel barge into Marlborough Sounds and anchored it in Kenepuru. Since then the New Zealand aquaculture industry has evolved from the activities of this group of innovative pioneers to a professional, specialised food production sector focused on environmental sustainability and food safety (Vitasovich, 2012).

In 2011, the New Zealand aquaculture industry employed over 3,000 personnel and generated over $400 million of revenue, out of which $298 million was produced in exports (Table 1) (New Zealand seafood exports, 2012).

Table 1.	New Zealand aquaculture statistics – production and revenue metrics for 2011.		
	Mussel	Salmon	Oyster
Harvested product (green weight tonne)	101,311	14,037	1,804
Export revenue NZ$ (millions)	218	63	17
Domestic revenue NZ$ (millions)	35	65	8

Source: New Zealand Seafood Exports (2012).

For the export market, the dominant form of mussels is fully cooked or blanched on a half shell. Percent export by value for each product category is shown in Table 2 (New Zealand Aquaculture, 2012). Thus, the product forms that the greenshell mussels are sold in are limited. Unlike oysters for example, mussel meat is cheap[1] and is the logical candidate for adding value. That is the subject of this thesis.

Table 2.	Product forms of New Zealand green shell mussel for export.
Product category	Exports by value (%)
Half shell frozen	83.6
Meat frozen	9.7
Whole frozen	3.6
Preserved/marinated	1.3
All other forms and nutraceuticals	1.7

Source: New Zealand Aquaculture (2012).

Scope and objective

The primary objective of this study was to develop a gelled form of a mussel product that is in its present embodiment called *Perna*. This work follows that of Dsa (2013) who developed the comminuted spreadable gourmet product (Figure 1) that is currently undergoing market evaluation by Future Cuisine Limited of Swanson, Auckland.

[1] In November 2014, Future Cuisine Limited of Swanson, Auckland, quoted about $9 per kilogram of mussel flesh, a very cheap form of high quality protein.

Figure 1. The spreadable ‘pâté’ known as *Perna* (Dsa, 2013).

The aim here is to make a product that is sliceable, designed as an entrée product to be accompanied by, for example cheeses and olives, and to command a higher price than the spreadable pâté form developed by Dsa. At its simplest, the Dsa product comprises cooked mussels, comminuted through a 4-mm mincer plate, blended with 2% added salt, 2% glucose, 1% iota-carrageenan (a hydrocolloid thickening ingredient) and 0.02% of a lactic acid starter culture. (This typically comprises one of more lactic acid bacterium (LAB), and one or more bacterium intended to carry out limited fat and protein hydrolysis to create flavour complexity.) This mixture is packed under vacuum, and fermented at 30°C for 96 hours. Under vacuum the product is nominally stable at ambient temperature, but is routinely stored at refrigeration temperatures mainly because the market expectation is that seafood products should be stored refrigerated.

Lactic fermentations are routinely applied to many foods internationally, but what sets *Perna* apart is a simple but non-intuitive cooking step before fermentation. Remarkably, that step has not been applied before, but is necessary in the case of mussels with their digestive biota and their endogenous hydrolytic enzymes. Failure to cook prior to fermentation at these relatively low salt concentrations results in disgusting, odorous slurry shown in Figure 2 (Qiang, 2012).

Figure 2. The unpleasant slurry created by fermenting raw mussels with LAB (Qiang, 2012).

Perna as a pâté was the starting point for this project and what follows in this chapter is a review of literature relevant to this project: food preservation, non-alcoholic fermentations, mussel properties, hydrocolloids, and the techniques for assessing the properties of the intended product.

Literature Review

Food preservation

The diverse and nutrient composition of foods makes them a suitable environment for the growth and propagation of food spoilage microorganisms and common food-borne pathogens. It is therefore essential that adequate preservation technologies are applied to maintain safety and quality (Aymerich, Picouet, & Monfort, 2008). The processes used in preservation are principally concerned with inhibiting microbial spoilage, although other methods of preservation are also used to minimise deteriorative changes such as colour and oxidation of fats.

A number of interrelated factors influence the hygienic storage life of foods, specifically temperature, oxygen concentration, endogenous enzymes, water activity, light and, most importantly, the presence of viable microorganisms. All of these factors, alone or in combination, can result in detrimental changes in the colour, texture, odour and flavour of foods. Although deterioration of foods can occur in the absence of microorganisms (e.g., proteolysis, lipolysis and oxidation), microbial growth is by far the most important factor in relation to the keeping quality of foods.

Methods of food preservation can be grouped into three broad categories based on control by temperature, by water activity and, more directly, by inhibitory processes. These inhibitory processes involve well-known techniques such as packaging, extending to modern techniques such as application of hydrostatic pressure, and ionising radiation (Zhou, Xu, & Liu, 2010). However, a particular method of preservation may involve several principles. Each control step can be regarded as a 'hurdle' against microbial proliferation and combination of processes – hurdle technology – can be devised to achieve particular objectives in terms of both microbial and organoleptic[2] quality. The most common hurdles used in food preservation are temperature (high or low), water activity, acidity, a reductive environment, chemical preservatives (e.g., nitrite, sorbate, lactate, sulphite), and competitive microorganisms which are known to be harmless or even nutritionally useful (e.g., LAB, and certain molds).

Among alternative food preservation strategies, particular attention has been paid to biological preservation techniques, which extend the storage life and enhance the hygienic quality. Biological preservation usually refers to the use of a natural or controlled microflora and its antimicrobial metabolites (García, Rodríguez, Rodríguez, & Martínez, 2010). The subject of food

[2] Organoleptic quality means quality as perceived by the human senses: taste, smell, vision, hearing and touch.

preservation is broad. The most relevant subtopic for this thesis is lactic fermentation, now discussed in more detail.

Preservation of plant and animal foods by fermentation

a) Fermentation history in a nutshell

Fermentation of foods is one of the oldest food preservation technologies known to humans with the earliest records dating back to 8,000 years before present. It was used by the civilisations in the Fertile Crescent (the Levant) in the modern day Iraq (Caplice & Fitzgerald, 1999). With time, it has evolved and has been refined and diversified. Today a large fraction of the human food supply is derived from this technology, which is used in households, and small- and large-scale food industries (Yann & Pauline, 2014).

b) Selected versus natural starters

Fermentation can be achieved either by an uncontrolled approach or by deliberate selection of a fermentative microflora in a so-called starter culture. Selection of starters involves a limited number of bacterial species and strains chosen for specific technological reasons, e.g. controlled acidification and flavour production. Selection has been achieved empirically, such that beneficial microorganisms were favoured for further use while spoilage and harmful pathogens were avoided (Holzapfel, Geisen, & Schillinger, 1995). This approach to fermentation has been largely developed over the past century. Starters can be used in the initial steps of the food manufacturing process and in maturation. Their regular use has led to standardized final products.

Natural starters – also called wild, indigenous, or adventitious starters – are made up of complex microflora, especially lactic acid bacteria (LAB) but also yeasts and molds that can originate from various sources such as the raw materials, processing tools, and the wider processing environment. Natural starters are mainly linked to the techniques of back slopping; however, they can also result spontaneously from the atmosphere or the raw materials (Yann & Pauline, 2014).

Back slopping is defined as inoculating the fermentable food with a small portion of a previously successful fermentation. Back slopping is traditionally used for sauerkraut fermentation to favour the dominance of the best-adapted strains, which shortens the fermentation time and reduces the risk of failure. The LAB responsible for the fermentation of Korean kimchi (fermented cabbage) has been well researched. In particular, *Leuconostoc mesenteroides* and *Pediococcus pentosaceus* start the fermentation, and the combination of *Lactobacillus plantarum* and *Lactobacillus brevis* further dominate depending on the temperature of incubation. Table 3 presents

some examples of natural and spontaneous starter microflora for several products from around the world.

c) Lactic acid bacteria, a generally useful class of fermentative microorganisms

Of the fermentative organisms used in food, the LAB is a particularly useful group. They often are naturally present in food products, and can act as powerful competitors to contaminating spoilage microorganisms by producing a wide range of antimicrobial metabolites such as organic acids – lactic being the best example, diacetyl, acetoin, hydrogen peroxide, reuterin, reutericyclin, antifungal peptides, and bacteriocins (Cleveland, Montville, Nes, & Chikindas, 2001).

The most relevant antimicrobial substance produced by LAB is lactic acid and the concomitant reduction of pH. The antimicrobial effect of organic acids in food ecosystems lies in the reduction of pH, as well as the nature of the undissociated form of the organic acid, which inhibits the growth of unwanted microorganisms (Ghanbari, Jami, Domig, & Kneifel, 2013).

Bacteriocins are a heterogeneous group of antibacterial proteins that vary in spectrum of activity, mode of action, molecular weight, genetic origin and biochemical properties. The production of bacteriocins by LAB is very significant for applications in food systems and thus, unsurprisingly, these have been most extensively investigated. Among numerous bacteriocins so far characterized, nisin is best defined, and is the only purified bacteriocin preparation approved for use in food products (Cleveland et al., 2001; García et al., 2010). For example nisin is used to decontaminate artificially contaminated pieces of raw pork and in combination with 2% of sodium chloride as an anti-listerial agent in minced raw buffalo meat (Stiles & Hastings, 1991).

Table 3. Fermented products obtained by spontaneous fermentation or by the use of natural starters.

Product	**Origin**	**Raw material**	**Main microflora[1]**	**Type of fermentation**
Bread	Various	Grain flour	LAB, yeasts	Natural
Cheese	Various	Milk	*Lactobacillus* spp, *Lactococcus* spp, *Ln* spp.	Natural
Soy sauce	China	Soybeans	*Aspergillus, S. cerevisiae, Bacillus spp.*, LAB	Spontaneous or natural
Kefir	Various	Milk	LAB, yeasts	Natural
Chocolate	South America	Cacao beans	LAB, yeasts	Spontaneous
Kimchi, sauerkraut	Korea, Europe	Radish, cabbage	LAB (*Lb plantarum, Lb brevis*), *Streptococcus faecalis, Lnmesenteroides, Pc pentosaceus*	Spontaneous
Kivunde	Tanzania	Cassava	LAB	Spontaneous or natural
Fufu, Lafun	Nigeria		LAB, *Bacillus*, coliforms, *Enterococcus, Klebsiella, Candida*	
Attieke	Ivory Coast		LAB (*Ln mesenteroides* subsp. *mesenteroides, Ec faecalis*)	
Gari	West Africa		LAB, *Alacaligenes, Corynebacterium, Lb plantarum*	
Agbelina	Ivory Coast, Ghana		*Lbs brevis, Lb plantarum, Ln mesenteroïdes, Candida krusei*	
Tempe (tempeh)	Indonesia	Beans, cereals	*Rhizopus* ssp.	Spontaneous
Olive	Italy, Greece,Turkey	Olive	*Ln, Pediococcus, Lb plantarum*	Spontaneous
Pickles	Turkey	Cucumber	*Lb plantarum, Lb pentosaceus, Ln mesenteroides, Pc cerevisiae*	Spontaneous or natural
Rabadi	India	Barley, buttermilk	LAB (*Ln mesenteroïdes, Ec faecium, Pc pentosaceus, Lb curvatus*), *Saccharomyces cerevisiae, S. kluyveri, Debaryomyceshansenii, Pichia*	Spontaneous
Selroti	Himalayas	Rice, maize, millet		
Injear	Ethiopia	Sorgo, tef	LAB	Spontaneous
Ekung, eup, hirring	India	Bamboo shoots	*Lb plantarum, Lb brevis, Lb casei, Lbfermentum, Lactococcus, Tetragenococcus*	Spontaneous
Gundruk, khalpi	Nepal	Cabbage, mustard and radish leaves	LAB (*Lb plantarum, Lb brevis, Lb pentosaceus*)	Spontaneous

[1] LAB: lactic acid bacteria; Ln: *Leuconostoc*; Lb: *Lactobacillus*; Pc: *Pediococcus*; Ec: *Enterococcus*; S: *Saccharomyces.*

Source: Yann & Pauline (2014)

In Figure 3, the nisin amino acid residues in red have a positive net charge and those in blue are hydrophobic. The amino terminus is the NH_2; Dha is dehydroalanine; Dhb is dehydrobutyrine; Lan is lanthionine; Mla is methylanthionine; and S is the thioether bridge (Peschel & Sahl, 2006). The other amino acids have the single letter code, e.g. L is leucine.

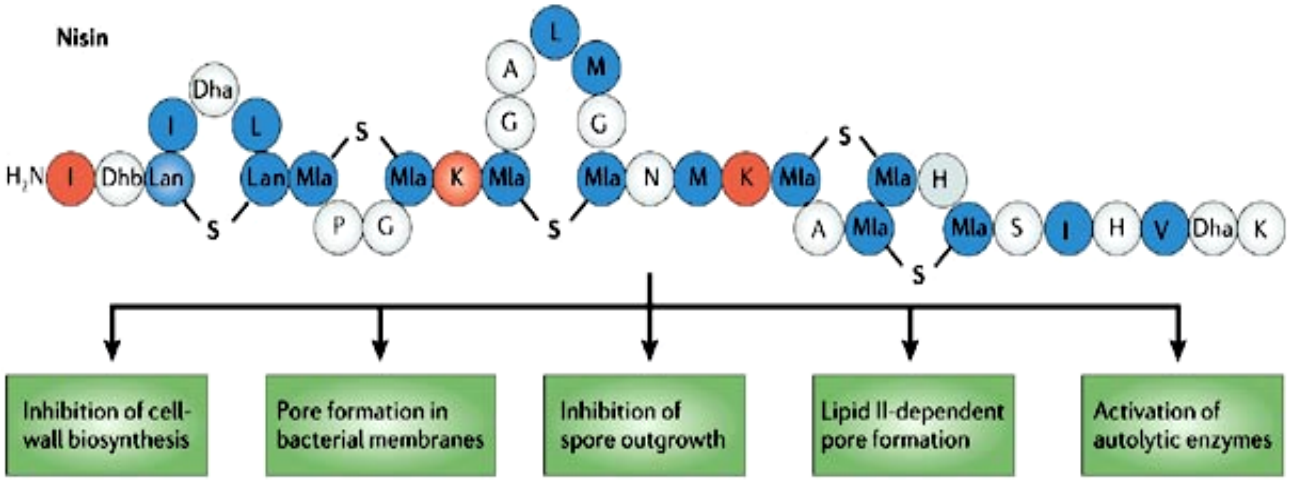

Figure 3. Molecular structure of nisin and its various modes of antimicrobial action (Peschel & Sahl, 2006).

Based on the reports on the application of LAB protective culture and/or their bacteriocins in seafood products, the recognized strengths of biological preservative agents can be summarized as follows: (1) promoting extended shelf life of food during storage time; (2) contributing to decreased risk for transmission of food-borne pathogens; (3) allowing reduced application of chemical preservatives and harsh physical treatments such as cooking, heating, refrigeration, etc., therefore helping to preserve the nutritional quality and organoleptic properties of foods; (4) is a cost-effective technology, and; (5) a useful response to the consumer demands of minimally processed, safe, preservative-free foods (Gálvez, Abriouel, López, & Omar, 2007). This explains the significance of fermentation as a biological preservative technique and has been summarised in Figure 4.

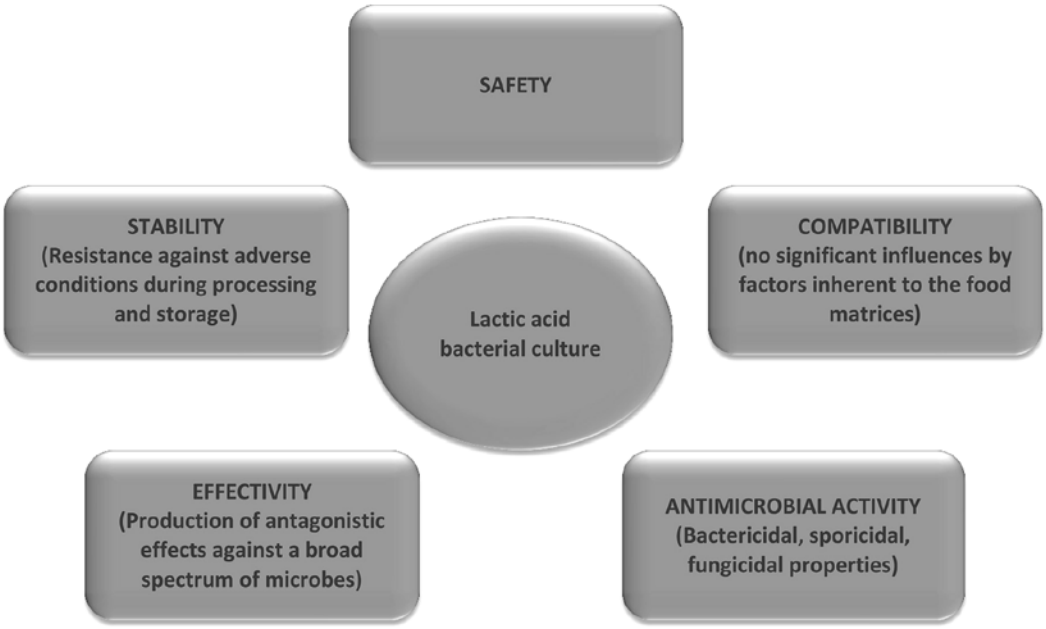

Figure 4. Principles of potential LAB protective cultures and/or their inhibitory metabolites (Ghanbari et al., 2013).

However, there are limitations in the use of LAB. The effectiveness of protective cultures and/or their inhibitory compounds in food can be limited by a range of factors such as: (1) the narrow activity spectrum of some antimicrobials; (2) the spontaneous loss of bacteriocinogenicity due to genetic instability; (3) limited diffusion behaviour in solid matrices; (4) inactivation through proteolytic enzymes or binding to food ingredients such as fats; (5) poor adaptation of the culture to refrigerated food environments; (6) low antimicrobial production and the development of bacteriocin-resistant bacteria. In addition, most LAB protective cultures are not capable of surviving heat treatment processes over 45°C and therefore must be added only after any heat treatment (Devlieghere, Vermeiren, & Debevere, 2004). (Note that heat treatment is commonly applied to milk (pasteurisation) before lactic fermentation, and to soy beans before sauce production (see below), but that appears to be the extent of heating before fermentation. As is known now, a heat step is essential for mussels (Figures 1 and 2) and almost certainly for any shell fish.)

What follows now is a description of lactic acid fermentation as it applies to specific perishable foods, such as milk, meat, fish and some vegetables and fruits.

d) Milk products

i) Yoghurt and cheese

Yoghurt production and often cheese production requires lactic fermentation of milk. In yoghurt production, the starter is generally a mixed culture of *Streptococcus thermophilus* and

Lactobacillus bulgaricus. The *Streptococcus* grows first because it is inhibited between pH values of 4.2 and 4.4, which is then followed by *Lactobacillus* growth, both adding flavour to the yoghurt. *Lactobacillus* can tolerate values as low as pH 3.5 (Caplice & Fitzgerald, 1999).

Most commercial lactic cheeses (Table 3) are produced with a starter culture such as mesophilic *Lactococcus lactis* subspp., *Lactis cremoris*, or thermophilic *Streptococcus thermophiles* and *Lactobacillus helveticus*, depending on the specific application. A secondary microflora can be added for a number of reasons: (1) to affect the texture of cheese (for instance *Propionibacterium* is used in the production of CO_2 in Swiss cheese); (2) molds, yeasts and bacteria other than LAB are used in some varieties of cheese (e.g., *Penicillium roqueforti* in blue cheeses); and (3) to impart flavour by the production of diacetyl compounds by *Lactococcus lactis* subsp.

ii) Fermented milk

The popularity of fermented milks has increased in recent years, not only because of their attractive taste but also because of the many health benefits perceived to be or genuinely caused by them. For example, kefir (Table 3) and kumiss are fermented milk drinks. They use the characteristic acid producing *Lactobacillus lactis* and *Lactobacillus delbrueckii* subsp. *bulgaricus* plus an alcohol-producing *Torula* species. *Lactobacillus acidophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* are used in the production of acidophilus milk and in Bulgarian buttermilk where the inoculated sterile milk is held at 37°C until a smooth curd develops (Caplice & Fitzgerald, 1999).

e) Vegetable and fruit products

There are more than 20 varieties of commercial raw vegetable and fruit fermentations sold in Europe, the most economically relevant of these are the fermentations of olives, cucumbers, and cabbage, the last known as kimchi and sauerkraut (Table 3) (Caplice & Fitzgerald, 1999). Under favourable anaerobic conditions, water activity, salt concentration and temperature, raw vegetables and fruits undergo spontaneous lactic acid fermentation. In some cases, an alcoholic fermentation takes place at the same time (Di Cagno, Coda, De Angelis, & Gobbetti, 2013).

i) Sauerkraut

Commercial sauerkraut fermentation generally relies on the natural LAB present on the cabbage leaf for initiating fermentation. Microbiological studies have shown that four species are typically present in sauerkraut fermentation of which *Leuconostoc mesenteroides* is the major species involved in the early stage and *Lactobacillus plantarum* becomes dominant in the latter, starting at about 5 to 7 days after the onset of fermentation. The correct sequence of organisms is

essential in achieving a stable product with flavour and aroma typical of sauerkraut (Plengvidhya, Breidt, & Fleming, 2004).

ii) Soy sauce

Soy sauce (Table 3), shoyu in Japanese, is used widely all over the world as a condiment and colouring agent. It is made by fermentation of four ingredients, soybeans, wheat grain, water and salt. The production of fermented soy sauce involves three major steps, namely, koji[3] production, brine fermentation, and refining. The microorganisms involved in soy sauce production and the biochemical and chemical changes to soybean and wheat during fermentation greatly influence the sensory attributes and quality of soy sauce (Isaac, 1994).

f) *Meat products*

Meat fermentation is a low energy, biological acidulation and preservation method which results in distinctive meat properties such as flavour, palatability, increased shelf life and a host of other desirable consumer attributes. Fermented sausages in particular are made as a result of lactic fermentation of a mixture of meat together with fat, salt, curing agent nitrite (sometimes derived from the reduction of nitrate), sugar and spices. These sausages are generally classified as dry or semi-dry. Dry sausages have a water activity of less than 0.90 as compared to semi-dry sausages being 0.95. Fermentation temperatures vary according to the individual product but they are generally less than 22°C for dry and mold-ripened sausages and 22 to 26°C for semi-dry varieties. Sausages produced using a starter generally has a final pH of 4.0 to 4.5 (Caplice & Fitzgerald, 1999).

According to Lucke (1994), most European fermented sausages formulated with nitrite have added starter culture of LAB (lactobacilli and pediococci) and catalase-positive cocci (*Staphylococcus carnosus* and *Micrococcus varians*). Yeasts and molds that are utilised as starters in meat fermentation include *Debaryomyces hansenii, Candida famata, Penicillium nalgiovense* and *Penicillium chrysogenum*. LABs producing bacteriocins have been demonstrated to reduce the count of *Listeria monocytogenes* by one log early in meat fermentation and this is one particular application where the use of bacteriocinogenic cultures appears to have potential value as an additional inhibitory hurdle (Caplice & Fitzgerald, 1999).

g) Seafood products

Seafood is highly nutritious, but also highly susceptible to spoilage due to microbiological and biochemical degradation (Mejlholm et al., 2008). As a result, the development of effective

[3] Koji is cooked soybeans that have been inoculated with a fermentation culture, *Aspergillus oryzae.*

processing treatments to extend the shelf life of seafood products is important. This is particularly important, because affluent consumers increasingly demand high quality but minimally processed seafood. In this context, lower levels of salt, fat, acid and sugar and/or the complete or partial removal of chemically synthesised additives has become commercially important (Ghanbari et al., 2013), although these demands often have no basis in fact. Lactic fermentation of seafood is one way of satisfying this demand.

i) LAB and seafood products

The diverse and rich nutrient composition of seafood provides an ideal environment for growth and propagation of spoilage microorganisms and common food-borne pathogens (Ghanbari et al., 2013). Biological preservation of seafood products is an alternative to, for example, retorting and freezing, to meet safety standards and to control microbial deterioration without negative impact on the sensory quality of the product. The selection of LAB possessing the GRAS (generally recognised as safe) status (U.S. Food and Drug Administration) as protective cultures is generally agreed as beneficial for extending the shelf life of seafood products (Françoise, 2010). Likewise, they also fulfil the QPS (qualified presumption of safety) requirements (Leuschner et al., 2010).

Usually, LAB are not considered as typical micro-flora of the aquatic environment, but certain genera, including *Carnobacterium*, *Enterococcus*, *Lactobacillus* and *Lactococcus*, have been found associated with fresh sea water fish (Table 4). Importantly – as noted above – their growth can also suppress potent spoilage germs by means of antagonistic and inhibitory activities either through the competition for nutrients or the production of one or more antimicrobial active metabolites (Ghanbari et al., 2013).

Table 4. Reports of LAB isolated from fish.

Type of lactic acid bacteria	Identified in these seafoods
Lactobacillus spp.	Arctic charr, Atlantic cod, Atlantic salmon, brown trout, herring, sturgeon fish, various fish
Carnobacterium spp.	Arctic charr, rainbow trout, brown trout, various fish
Aerococcus spp.	Atlantic salmon
Enterococcus spp.	Common carp, brown trout
Lactococcus spp	Common carp, brown trout
Leuconostoc spp.	Arctic charr
Pediococcus ssp.	Common carp, rohu
Streptococcus spp.	Arctic charr, Atlantic salmon, carp, eel, Japanese goldfish, rainbow trout, various salmonids, turbot yellowtail
Vagococcus spp.	Brown trout
Weissella hellencia	Flounder

Source: Ghanbari et al., 2013

There are two categories of fermented fish products – high salt products and low salt products. The former are a group of fermented foods with salt content of 13% w/v or higher to prevent putrefaction and development of pathogens such as those responsible for botulism. The substrates for such fermentations include soy and other grains (fermented by *Aspergillus oryzae*), or small fish. High salt products contain a mixture of savoury, amino acids and peptides that are important condiments, particularly for those unable to afford much meat in their diet (Steinkraus, 1996). Fermentation by this method usually takes longer to complete, anytime from a month to a year (Buisson, 1978). For instance in Thailand, a product called *Nam-pla* (sauce) is made from the fish species *Stolephorus* spp., *Rastrelliger* spp., *Cirrhinus* spp. and takes 5 months to a year for fermentation to complete.

The low salt product is characterised by a marked increase in the microbial count of lactic acid bacteria whose growth is supported by the addition of fermentable carbohydrate. As an example of such a product, fermented fish mince (FFM) comprises minced fish, minced steamed rice, minced garlic and salt. In its Southeast Asian expression, the mixture is tightly packed in banana leaves and/or plastic bags to eliminate air and left to ferment for up to 5 days at 30°C (Riebroy, Benjakul, & Visessanguan, 2008; Riebroy, Benjakul, Visessanguan, & Tanaka, 2007). FFM is routinely produced in 'cottage' - type businesses and are also a household activity for regular consumption and rely on the LAB that may be native to the fish and/or may be associated with processing equipments. Failure to ferment will result in spoilage of fish meat which in turn may cause food poisoning (Khem, 2009).

Proteolysis[4] is one of the significant biochemical changes occurring during fermentation of fish mince. It strongly affects texture and flavour development owing to the formation of low molecular weight compounds such as peptides, amino acids, aldehydes, organic acids and amines at the expense of the original protein polymers (Visessanguan, Benjakul, Riebroy, & Thepkasikul, 2004). The oxidation of fish fats also contributes to the changes in flavour, colour, and nutritive value (Riebroy, Benjakul, Visessanguan, Kijrongrojana, & Tanaka, 2004). Hence it is imperative that fat oxidation is controlled during fish fermentation.

Dsa (2013) showed that lactic fermentation can be applied to mussels, but – as he discovered – only after cooking (Figures 1 and 2), thus paving the way for lactic fermentations to be applied to invertebrate marine species where the gut biota and digestive enzymes are necessarily included in the fermentation mixture. The gut biota and digestive enzymes are not included in

[4] Proteolysis is the breakdown of proteins in to smaller polypeptides or amino acids. It is typically catalysed by an enzyme called proteases but may also occur at low pH and/or high temperature.

lactic fermentation of fish skeletal muscle. Dsa's discovery has opened up a previously unrealised commercial opportunity.

Fermented mussels, a commercial opportunity

Most of the edible shellfish in New Zealand waters such as mussels, cockles, oysters, pipi and scallops belong to a group of molluscs known as bivalves. The term bivalve refers to their two hinged shells that are also called valves. In mussels, both shells are usually the same shape, while in oysters and scallops one valve is flat and the other curved (Wassilieff, 2012).

Bivalves are adapted to living in a confined space and feeding by straining plankton from the water. They have no distinct head or radula (rasping tongue). About 440 species of bivalves have been identified in New Zealand waters. They are generally prolific with some species reaching huge densities of over 20,000 per square metre (Wassilieff, 2012).

Most bivalves are sedentary or slow-moving animals. Some, such as pipi and cockles, spend their entire life buried in seafloor sediment, while others like the oyster and mussel remain anchored to rocks and solid structures with strong, elastic threads secreted from a gland situated in their foot. When young, mussels can separate themselves from the threads and move about a little (Wassilieff, 2012).

a) Mussels

New Zealand has 22 species of mussels, of which the commercially farmed green shell mussel (*Perna canaliculus*) is the most common and popular as a food. Along with the blue mussel (*Mytilus galloprovincialis*), it is common around rocky coasts from low tide to subtidal depths (Wassilieff, 2012). Horse mussel (*Atrina zelandica*) is the largest living shellfish found in New Zealand. They live for about six years and usually grow 30 to 35 centimetres long. One specimen at the Auckland Museum measures 405 millimetres. (However, it is no match for the 1.5 metre fossil of *Magadiceramus rangatira* giant mussel that lived in New Zealand waters 100 million years ago (Wassilieff, 2012).)

b) Perna canaliculus, the New Zealand green shell mussel

The New Zealand green shell mussel, also known as green lipped mussel is an iconic New Zealand native. As outlined earlier, significant aquaculture production of green shell mussels began in New Zealand in the late 1970s and it has expanded exponentially since then. Mussel farms in New Zealand are located in coastal waters with suitable depths and tidal flows and use a longline aquaculture system. Harvesting is undertaken at sea and the mussels are transported and stored chilled for land based processing (Tuckey, Day, & Miller, 2013).

i) Farming of green shell mussels

Perna canaliculus is a dioecious broadcast spawner, but at different times for different populations. For example, at Ninety Mile Beach in Northland, the mussel mostly spawn between June and December, while two distinct spawning periods in early summer and autumn to spring occur in the Marlborough Sounds at the north end of the South Island. These variations in spawning activity have been attributed to regional temperature differences. For instance, the water temperature at Ninety Mile Beach rarely falls below 14°C, whereas temperatures in the Marlborough Sounds are between 10 and 11°C in winter and 20 and 21°C in summer. In general, gametes can be released throughout the year, but gonadal development only occurs at temperatures above 11°C and is also closely related to food availability ("New Zealand's green-lipped", 2013).

The mussel farmers in New Zealand face challenges at several steps of the farming process. Some of the most significant challenges include unpredictability of spat supply, resettlement of spat away from nursery lines, predation of spat by fish, organisms other than mussels (seaweeds like *Undaria*, sea squirts etc.) that foul long lines, and accumulation of toxins within mussels ("New Zealand's green-lipped", 2013). There are times when mussels are not harvested at all due to bad weather conditions and at times like these cooked frozen mussels are useful.

ii) Mussel properties

According to promotional literature from New Zealand Greenshell™ mussels website, "*The distinctive jade colour of the shell* (Figures 5 and 6) *adds visual appeal to a plate, with chefs and consumers worldwide experiencing the culinary versatility, extensive nutritional benefits, succulent plump meat and sweet tender taste of the New Zealand green shell mussel. It is fast becoming the worlds most sought after shellfish. They are grown in a clean and pristine environment with world leading food safety programmes, making them one of the safest mussels in the world*". Undoubtedly many of these claims are true but may well be matched or exceeded by other species of mussel grown elsewhere in the world.

Figure 5. The New Zealand green shell mussel.

Figure 6. The byssus, more commonly known as the beard, is used by the mussels to attach themselves to hard surfaces.

iii) Nutritional benefits

New Zealand green shell mussels are associated to a wealth of nutritional benefits being high in iron, protein and essential omega-3 fatty acids and low in fat, calories and cholesterol. It is a great source of haem iron[5], containing over 3 times the haem iron of steak on a weight basis. Ten medium sized mussels that is approximately 100 grams, provides half of the daily iron and quarter of the daily protein needs for an adult ("New Zealand's green-lipped", 2013).

[5] Haem iron is one of the two forms of iron which is easily absorbed by the body to carry out various bodily functions, including the transport of oxygen in the blood.

In addition, extracts from greenshell mussels have shown some success in the treatment of osteoarthritis[6] (Tuckey et al., 2013). A study that included 50 patients were administered randomly either with non-polar lipid extract from the New Zealand green shell mussel (called green shell oil capsules henceforth) or fish oil capsules. Reduction of pain was statistically evident at four weeks among the subjects who took green shell oil capsules compared with fish oil. These results propose that extracts from New Zealand green shell mussel might offer a potential alternative therapy with no side effects for osteoarthritis patients (Szechiński & Zawadzki, 2011; Zawadzki, Janosch, & Szechinski, 2013).

iv) Product forms

As reported earlier, the product range of commercial offerings of green shell mussels is limited (Table 2 and reproduced here as an image) and there may be an opportunity to develop mussel products that could expand the range and thereby give higher returns to the industry. As it stands, there is currently a large commodity focus in the industry.

Table 2. Product forms of New Zealand greenshell mussel for export.

Product category	Exports by value (%)
Half shell frozen	83.6
Meat frozen	9.7
Whole frozen	3.6
Preserved/marinated	1.3
All other forms and nutraceuticals	1.7

Source: New Zealand Aquaculture (2012).

c) The challenge

Based on pilot experiments by an AUT undergraduate John Qiang, Gerald Dsa, an AUT postgraduate student, developed a fermented mussel product that was unusual in that the mussels were fully cooked before fermentation. In outline, the mussels are conventionally cooked in boiling water, minced through a 4 mm plate, and mixed with 2% salt, 2% glucose, 0.01% lactic acid starter culture and a thickening agent like carrageenan. The mixture is packed under vacuum, and held at 30°C for 4 days by which time fermentation is complete as judged by pH of around 4.5. Cooking is itself a preservation method (although only short term), and it was counterintuitive to ferment cooked mussels. However, it was essential to cook the mussels prior to fermentation. Fermentation

[6] Osteoarthritis is a chronic condition in which the material that cushions the joints, called cartilage, breaks down. This causes the bone to rub against each other causing pain, loss of mobility and reduced quality of life.

of raw mussels yielded undesirable slurry (as shown previously in Figure 2) with intense sulphur smell of mussel mince. This outcome was thought to be due to the digestive enzymes of the mussel and enzymes derived from the mussel gut microflora. Cooking completely solved this problem. The process outline described yields a spreadable 'pâté' that can be made coarse or fine depending on the extent of mincing.

This spreadable 'pâté' is, however, only one form of this product. Dsa (2013) attempted to create a gel structure that could be sliced rather than spread. The reasoning behind this aim was that a spread would limit the market opportunities. As a sliceable gel, the food might be presented as part of a gourmet meal, which could command a higher price than a spread. In the event Dsa was unsuccessful in creating a gel in the limited time available, so that is the primary objective of the current research. Edible gels are created using hydrocolloids which is the subject of next section, focusing particularly on those used in the current study.

Hydrocolloids

a) Definition

Food hydrocolloids are high molecular weight hydrophilic biopolymers used as functional ingredients in the food industry to control the microstructure, texture, flavour and shelf-life of the food products (Dickinson, 2003). They are often characterised by the property of forming viscous dispersions and/or gels when dispersed in water (Cevoli, Balestra, Ragni, & Fabbri, 2013). Presence of a large number of hydroxyl (-OH) groups markedly increases their affinity for binding water molecules rendering them hydrophilic compounds. They are widely used in dairy and bakery products, canned foods, salad dressings, beverages, sauces, soups and other processed foodstuffs.

b) Properties

Hydrocolloids have a wide array of functional properties in foods including thickening, gelling, emulsifying, stabilization, coating, inhibiting ice and sugar crystal formation and the controlled release of flavours. In addition, they produce a dispersion, which is intermediate between a true solution and a suspension, and exhibit the properties of a colloid. Considering these two properties, they are aptly named 'hydrophilic colloids' or 'hydrocolloids' (Saha & Bhattacharya, 2010). Their functional properties are also affected by its interactions with other food components like proteins, polysaccharides, lipids, sugars, and salts (Cevoli et al., 2013).

c) Sources

Hydrocolloids are polysaccharides extracted from plants, seaweeds and microbial sources, as well as gums derived from plant exudates, and modified biopolymers made by the chemical or enzymatic treatment of starch or cellulose (Dickinson, 2003).

d) The phenomenon of gel formation

While all hydrocolloids thicken and impart stickiness to aqueous dispersions, a few biopolymers also have another major property of being able to form gels. Gel formation is the phenomenon that involves cross-linking of the polymer chains to form a three-dimensional network that traps or immobilises the water within it to form a rigid structure that is resistant to flow. In other words, it becomes viscoelastic and exhibits characteristics of both a liquid and a solid. The textural properties like elastic or brittle, long or spreadable, chewy or creamy and sensory properties like opacity, mouth feel and taste of a gel vary widely with the type of hydrocolloid used. Hence, to design a specific food formulation, knowledge of the conditions required for gelling of particular hydrocolloid dispersion, the characteristics of the gel produced and the texture it confers are very important characteristics (Saha & Bhattacharya, 2010).

e) Types

The important hydrocolloids that find application in food as gelling agents are alginate, pectin, carrageenan, gellan, gelatine, agar, modified starch, methyl cellulose and hydroxypropyl methyl cellulose. Hydrocolloids that are commonly used as thickening agents are starch, xanthan, guar gum, locust bean gum, gum karaya, gum tragacanth, gum Arabic and cellulose derivatives (Saha & Bhattacharya, 2010).

f) Hydrocolloids used in this project

Of the many gelling hydrocolloids, two were chosen for testing in this project, carrageenans and gelatine. The former was chosen because in a seafood product it would be commercially useful to have all ingredients from marine sources, where possible. Thus, salt, mussels, and gelling agent would all be derived from the sea. However, when it became clear that the gels from carrageenan – such as they were – were unable to create the required rigid structure, gelatine was tested in the common knowledge that high concentrations of gelatine give very rigid gels. (Moreover, fish-derived gelatines are available, but because none was available in New Zealand, typical animal derived gelatine was used for proof of concept.) Agar and alginate are also derived from the sea, but time did not allow testing of these two.

Another reason for choosing these two is the temperature range in which these hydrocolloids disperse and form gels. Table 5 derived from Sufferling (no date), shows the dissolution temperatures for some hydrocolloids. Furthermore, added culture is sensitive to temperatures of above 45°C which may cause inactivation resulting in no or inadequate fermentation. Carrageenans and gelatine were the best option from that available group due to their ability to be in a liquid state even at 40°C when the culture was added. Thus, the review of hydrocolloids was restricted to carrageenans and gelatine (Table 6).

Table 5. Typical dissolution temperatures of several hydrocolloids.

Hydrocolloid	Temperature or range of dissolution (°C)
Starch	80 to 100
κ-Carrageenan	70 to 80
ι-Carrageenan	70 to 80
λ-Carrageenan	40
Gelatine	60 to 80
High methoxy pectins	35 to 100
Low methoxy pectins, guar, alginates, xanthan	30 to 50
Locust bean	90 to 110

Source: Sufferling, n.d.

i) Carrageenans

Carrageenans are a group of sulphated galactose polymers extracted from certain species of red seaweeds, and the classification into κ-, ι- and λ-carrageenan depends on the pattern of sulphation. In general, κ-carrageenan forms hard, strong and brittle gels which are freeze/thaw unstable. This is in contrast to ι-carrageenan that forms soft and weak gels that are freeze/thaw stable. The first step in the gelation of carrageenan is the transition from a disordered (random coil) to an ordered (helical) state. The helical conformation is promoted by the addition of salts or by lowering the temperature. Monovalent cations (Na^+, K^+, Rb^+, Cs^+ and NH_4^+) promote the aggregation of κ-carrageenan double helices to form so-called aggregated 'domains' (Figure 7). Gel formation in ι-carrageenan gels is believed to take place at the helical level by branching and association through incomplete formation of double helices. The number of branching points plays an important role in the gel strength of ι-carrageenan gels (Banerjee & Bhattacharya, 2012; Renard, van de Velde, & Visschers, 2006).

Table 6. List of hydrocolloids used in this project and their properties.

Properties	**Iota (ι) carrageenan**	**Kappa (κ) carrageenan**	**Gelatine**
Origin	Polysaccharide obtained from red seaweeds.		Protein obtained from collagen in animals.
Texture	Thermoreversible, soft shear-thinning, elastic gel with calcium.	Thermoreversible, firm, brittle gel with potassium.	Thermoreversible, soft, elastic gel; melts in mouth.
Clarity	Slightly turbid	Clear/slightly tan	Clear, transparent.
Dispersion	Cold water, dispersion is improved by mixing with sugar (3-5x) or small amounts of alcohol.		Soak in cold water.
Hydration (dissolution)	>70°C; for high sugar concentrations: add sugar after hydration.	>70°C	>60°C
pH	4-10		4-10
Setting	40-70°C, higher temperature with increasing electrolyte concentration.	30-60°C, higher temperature with increasing electrolyte concentration.	<15°C, slow (hours)
Melting	5-10°C above setting temperature.	10-20°C above setting temperature.	25-40°C
Promoter	Calcium yields soft and elastic gels.	Potassium, milk proteins.	Transglutaminase (1-3%), milk, sugar, low alcohol concentration.
Inhibitor	Hydrolysis of solution at low pH with prolonged heating; gels are stable.	Salts; hydrolysis of solution at low pH with prolonged heating; gels are stable.	Salts, acids, prolonged heating, proteases present in fruits, high alcohol concentration, and tannins can cause precipitation.
Tolerates	Salt		Alcohol up to ~40%.
Viscosity of solution	Medium	Low	Low
Typical concentration	0.5-2% for gel.		1-9% depending on Bloom number.
Syneresis	No	Yes; can be reduced by addition of locust bean gum.	No

Source: Lersch, 2014

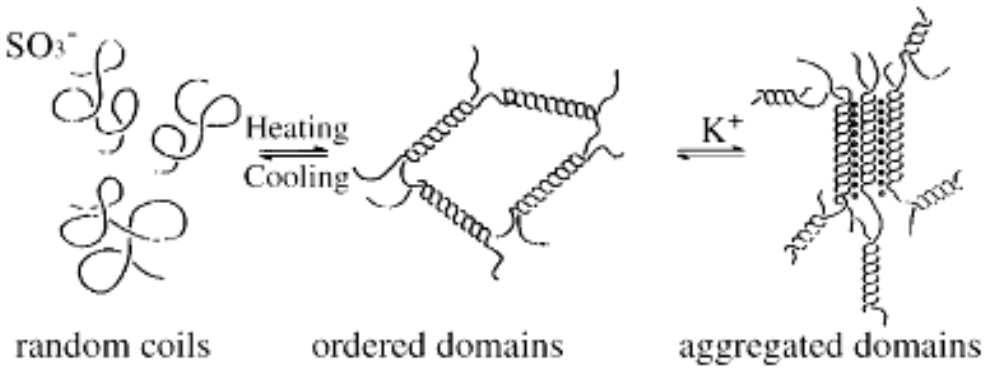

Figure 7. Gelation of κ-carrageenan (Adapted from Renard et al., 2006).

Carrageenans find maximum application in dairy desserts like puddings, milk shakes, ice cream and chocolate milk because of its ability to form gels in milk at much lower concentrations compared to any other gelling agent (Puvanenthiran, Goddard, McKinnon, & Augustin, 2003). κ-Carrageenan forms gels in milk at low concentrations because of the electrostatic attraction between positively charged region of κ-casein and negatively charged sulphate group of κ-carrageenan. Another important application of carrageenans is in brine-injected meat. They can be dispersed in brine resulting in a low viscosity and therefore injectable mixture. The result is gel formation when the ham has been cooked and cooled (Saha & Bhattacharya, 2010). Table 7 shows the applications of carrageenans in food.

Table 7. Applications of carrageenans and gelatine in food.

Food product	**Carrageenans**	**Gelatine**
Bakery products	No	Yes a lot
Beverages	Yes	Yes
Confectionary	No	Yes a lot
Convenience: sauces, dressings, soups, marinades	Yes	No
Dairy, sweet drinks, desserts	Yes a lot	Yes a lot
Fruit preparations, jams, marmalades	Yes	Yes a lot
Ice-cream	Yes	No
Meat and poultry processing	Yes a lot	Yes a lot

Source: Lersch, 2014

ii) Gelatine

Gelatine is an important industrial gelling biopolymer typically derived from beef or pork connective tissue, which is rich in collagen. Fish frames and the contents of fish swim bladder is another source. Gelatine is used to increase the viscosity of aqueous systems and to form aqueous

gels. It's useful properties include thermoreversibility, a characteristic rheology described as melt-in-the-mouth, and an excellent release of flavour (Choi & Regenstein, 2000). Gelatine's single largest use is in gel desserts. Other uses include thickening yoghurts, low fat spreads, and in sugar confectionery. Gelatine has also been used in flavoured gelled milk desserts, either alone or in combination with carrageenan, and also in dessert creams (Saha & Bhattacharya, 2010). Table 7 illustrated earlier talks about the applications of gelatine in food. Estimated world usage of gelatine is about 200,000 tonne per year with U.S. usage being about 30,000 tonne for food and about 10,000 tonne per year for pharmaceutical applications (Choi & Regenstein, 2000).

Being a cold-setting thermoreversible gelation process, gelatine behaves more like a typical polysaccharide gel than a globular protein gel (Renard et al., 2006). Gelatine melts when heated and solidifies when cooled. It forms a semi-solid colloidal gel in combination with water (Banerjee & Bhattacharya, 2012). Upon cooling, gelatine exhibits a coil-to-helix or disordered-to-ordered transition. Figure 8 illustrates a model of gelatine gel formation. Gelation during cooling is governed by the partial reformation of the original triple helices found in collagen. In the first step, a polypeptide chain takes an orientation to induce a reactive site. Later, condensation of two other chains near the reactive site occur giving rise to triple helix formation (Renard et al., 2006).

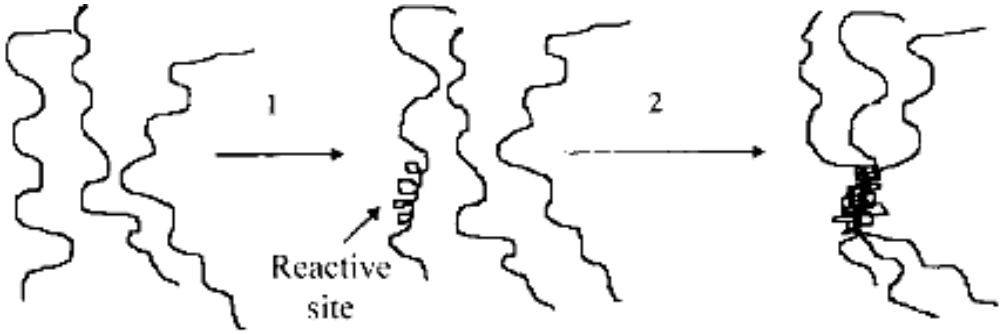

Figure 8. Model describing the cooperative character of gelatine gelation. (Adapted from Renard et al., 2006).

Having reviewed the hydrocolloids to be tested in this study, it remains to outline the main analyses that were applied to the fermentation outcomes. These were texture and colour, pH, titratable acidity, residual glucose concentration, and microbial analysis.

Texture analysis

Texture is an attribute that affects processing, handling, storage life and consumer acceptance of products. Texture analysis is the mechanical testing of food, cosmetics, pharmaceuticals, adhesives and other consumer products in order to measure their physical properties. Because of its adaptability, texture analysis has become a routine in many industries to

measure a specific or range of properties relating to the way a material behaves, breaks, flows, sticks, bends, etc. Analysis of texture involves compression, tension or torsion, or combinations of these three. In the case of food, compression is the most common way force is applied to an object, because the dominant force in mastication is compression (Stable micro systems, n.d.).

As is described in more detail in Chapter 2, the equipment used was a TAXT texture analyser, in which the compressive force is applied by a cylindrical probe with a known cross sectional area, thus applying a pressure. The speed of application of this pressure is controlled by software and resultant forces are monitored by a load cell. Data collected are force, distance and time from which a range of textural parameters can be calculated, like work, elasticity etc.

Colour analysis

Colour analysis of foods is dominated by measurement of reflected light because that is what the eye sees and the brain perceives. The instrument used to measure light here was a ColorFlexEZ spectrophotometer. In outline, a white light with a defined intensity and spectrum is reflected from the food and is separated into its component wavelengths by a dispersion grating. The relative intensities of the light at different wavelengths along the visible spectrum (400 to 700 nm) are then analysed to produce numeric results indicative of the colour of the sample. L*, a* and b* indicates lightness, redness/greenness, and yellowness/blueness, respectively (HunterLab, 2013). More details are presented in Chapter 2.

Titration and residual glucose concentration

As will be further discussed at the end of this chapter, the way that mussels were boiled prior to mincing, blending of ingredients and fermentation, had a marked effect on the final pH of the *Perna*. This suggested that boiling time affected the buffering capacity of the mussel proteins and was explored by titration. Titration is the progressive addition of one solution of a known concentration called a titrant – acid or alkali in the present study – to a known volume of another solution of unknown concentration – alkaline or acidic moieties in *Perna* – until the reaction reaches an endpoint (Acid-base titrations, n.d.). As an example of titratable acidity, titration begins with a known volume of fermented mussel slurry sample and a known concentration of sodium hydroxide. The sodium hydroxide is titrated into the sample. A pH meter measures the equivalence point or the point at which all of the available hydrogen ions in the sample have reacted with the sodium hydroxide. The endpoint chosen was pH 8.

An adjunct to this work was determination of residual glucose concentration. The rationale for this assay was that if *Perna* failed to achieve a low pH was it because fermentation was limited – resulting in high residual glucose – or because of increased buffering capacity of mussels that

were boiled for a long time? The glucose concentration in *Perna* was determined by the use of blood glucose meter such as are used domestically by diabetics.

Microbial analysis

The microbial study was done to better understand the microorganisms present in *Perna* before and after fermentation. To achieve this, aerobic, anaerobic, and LAB plate count were routinely conducted for the boiling time experiments.

Thesis plan

As was discussed in the section *Product forms*, the main aim of this thesis was to make a fermented mussel gel and that work is reported in Chapters 4 (Tables 26 and 27) and 5 (Tables 30, 31 and 38). However, some early experiments with mussels had pH of as low as 3.7, which is not ideal for this type of product. The lower the pH, the more acidic smell was generated along with excessive syneresis. Therefore, it was important to attain a pH mark of about 4.5 after fermentation, and surprisingly that was linked to the time the mussels were initially boiled. Long boiling time resulted in higher pH and lower mussel microbial load in the final fermented product. All this work is presented in Chapter 3. Finally, the conclusions are drawn in Chapter 6. The plan of this is presented in Table 8.

Table 8. Thesis plan.

Chapter	Outline of contents
1	Introduction
2	Materials and methods, describing generic techniques.
3	Boiling time experiments relating to final pH.
4	Gelation experiments with carrageenans.
5	Gelation experiments with gelatine.
6	Conclusion and suggestions for future work.

Chapter 2

Materials and Methods

This chapter reports the most typical and common methods used for the study. Further details of variations in these methods are reported when each experiment is described in later chapters.

Equipments

A Kenwood domestic mixer (Havant, UK) was used to mince cooked mussel meat through a 4 mm- then a 3 mm-plate after cooking. Capped plastic containers with an internal diameter of 3.8 cm were frequently used to pack *Perna* after addition of all ingredients and before fermentation (Figure 29). These red capped containers were mainly used for carrageenan work and held about 50 g of *Perna*. For gelatine work, rectangular polyethylene containers (Pam's brand, Pak'n Save; and Homebrand, Countdown, Auckland) were used (Figures 42 and 43). These held 200 g and 300 g respectively. A digital thermometer was used for gelatine experiments to eliminate the risk of mercury contamination.

Vacuum barrier bags measuring 200 by 250 mm (D. M. Dunningham Limited, Auckland) were used to pack all *Perna* products, using a vacuum packer Model DZ400-ZD from China and sold by William Lin, Waitakere. On Day 5, following fermentation and refrigeration, a conventional pH meter (Meterlab PHM-201, U.K.), was used to confirm successful fermentation.

An Ultra-Turrax drive fitted with a T25 dispersing element (IKA Werke, Staufen, Germany) was used to disperse *Perna* in to deionised water for further testing (pH, titratable acidity and alkalinity). A blood glucose meter (ACCU-CHEK Advantage, Auckland) was used to determine residual glucose concentration in *Perna*.

Two other important items of equipment, a colour meter and a texture analyser, are described in later sections of this chapter.

Chemicals, microbial media and fermentation culture

NaCl, KCl, $CaCO_3$, and 1M NaOH were standard laboratory analytical grades. Glucose was as the monohydrate such as would be used in commercial applications. Lactic acid was as a 90% solution in the carrageenan work (The British Drug Houses Ltd., England) and 88 to 90% solution in gelatine work (Scharlau, Germany). Two types of carrageenans were variously used, κ-carrageenan (Gelcarin PS9111) and ι-carrageenan (Gelcarin GP379) both from Hawkins Watts, Auckland. Both have the capacity to thicken and form gels, but they differ in their gel textures, the former being harder. Davis gelatine ((Figure 31) Davis Food Ingredients, Auckland) was used in all

the gelatine experiments which were bought from a local supermarket. The lactic fermentation culture was BFL-F02 from Chr. Hansen, Melbourne, Australia. This is described as a rapid fermentation culture containing two bacteria species, *Pediococcus pentosaceus* and *Staphylococcus carnosus.*

Media used in the microbial analysis of *Perna* was peptone water, plate count agar, and Lactobacilli MRS Agar (BD Difco, New Jersey).

Source of mussels

There were two types of green shell mussels used for this project. The first was live mussels (Figure 5) cooked within four hours of purchase and the other was commercially cooked and frozen mussels devoid of shells (Figure 32). Both types were from a local supermarket (Countdown, Auckland). The source of mussels for Auckland supermarkets was from farms where spawning occurs around July, such that the flesh condition is poor between July and November. For this reason the chosen mussel processor, Future Cuisine of Swanson, freezes cooked mussel meat prior to July as a buffer for the spring months and are used for gelatine work. The environment may pose other barriers apart from poor flesh condition such as bad weather because of which mussels are not harvested, accumulation of toxins, insufficient spat supply and many more which drastically reduce the supply of live mussels throughout the region affected.

Preparation of mussel meat in the laboratory for boiling time experiments

The live mussels were cooked in batches of 1.0 kg in 1.5 L of tap water at a rolling boiling in a covered pot on a domestic glass-top hot plate (Fisher and Paykel, Auckland) for different times, the reason for which will be described in later sections. These conditions were strictly adhered to. The most common boil times for this project were 2, 5, 7 and 10 min. After boiling, the mussels were cooled at ambient temperature on the laboratory bench. Cooked mussel meat was isolated from the shells, and the beard discarded. A typical yield was about 200 g from a kilogram of live mussel. The cooked mussel meat was then minced twice, first through a 4.0 mm plate and then through a 3.0 mm plate in the Kenwood mixer. This mince was then blended with other ingredients to be described in other sections.

pH determination in boiling time and other experiments

pH, which was an important measure of the success of fermentation, was routinely measured (Rushing, n.d.). There were two methods of preparing *Perna* for pH testing, the first to simply confirm that fermentation had occurred. For this, 2-g sample of *Perna* was dispersed by shaking in 18 mL of deionised water. In a typical experiment, each of the three containers per treatment was

sampled to yield one mean value. Between measurements, the pH probe is rinsed with deionised water and blot dried. The second method was used for boiling time experiments. Ten grams of accurately weighed *Perna* was dispersed in 80 mL of deionised water using Ultra-Turrax T25 dispersing element. The drive was set at 13,500 rpm for 30 sec. The dispersing element was then rinsed with deionised water into the mussel slurry, and the final volume made up to a 100 mL. The fermented mussel slurry obtained was left at ambient temperature for 20 min, with gentle magnetic stirring. The idea was to get an accurate equilibrium value of pH before fermentation, after fermentation, and during titration of both these conditions with acid and alkali respectively, as described in the next section.

Titratable acidity and alkalinity in boiling time experiments

From the 100 mL of mussel slurry, 50 mL of magnetically stirred slurry was used for titration, either to titrate unfermented *Perna* to pH 3.5 with 0.05 M HCl, or to titrate fermented *Perna* to pH 7.0 with 0.05 M NaOH (Acid-base titrations, n.d.). In the latter case, phenolphthalein was used as an indicator, although the colour change at pH 8 was difficult to see. No indicator was used for the acid titrations. Readings from the pH meter were monitored with typically 20 values as pH changed by the progressive addition of 1 mL aliquots of acid or alkali. Titrations were done in duplicate for every boil time treatment (Table 10) and there were three replicates of the boil times, from different mussel purchases.

Determination of residual glucose concentration in boiling time experiments

The residual glucose concentration of unfermented (Day 0) and fermented (Day 5) *Perna* was determined by means of the blood glucose meter (ACCU-CHEK Advantage) applied in duplicate to the 100 mL slurries previously described. A test strip was inserted in the meter and a drop of mussel slurry with the help of a dropper was put through. The glucose value is displayed in mmol/L within 30 seconds. These meters are designed for human blood with a buffered pH of around 7.0. Realising that *Perna* was a very different matrix; the diabetes meter would require calibration by the method of standard addition which was beyond the scope of this research.

Microbial analyses in boiling time experiments

For LAB determination, peptone water was made by dissolving 15 g of the medium in one litre of deionised water. To determine total bacterial population, plate count agar was made by suspending 23.5 g of the medium in one litre of distilled water. It was heated with frequent agitation and boiled for about a minute for complete dissolution. The final pH of both the media was adjusted to 7.2 ± 0.2 using 1 M NaOH. Lactobacilli MRS agar was prepared by suspending 70 g of the medium in one litre of distilled water. To distinguish acid-producing bacteria from other

microorganisms, 0.3% (w/v) $CaCO_3$ was added to the MRS agar mixture. It was heated with frequent agitation and boiled for about a minute for complete dissolution. The final pH was adjusted to 6.5 ± 0.2 using 1 M NaOH. All media were sterilised before use by autoclaving at 121°C for 15 minutes.

For sample preparation, a 10-g sample of *Perna* was macerated with 90 mL of 0.1% sterile peptone water for 2 min to achieve dispersion. Decimal serial dilutions of the macerated mixture were made up to six fold with 0.1% sterile peptone water.

For aerobic plate count, 0.1 mL aliquots of each dilution were spread in duplicates on to the surface of plate count agar and incubated at 30°C for 48 h. For anerobic plate count, 0.1 mL aliquots of each dilution were spread in duplicate on the plate count agar and incubated at 30°C for 48 h using an anaerobic jar (BD GasPak™ EZ). For isolation of lactic acid bacteria, 0.1 mL aliquots of each dilution were similarly spread twice on MRS agar. The plates were incubated under anaerobic conditions at 30°C for 48 h. Plates with between 30 to 300 colonies were counted. Bacterial count per mg of *Perna* (present in 0.1 mL aliquots used to plate out) was calculated by multiplying the average number of colonies per plate by the reciprocal of the dilution. Total bacterial and LAB counts were finally reported as log colony-forming units per gram of *Perna* (cfu g-1).

Colour determination for many experiments

Colour was routinely measured in International Commission on Illumination (CIE, 1999) colour space using a ColorFlexEZ spectrophotometer (HunterLab, 2013)

An empty container with its cap attached was placed base-down on the exposure port and covered with the black shroud. Several readings were taken and the mean values of L*, a* and b* were calculated. These data were the blank values. The colour of containers with *Perna* was measured in the same way (Figure 9), and the blank values were subtracted before further calculations of means etc.

Figure 9. *Perna* being measured for colour using a ColorFlexEZ spectrophotometer. A black shroud (not shown) was routinely used to shield the objects being measured from ambient light.

Texture analysis

Texture analysis was routinely applied in many experiments. It was done using the TAXT Plus Texture Analyser (Stable Micro Systems, U.K.) in compression mode. The samples were placed on the platform base of the instrument and the probe descended to compress the sample once and then retreat back to the origin (Figure 11) (Stable micro systems, n.d.). The samples were equilibrated to room temperature before the analysis.

To determine the hardness of *Perna* and other materials, a cylindrical stainless steel probe (Figure 10), 6 mm in diameter, was used for all experiments. A custom knife blade (Figure 10) was used to determine the slicing properties of the gelatine gel. It was made by Mr Jim Crossen of AUT Engineering. The blade was a 9 mm Tajima LCB-30 Endura purchased locally from a hardware store. It was set at an angle slicing the gel at 45°. A fresh blade was used for every batch of samples.

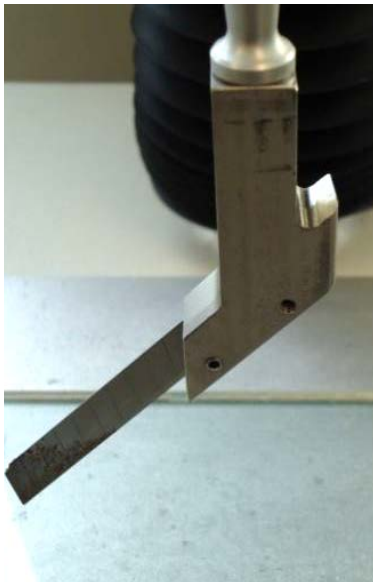

Figure 10. The cylindrical stainless steel probe (left) and a custom made knife blade (right) used in the texture analysis.

The settings of texture analysis were constant for all experiments (Table 9) but gelatine work wherein the test speed was changed to 5.0 mm sec^{-1} and distance of penetration to 10.0 mm. In outline, the probe descended at 5.0 mm sec^{-1} until contact with the *Perna* triggered (0.0890 N) a descent at the test speed (2.0 mm sec^{-1}), until 5.0 mm of penetration was attained.

Table 9. Most common parameters for texture analysis.

Name	Setting
Test mode	Compression
Load cell	50.0 N
Pre-test speed	5.0 mm sec^{-1}
Trigger type	Force
Trigger force	0.0890 N
Test speed	2.0 mm sec^{-1}
Post-test speed	10.0 mm sec^{-1}
Target mode	Distance
Distance	5.0 mm

The red top containers (Figure 29) used for carrageenans experiment had an internal diameter of 38 mm which allowed three penetrations per container, arranged in a triangular pattern. The Pam's and Homebrand containers (Figures 42 and 43) used for gelatine work were bigger, allowing three penetrations in a row per container, for the hardness and sliceability test. Data

recorded were force and time, the latter being converted to distance knowing the test speed. The sliceability was the peak cutting force required.

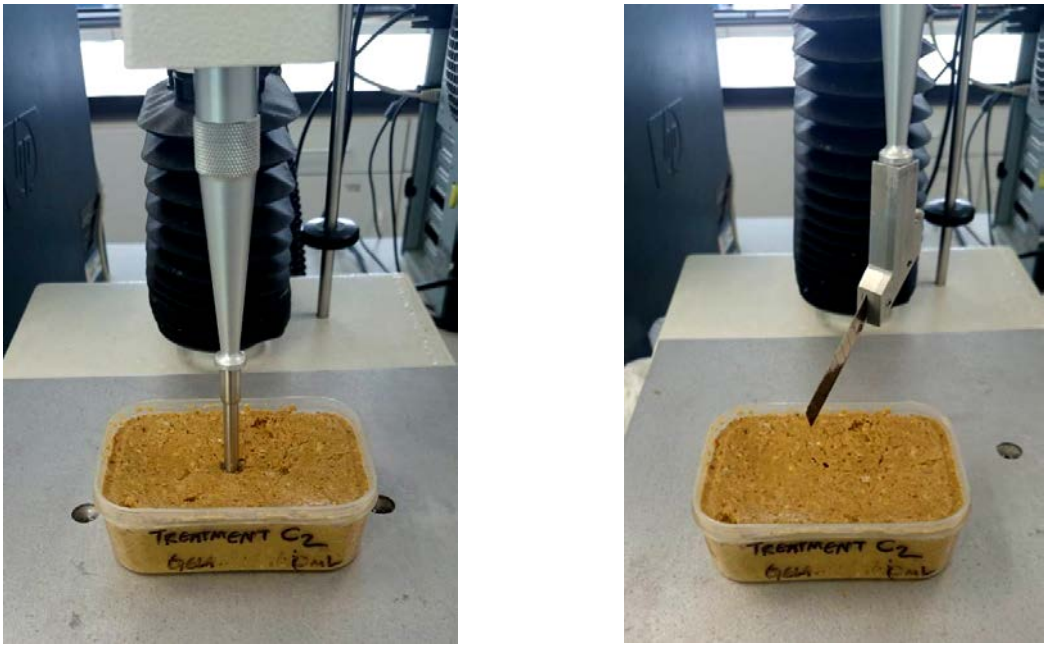

Figure 11. The two probes used for the texture analysis of fermented *Perna*.

Data analysis

All data were entered into Microsoft Excel and manipulated by standard routines in that program, yielding means and standard deviations for tables and graphs.

Chapter 3

Boiling Time Work

Although the main aim of this work was to prepare a gelled form of *Perna* with carrageenans or other gelling agent, several experiments showed a disturbing phenomenon where it appeared that a residual native microflora of the cooked minced mussel may have been detrimentally competing with the added lactic acid-generating microflora. The phenomenon was adverse appearance, strong acidic smell and syneresis. This might have been due to mussel physiological state or insufficient boiling time. The latter possibility is explored in this chapter.

A pilot experiment on boiling time effects on *Perna*

To explore the effects of boiling time on the organoleptic properties of *Perna*, four boiling times were arbitrarily chosen. The boiling times were 2, 5, 7 or 10 minutes prior to *Perna* preparation as discussed in Materials and Methods. In a typical experiment, the mussel minces were blended with 2% ι-carrageenan, 2% sodium chloride, 2% glucose monohydrate and 10% of deionised water[7] (Table 10), where the treatment names reflect boiling times. Water was added to avoid clumping and to ensure an even distribution of ingredients within the mince. LAB culture of 0.02% was then added to the blend and mixed for a minute. The resultant unfermented *Perna* was then stuffed in to red-capped plastic containers which held about 50 g and vacuum packed immediately, but with the caps barely tightened. Subsequently the containers were incubated at 30 ± 2°C for 96 hours followed by refrigeration for a minimum of 24 hours (by then Day 5) before the analyses were conducted.

Table 10. Typical formulation of *Perna* for boiling time experiments.

Treatment code	Boiling time (min)	Mussel mince (g)	ι-Carrageenan (g)	NaCl (g)	Glucose (g)	LAB (g)	Water (mL)
Treatment 2	2	200	4.0	4.0	4.0	0.04	20.0
Treatment 5	5	200	4.0	4.0	4.0	0.04	20.0
Treatment 7	7	200	4.0	4.0	4.0	0.04	20.0
Treatment 10	10	200	4.0	4.0	4.0	0.04	20.0

[7] It is realised that these quantities are not final concentrations. For example the final concentrations of NaCl and glucose from Table 10 would be about 1.72 % (w/w).

Results and Discussion

At Day 5 – one day after fermentation – all *Perna* preparations were light brown, with the 2 minute treatment (Treatment 2) having a strong acidic smell. Smells from longer boiling times were better (Table 11). There was an unexpected major difference in pH, which increased with the increase in boiling time (Table 11). Looking forward to Tables 26 and 27 in Chapter 4, where the pH values were all below 4.0, suggests a cause for the low pH, which is short boiling time. Whether this low pH was a microbial effect or was due to some buffering effect linked to protein denaturation was not known.

Table 11. Key results of the pilot boiling time experiment.

		Perna properties		
Treatment code	Boiling time (min)	Appearance	Smell (acidic)	pH[1]
Treatment 2	2	Light brown	Strong	3.95 ± 0.03
Treatment 5	5	Light brown	Intermediate	4.76 ± 0.05
Treatment 7	7	Light brown	Mild	4.85 ± 0.05
Treatment 10	10	Light brown	Mild	5.30 ± 0.14

[1] pH ± standard deviation is from 3 replicate vials.

The colour of *Perna* was also measured and the data are in Appendix 1. The boiling time had no effect on the colour of fermented *Perna*.

Replicated experiments for boiling time – Part 1

In light of the result in Table 11, this work was repeated twice in autumn (May, 2014) and in addition, the titratable alkalinity of the unfermented *Perna* was determined. (Unfermented *Perna* comprised of minced cooked mussel, salt, glucose, ι-carrageenan, water and culture before vacuum packing.) The idea was that buffering capacity of mussel proteins may change with boiling time. Titratable alkalinity was done by monitoring pH as 0.05 M HCl was progressively added to stirred slurry of 1 part unfermented *Perna*, 9 parts water as described in Materials and Methods. After fermentation, titratable acidity was similarly measured for fermented *Perna*. The colour of fermented *Perna* was also measured and the data are in Appendices 2 and 3.

Titratable alkalinities for the repeat experiments are shown in Figures 12 and 13.

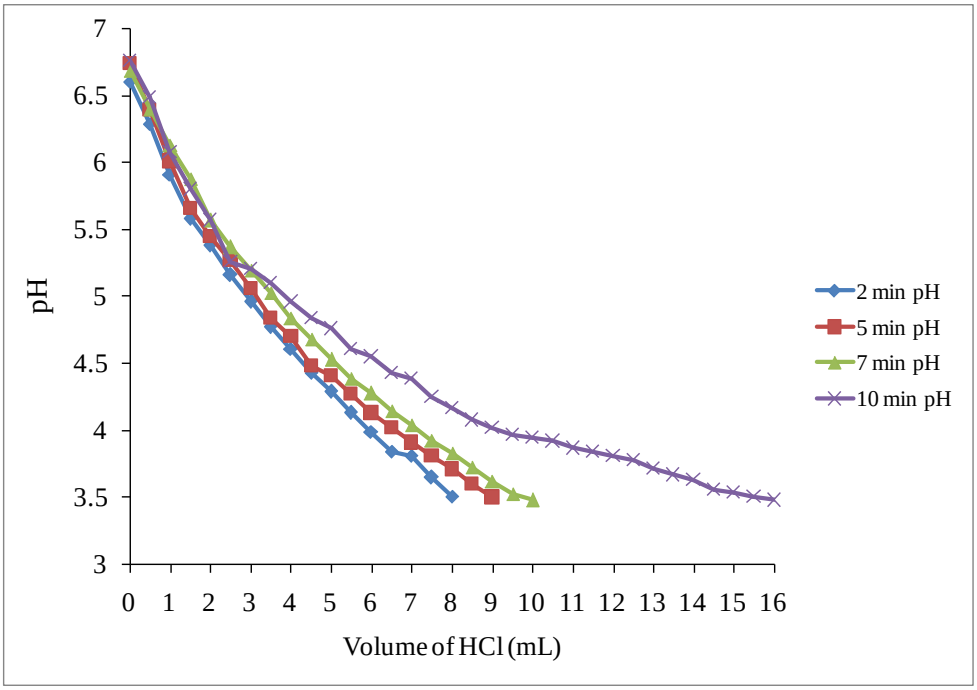

Figure 12. Pattern of the buffering capacity of unfermented *Perna* (Replicate 1). The starting pH was about 6.75 and titration continued to pH 3.50.

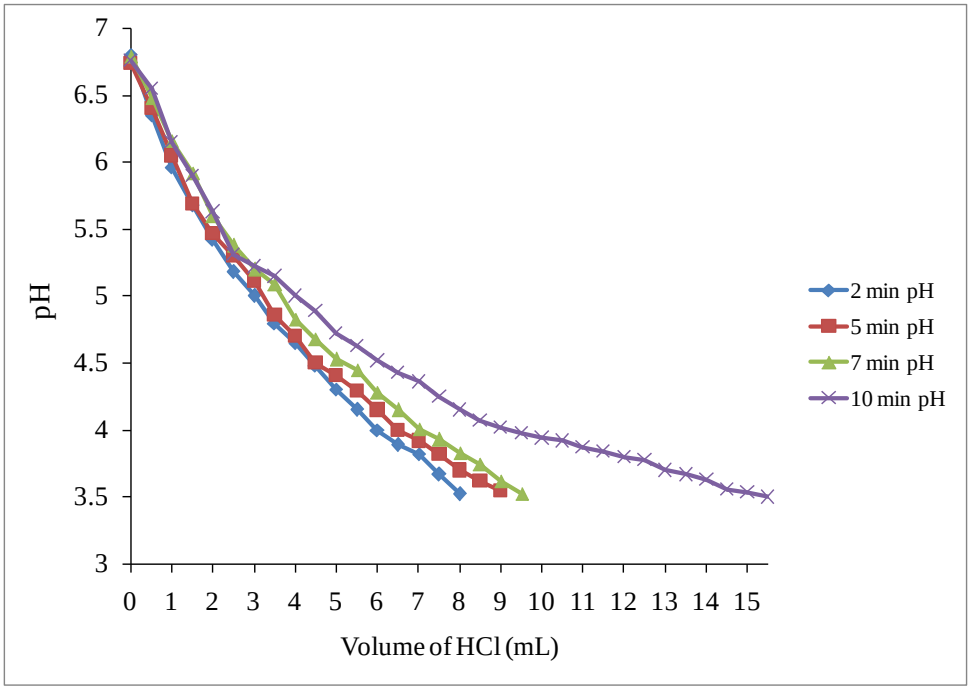

Figure 13. Pattern of the buffering capacity of unfermented *Perna* (Replicate 2). The starting pH was about 6.75 and titration continued to pH 3.50.

The pattern of titratable alkalinity was similar in the replicates. Buffering capacity clearly increased with boiling time, and can be summarised in Table 12, expressed as μmole of HCl per gram to titrate from the initial pH (about pH 6.75 for all) to a final pH of 3.50. Titration of protein with an acid involves progressive neutralisation of the basic amino acids, lysine, arginine, and histidine and protonation of the acidic amino acids, aspartic acid and glutamic acid. Boiling is presumably resulting in increased availability of basic amino acids and/or reduced availability of acidic amino acids. However, the details are unknown.

Table 12. Titratable alkalinity of unfermented *Perna* to a final pH of 3.5.

	Titratable alkalinity (μmole HCl g^{-1})	
Treatment code	Data from Figure 12	Data from Figure 13
Treatment 2	80	80
Treatment 5	90	90
Treatment 7	100	95
Treatment 10	160	155

All these results suggested a cause for the differences in final (fermented) *Perna* pH with boiling time, highest for Treatment 10 (Tables 11, 13 and 14), because of higher buffering capacity. Although the pH range in Table 11 on one hand and Tables 13 and 14 on the other was different, the trends were the same, and results for smell were almost similar. The more acidic treatments were more strongly sensed by smell. Given that consumers who dislike *Perna* found the flavour (and smell is a component of this) too strong, the results suggest that *Perna* fermentations must be controlled to prevent excess acidity, however that may be achieved.

Table 13. Boiling time experiment, Replicate 1.

		Perna properties (after fermentation)		
Treatment code	Boiling time (min)	Appearance	Smell (acidic)	pH[1]
Treatment 2	2	Light brown	Strong	4.05 ± 0.02
Treatment 5	5	Light brown	Intermediate	4.46 ± 0.02
Treatment 7	7	Light brown	Mild	4.51 ± 0.06
Treatment 10	10	Light brown	Mild	4.60 ± 0.13

[1] pH ± standard deviation is from 3 replicate vials.

Table 14. Boiling time experiment, Replicate 2.

Treatment code	Boiling time (min)	*Perna* properties (after fermentation)		
		Appearance	Smell (acidic)	pH[1]
Treatment 2	2	Light brown	Strong	4.32 ± 0.02
Treatment 5	5	Light brown	Strong	4.17 ± 0.02
Treatment 7	7	Light brown	Mild	4.44 ± 0.03
Treatment 10	10	Light brown	Mild	4.55 ± 0.02

[1] pH ± standard deviation is from 3 replicate vials.

To summarise the possible cause(s) of this phenomenon, there are at least two, not mutually exclusive possibilities: mussel seasonal condition and insufficient boiling. The insufficient boiling may have two aspects, a detrimental contribution from residual mussel gut enzymatic activity and microbial species that survived boiling temperatures. Another aspect to final pH is the nature of the culture's LAB and the available fermentable carbohydrate but that is beyond the scope of this research.

The pH phenomenon was also explored in another way. The pattern of titratable acidity of fermented *Perna* was measured at Day 5 in the same way as titratable alkalinity was measured at Day 0 except that the titrant was 0.05 M NaOH (Figures 14 and 15).

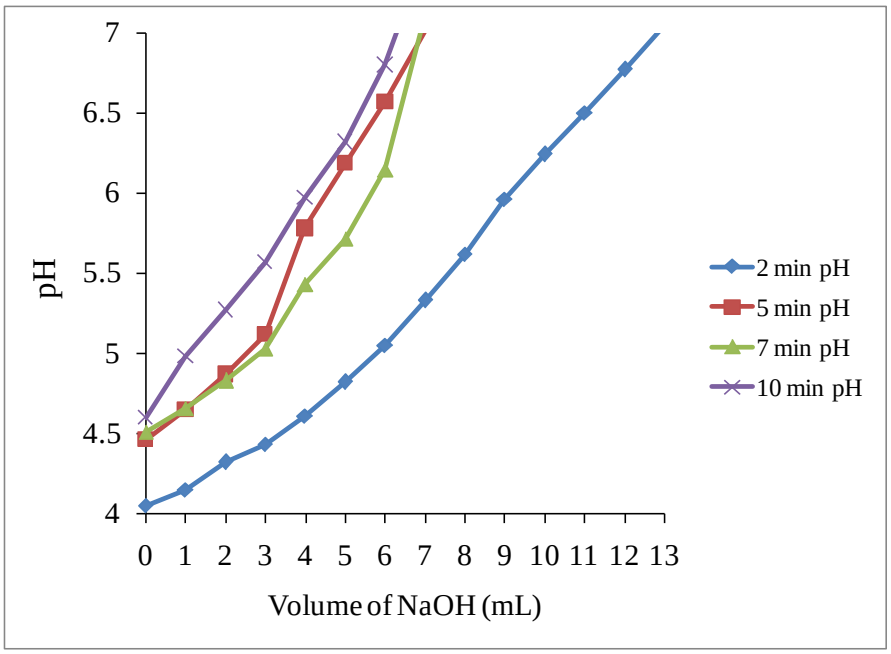

Figure 14. Pattern of the buffering capacity of fermented *Perna* (Replicate 1). The starting pH was different for different treatments and titration continued to pH 7.0.

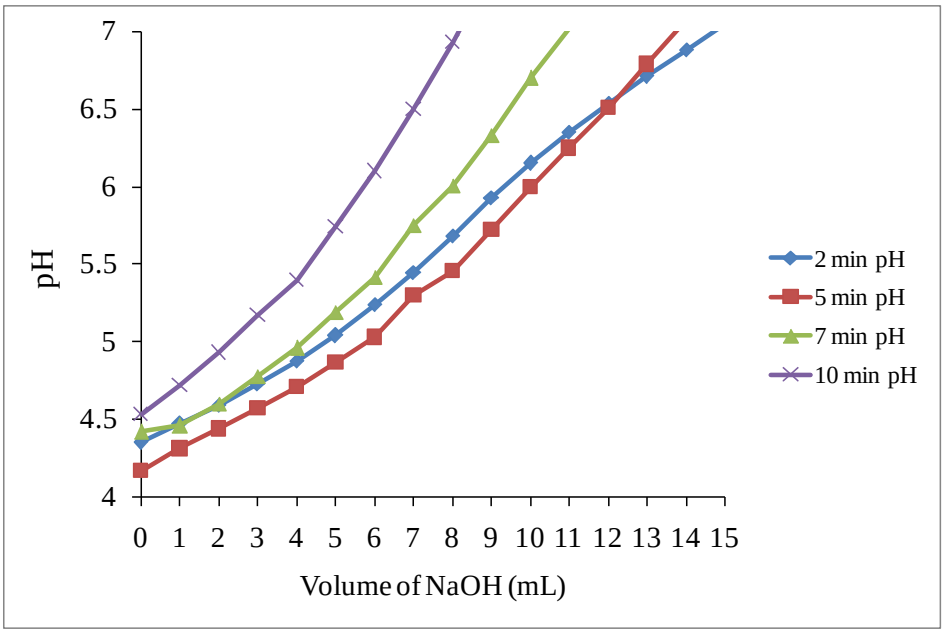

Figure 15. Pattern of the buffering capacity of fermented *Perna* (Replicate 2). The starting pH was different for different treatments and titration continued to pH 7.0.

Treatment 10 was shown to have the greatest buffering capacity for the titratable alkalinity experiments (Figures 12 and 13), so the expectation was that more alkali would be required to approach a neutral pH from Treatment 10. This was not the case. Inspection of Figures 14 and 15 shows the slope of the titration line for the two Treatment 10s was generally steeper than for the other three treatments. In other words less alkali was needed to approach neutrality. This phenomenon can be viewed in another way. In Tables 13 and 14, the starting pH was different so the mole of NaOH required to cause a pH change of 2 units was arbitrarily chosen (Table 15) at a starting pH that could be applied to all curves. This was pH 4.60, the final pH of Treatment 10 in Table 13.

Table 15. Titratable acidity (μmole NaOH g^{-1}) through 2 pH units from 4.60 to 6.60.

Treatment code	Data from Figure 14	Data from Figure 15
Treatment 2	80	105
Treatment 5	50	95
Treatment 7	61	80
Treatment 10	60	70

The results are not completely clear but there is an indication that less alkali is needed for Treatment 10. To better understand this phenomenon, it is re-examined in the next section, Replicated experiments for boiling time – Part 2.

To gain another insight into the pH phenomenon, it was decided to measure the glucose concentration in *Perna* 1 + 9 slurries in all the replicates at Day 0 and Day 5. This was done using the simple technology of a domestic diabetes meter as described in Chapter 2. The values at Day 0 (Figure 16) were around a plausible 0.018 g per g of *Perna* given that the nominal concentration of glucose was 2 g per 100 g of *Perna* (see also Footnote 7 on page 33). At Day 5 it was expected that the concentration would be lower in all treatments and that was usually true (Figure 17). However, there was a clear trend to lower apparent glucose concentration as boiling time increased. Increased boiling time results in higher pH so at the very least, high residual glucose concentration might occur in Treatment 10. This was not the case. The explanation for this paradox may be that the meters are calibrated for blood at pH 7 and have a systemic tendency to return higher and inaccurate values at progressively lower pH values as previously described by Young et al. (2004). It is concluded that the meters are unreliable for work with fermented *Perna* and glucose monitoring was discontinued. Further work would require calibration by the method of standard addition, or by a different method to counter the pH effect, both of which were beyond the scope of this research.

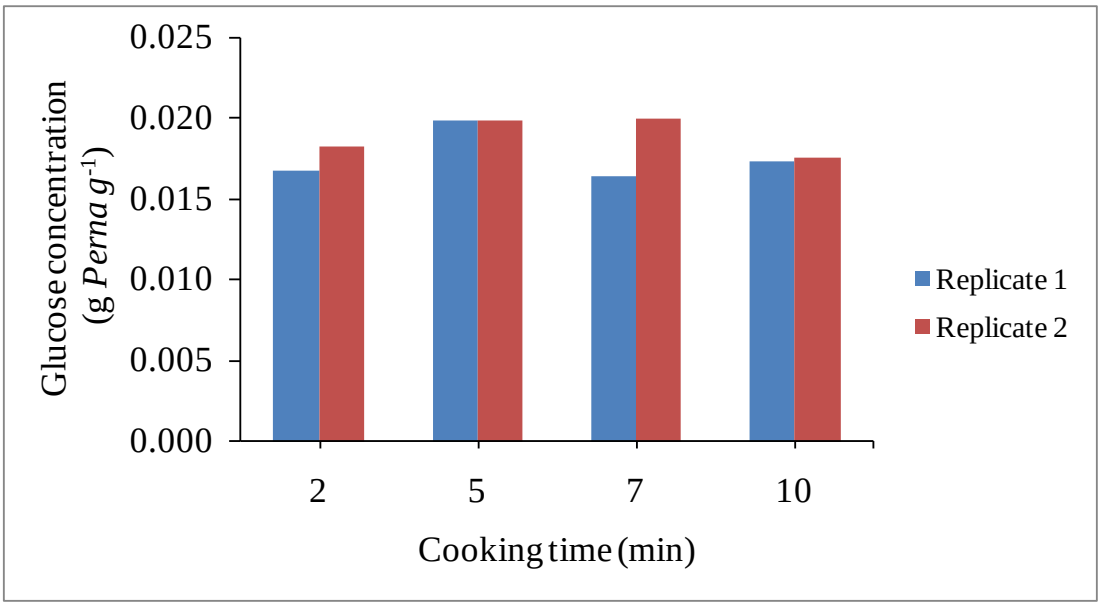

Figure 16. Glucose concentration of unfermented *Perna* at Day 0 as determined from slurries using a diabetes meter.

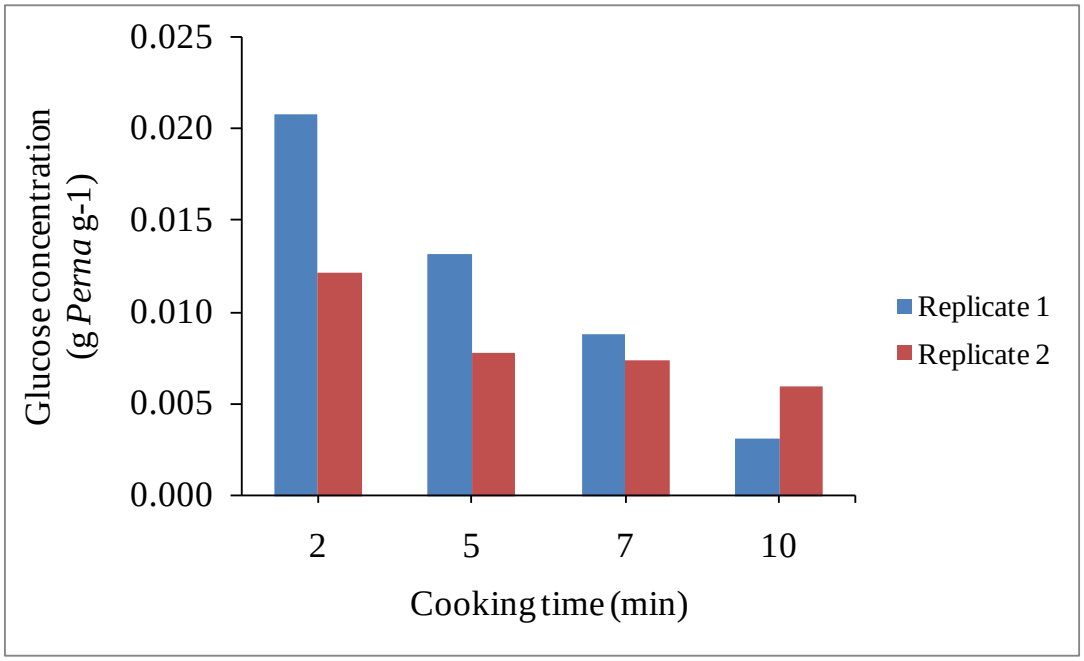

Figure 17. Glucose concentration of fermented *Perna* at Day 5 as determined from slurries using a diabetes meter.

Total bacterial populations including aerobic, anaerobic, and LAB counts of *Perna* at Days 0 and 5 were measured. Serial dilutions of *Perna* were prepared at these times and spread-plated in duplicates on plate count and MRS agars and incubated according to the procedure outlined in Materials and Methods.

Before incubation, the microbial population of the unfermented *Perna* together with the added LAB starter culture was about 10^6 cfu g^{-1} in Treatments 2 and 5, and perhaps a little lower in the other two (Figure 18). After fermentation, the total bacterial population of *Perna* increased about 80 times (Figure 19). While it is not clear why the pH differed with varying boiling times, the microbial contents of *Perna* at Day 5 for the treatments were more or less the same but with a suggestion they may be lower in Treatment 10. However the results were not remarkable and do not explain the pH effect.

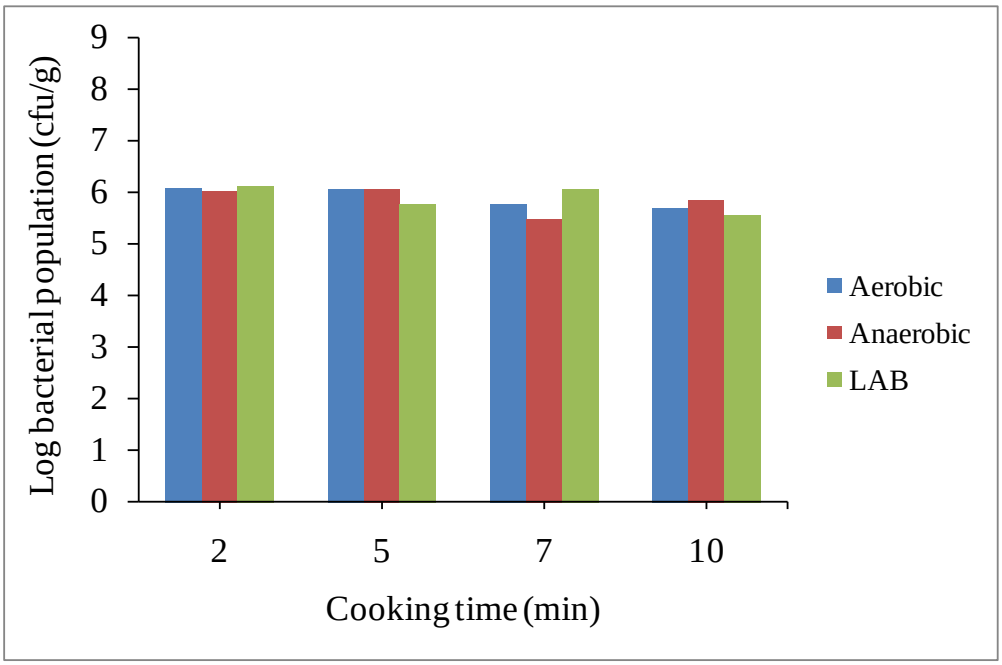

Figure 18. Total bacterial population of unfermented *Perna*.

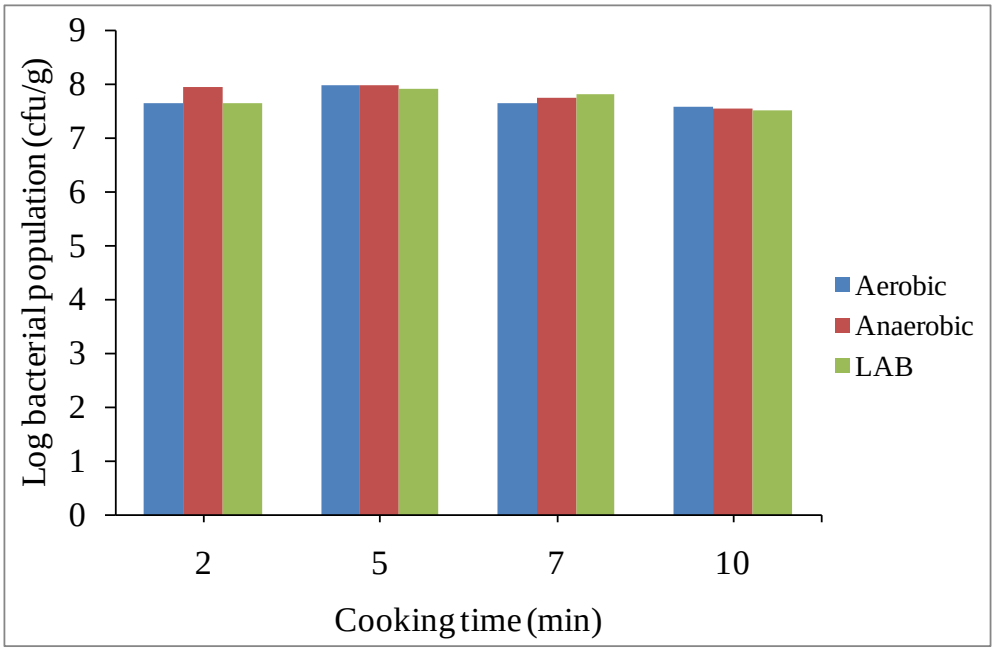

Figure 19. Total bacterial population of fermented *Perna*.

The colour for *Perna* made in these two experiments and in the pilot experiment were unaffected by boiling time as is clear from histograms of L*, a* and b* in Appendices 1, 2 and 3. Inspection of those graphs show that a* values were slightly negative in experiments reported in Tables 11 and 13 – that is greenish – and positive in Table 14 work. The likely explanation for this is the variable ratio of male to female mussels, the former cream-fleshed and the latter orange-

fleshed. However, numbers or masses by gender were not recorded at the time of *Perna* preparation and nothing could be done to confirm or reject this model.

Replicated experiments for boiling time – Part 2

To confirm that the previous boiling time results were truly reproducible, the experiments were repeated twice. One rationale for doing this was to see if the phenomenon were a seasonal effect because all the boiling time experiments spanned about 6 months. This experiment was done in later spring months (October, 2014) when the mussels are bigger and healthier, generally in a better condition than those used in earlier experiments. In this section, all analyses including buffering capacity and residual glucose monitoring were performed after fermentation.

Results and Discussion

Tables 16 and 17 show that as boiling increased, the final pH increased, as previously reported in Tables 11, 13 and 14. The smells of *Perna* were also reproducible in that lower pH *Perna* had a stronger acidic smell.

Table 16. Boiling time experiment, Replicate 3.

Treatment code	Boiling time (min)	*Perna* properties (after fermentation)		
		Appearance	Smell (acidic)	pH[1]
Treatment 2	2	Light brown	Strong	3.77 ± 0.02
Treatment 5	5	Light brown	Intermediate	3.78 ± 0.03
Treatment 7	7	Light brown	Mild	3.96 ± 0.02
Treatment 10	10	Light brown	Mild	4.04 ± 0.02

[1] pH ± standard deviation is from 3 replicate vials.

Table 17. Boiling time experiment, Replicate 4.

Treatment code	Boiling time (min)	*Perna* properties (after fermentation)		
		Appearance	Smell (acidic)	pH[1]
Treatment 2	2	Light brown	Strong	3.91 ± 0.01
Treatment 5	5	Light brown	Strong	3.71 ± 0.01
Treatment 7	7	Light brown	Mild	4.15 ± 0.06
Treatment 10	10	Light brown	Mild	4.10 ± 0.03

[1] pH ± standard deviation is from 3 replicate vials.

The pattern of titratable acidities of fermented *Perna* at Day 5 is shown in Figures 20 and 21.

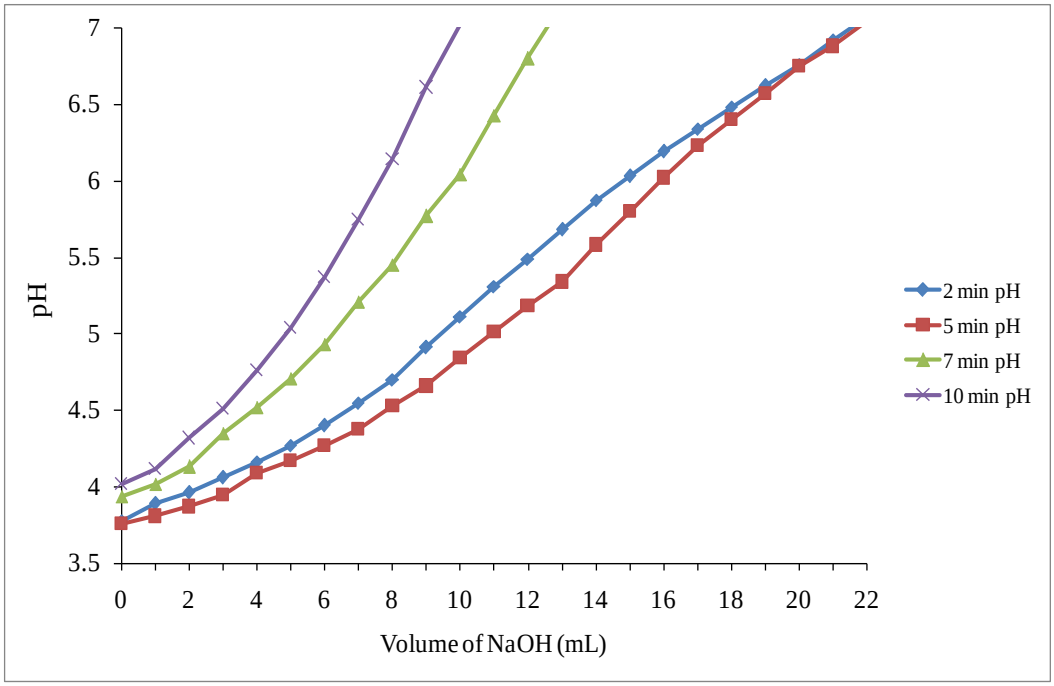

Figure 20. Pattern of the buffering capacity of fermented *Perna* (Replicate 3). The starting pH was different for different treatments and titration continued to pH 7.0.

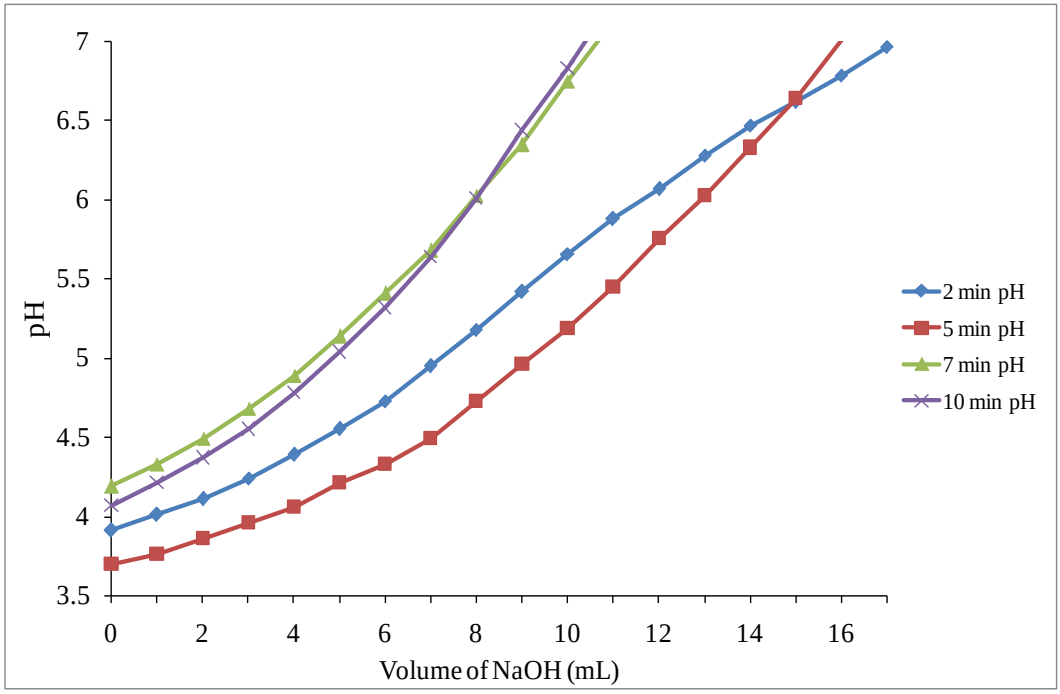

Figure 21. Pattern of the buffering capacity of fermented *Perna* (Replicate 4). The starting pH was different for different treatments and titration continued to pH 7.0.

The buffering capacity can be summarised in Table 18, where the μmole of NaOH per gram to titrate through two pH units starting from 4.60 to 6.60, as done in Table 15. The pattern was the same as previous replicated experiments in that more alkali was required at shorter boiling times.

Table 18. Titratable acidity (μmole NaOH g-1) through 2 pH units from 4.60 to 6.60.

Treatment code	Data from Figure 20	Data from Figure 21
Treatment 2	120	95
Treatment 5	105	75
Treatment 7	70	70
Treatment 10	55	65

The colour of *Perna* in these two experiments was measured in the L*, a*, b* space using a Hunter meter. The results thus obtained are shown in Appendices 4 and 5, but are unremarkable.

Summary of boiling time experiments

The pH of *Perna* dropped significantly if mussels were cooked for shorter times, even achieving values below pH 4. What exactly caused this pH change is yet to be understood. One underlying principle can be the seasonal effect on the appearance of green shell mussels. Due to which the boiling time experiments were carried out within a span of 6 months to understand the pH effect. However, the useful empirical observation was that if the mussels were cooked between 7 and 10 min, then the pH of about 4.5 could be achieved for *Perna* using the particular culture chosen, BFL-F02. And this is a likely desired pH for this product, because the smell of *Perna* was especially strong for the highly acidic treatments, but was reduced with an increase in pH. This strongly suggests that the pH must be controlled to achieve a desirable sensory quality of the final product.

Finally, the different boiling times had no effect on the colour of the final product. This can be clearly seen in the colour graphs situated in Appendix. The only clear effect on the colour of *Perna* is the ratio of male to female mussels which is beyond the scope of this research.

After examining the effects of various boiling times on the organoleptic properties of *Perna,* it was time to resume the course of this thesis which was to make a gelled mussel product and this is discussed in the chapters to come.

Chapter 4

Carrageenan gels

This chapter will explore the usage of kappa and iota carrageenans as a potential gelling agent in the production of *Perna*. But first we embark on the gelation properties of these hydrocolloids at near neutral and acid conditions which are discussed next.

Evaluation of carrageenans gelation under neutral and acid conditions

As discussed in the Introduction, it was of interest to find out the gelation properties of carrageenans under initially neutral and finally acid conditions that occur in *Perna* production. These comprised combinations of κ- or ι-carrageenans, with NaCl or KCl, in deionised water[8] or lactic acid buffer to study their texture. Details of texture analysis are presented later in this chapter.

Lactic acid buffer was prepared by the addition of 16.2 mL of 90% lactic acid into 1800 mL of distilled water and pH adjusted to 4.5 using NaOH pellets. The final volume of the buffer was made to 2 L yielding a final lactate / lactic concentration of 80 mM.

The mixtures were made by adding the carrageenans and salt to deionised water or lactate buffer at 80°C (Tables 19 and 20). During the 15 min it took for the carrageenans to disperse, mixtures were magnetically stirred. After dissolution they were distributed amongst three 50 mL red-capped plastic containers, capped, then left to cool to ambient. This was followed by overnight storage at 4°C for gelling to take its course. Before inspection and measurements were made, the containers were equilibrated to ambient temperature. The measurements were colour and texture analysis, described later in this chapter.

[8] All water used in formulations was deionised. Tap water was used where specified.

Table 19. Treatments with carrageenans under nominally neutral conditions.

Treatment code	Deionised water (mL)	Carrageenan (κ or ι) (g)	KCl (g)	NaCl (g)
A	150	3.00	3.00	0.00
B	150	3.00	0.00	3.00
C	150	3.00	0.00	0.00
D	150	2.25	2.25	0.00
E	150	2.25	0.00	2.25
F	150	2.25	0.00	0.00
G	150	1.50	1.50	0.00
H	150	1.50	0.00	1.50
I	150	1.50	0.00	0.00
J	150	0.75	0.75	0.00
K	150	0.75	0.00	0.75
L	150	0.75	0.00	0.00

Table 20. Treatments with carrageenans in lactate buffer of pH 4.5.

Treatment code	Lactic acid buffer (mL)	Carrageenan (κ or ι) (g)	NaCl (g)
M	150	3.00	3.00
N	150	3.00	0.00
O	150	2.25	2.25
P	150	2.25	0.00
Q	150	1.50	1.50
R	150	1.50	0.00
S	150	0.75	0.75
T	150	0.75	0.00

Results and Discussion

As discussed in the Introduction (Chapter 1), carrageenans were one of the two gelation agents of choice, the other being gelatine. In the current way *Perna* is made, both carrageenans and gelatine necessarily have to be added before fermentation at which time the pH is around 6.5 (results shown later), nominally neutral conditions. Carrageenans and gelatine gels are both thermally labile and might have to be added at high temperatures at the time of mixing, which could potentially deactivate the added culture. For this reason, the gelation properties of these gels were explored at ambient temperatures, the subject of this chapter being carrageenans only. For

nominally neutral conditions the carrageenan gels were made with deionised water. An acidic pH was also examined, the rationale being that if carrageenan were to be a successful gelation agent for *Perna*, the gel would have to be stable at pH 4.5. A lactic/Na lactate buffer was used to achieve that pH. In this work, two types of carrageenan were used, the κ- and the ι-carrageenan. NaCl was always tested as a gelation promoter, and KCl was tested out of curiosity at neutral pH only. A third type, λ-carrageenan, does not form gels, and hence was not used in this work.

i) Properties of κ-carrageenan gels at nominally neutral pH

Table 21 shows the characteristics of κ-carrageenan gels at pH 6.5 where the added salts were KCl, NaCl or neither. The higher the carrageenan concentration, the firmer the gel as expected, but the addition of salt was not necessary for gel formation. Syneresis, which would almost certainly be undesirable in *Perna*, generally decreased with increasing gel concentration. The syneresis from 1.50 g of κ-carrageenan (in 150 mL) with neither salt was zero, indicating that gel formation with salts – such as will occur in *Perna* – would be likely to generate syneresis if the κ-carrageenan concentration were low. Importantly, the syneresis with NaCl was the least of the three salt treatments at higher κ-carrageenan concentrations.

Table 21. Properties of κ-carrageenan gels under nominally neutral conditions with a final volume of 150 mL. Syneresis is scaled where 0 = none and 3 = abundant.

Treatment code	κ-Carrageenan (g)	Salt used	Gel properties	
			Appearance	Syneresis
A	3.00	KCl	Firm	1
B	3.00	NaCl	Firm	0
C	3.00	None	Firm	1
D	2.25	KCl	Semi-solid	2
E	2.25	NaCl	Semi-solid	1
F	2.25	None	Semi-solid	2
G	1.50	KCl	Semi-solid	3
H	1.50	NaCl	Semi-solid	3
I	1.50	None	Semi-solid	0
J	0.75	KCl	No gel (watery)	No gel
K	0.75	NaCl	No gel (turbid)	No gel
L	0.75	None	No gel (watery)	No gel

As for many experiments, colour was measured, although the light colour of κ-carrageenan gels would be swamped by the intense colour of *Perna*. These colour data are shown in Appendix 6. Generally, higher concentrations reflected more light, and were greener and yellower. Colour differences between salt treatments were minimal.

Of more practical interest were the texture properties, in particular hardness (Figure 22). Higher the concentration, harder the gel gets, although NaCl treatments were inferior to KCl treatments.

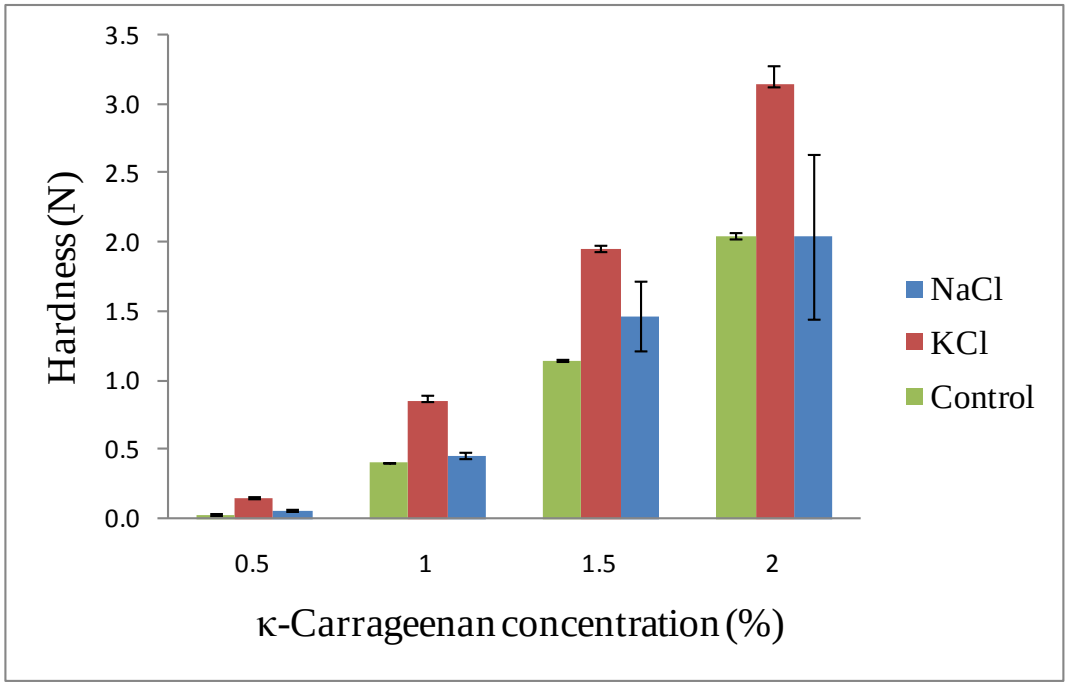

Figure 22. Hardness of κ-carrageenan gels at pH 6.5.

ii) Properties of κ-carrageenan gels at pH 4.5

Whereas a gel will form under nominally neutral conditions, such as will be encountered in the preparation and early stages of *Perna* fermentation, those gel properties may change at the final *Perna* pH of about 4.5. Table 22 shows the characteristics of κ-carrageenan gels at pH 4.5. In this work, KCl was omitted because it is unlikely to be used in commercial versions of this product.

As expected, higher the concentration of carrageenan, firmer the gel. No syneresis was encountered with the highest concentration of κ-carrageenan and salt (Treatment M). This combination could be the key to forming *Perna* gel.

Table 22. Properties of κ-carrageenan gels under acid conditions with a final volume of 150 mL. Syneresis is scaled where 0 = none and 3 = abundant.

Treatment code	κ-Carrageenan (g)	Salt used	Gel properties	
			Appearance	Syneresis
M	3.00	NaCl	Firm	0
N	3.00	None	Firm	1
O	2.25	NaCl	Semi-solid	1
P	2.25	None	Semi-solid	2
Q	1.50	NaCl	Semi-solid	3
R	1.50	None	Semi-solid	3
S	0.75	NaCl	No gel (watery)	No gel
T	0.75	None	No gel (watery)	No gel

The colour of the gels was measured and the data are in Appendix 7. The colour profile was closely similar to the κ-carrageenan gels formed at pH 6.5. (No colour measurements were made with the later ι-carrageenan gels, because their light colour would similarly be swamped by the intense colour of *Perna*.)

The hardness of the gels at the approximate pH of the fermented *Perna* was of particular interest. Like the previous experiment, gel hardness was directly proportional to the concentration of κ-carrageenan, with NaCl having little to no effect (Figure 23).

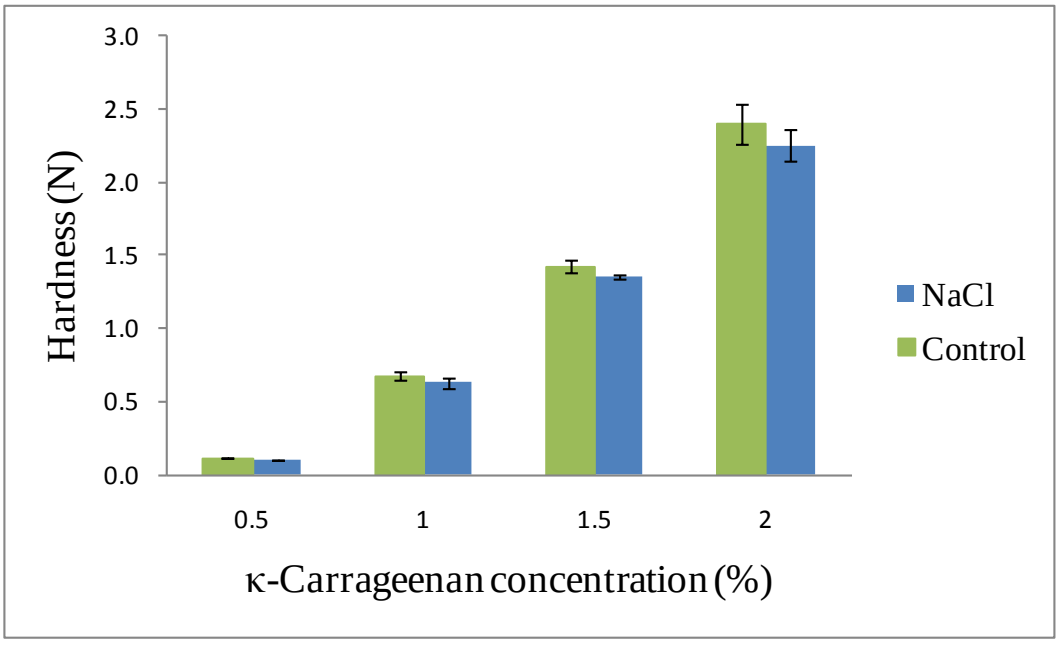

Figure 23. Hardness of κ-carrageenan gels at pH 4.5.

A comparison of the hardness of gels obtained at pH 6.5 and 4.5 is illustrated in Figure 24. κ-Carrageenan gels formed at lower pH were about as hard as those formed at near neutral pH (No analysis of variance has been done because the results are obvious in Figure 24). This gives good reason for κ-carrageenan gels being stable at acidic pH of 4.5.

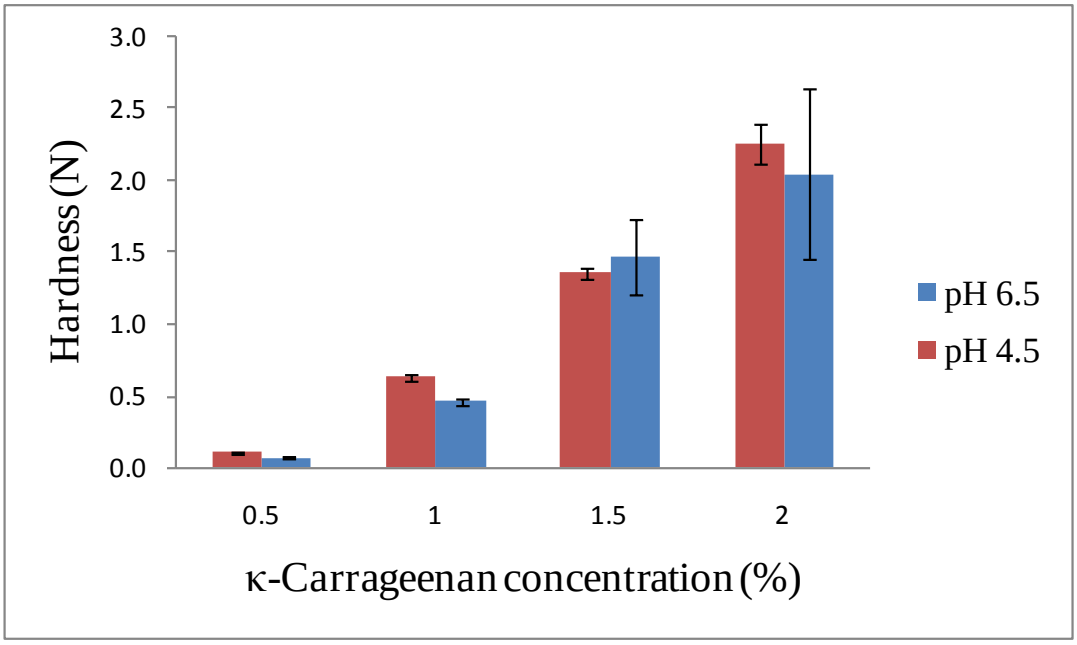

Figure 24. Hardness comparison of κ-carrageenan gels made at pH 6.5 and 4.5.

iii) Properties of ι-carrageenan gels at nominally neutral pH

Previous experiment was repeated but this time with ι-carrageenan as the preferred gelling agent. Table 23 shows the typical characteristics of ι-carrageenan gels at pH 6.5. The ι-carrageenan gels formed were semi-solid and elastic as opposed to the gels formed with κ-carrageenan. The hardness of gels increased with the concentration (Figure 25) and no syneresis was observed in any of the treatments. Moreover, NaCl and KCl had little to no effect on the hardness and syneresis of the gels. As mentioned earlier, colour analysis was not performed for any of the gels made with ι-carrageenan.

Table 23. Properties of ι-carrageenan gels under nominally neutral conditions with a final volume of 150 mL. Syneresis is scaled where 0 = none and 3 = abundant.

Treatment code	ι-Carrageenan (g)	Salt used	Gel properties	
			Appearance	Syneresis
A	3.00	KCl	Semi-solid	0
B	3.00	NaCl	Semi-solid	0
C	3.00	None	Semi-solid	0
D	2.25	KCl	Semi-solid	0
E	2.25	NaCl	Semi-solid	0
F	2.25	None	Semi-solid	0
G	1.50	KCl	Slurry	0
H	1.50	NaCl	Slurry	0
I	1.50	None	Slurry	0
J	0.75	KCl	No gel (watery)	No gel
K	0.75	NaCl	No gel (watery)	No gel
L	0.75	None	No gel (watery)	No gel

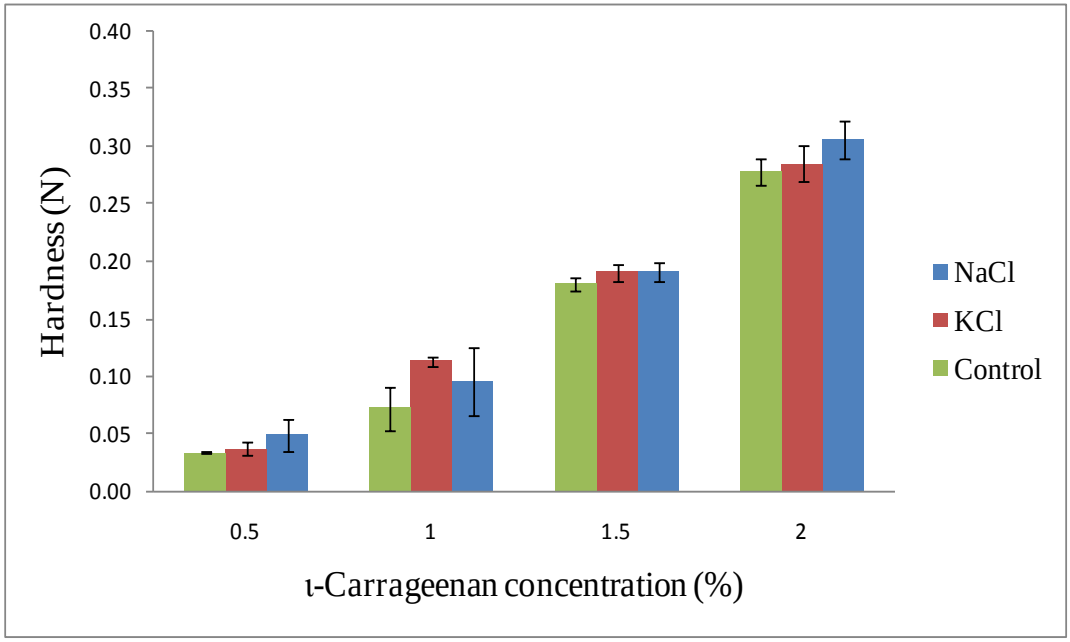

Figure 25. Hardness of ι-carrageenan gels at pH 6.5.

iv) Properties of ι-carrageenan gels at pH 4.5

Table 24 illustrates the usual properties of ι-carrageenan gels at pH 4.5 in the presence and absence of NaCl. As expected, hardness of the gels increased with the concentration and once more NaCl had no marked effect on its hardness (Figure 26). No syneresis was observed in any of the treatments.

Table 24. Properties of ι-carrageenan gels under acid conditions with a final volume of 150 mL. Syneresis is scaled where 0 = none and 3 = abundant.

Treatment code	ι-Carrageenan (g)	Salt used	Gel properties	
			Appearance	Syneresis
M	3.00	NaCl	Semi-solid	0
N	3.00	None	Semi-solid	0
O	2.25	NaCl	Semi-solid	0
P	2.25	None	Semi-solid	0
Q	1.50	NaCl	Slurry	0
R	1.50	None	Slurry	0
S	0.75	NaCl	No gel (watery)	No gel
T	0.75	None	No gel (watery)	No gel

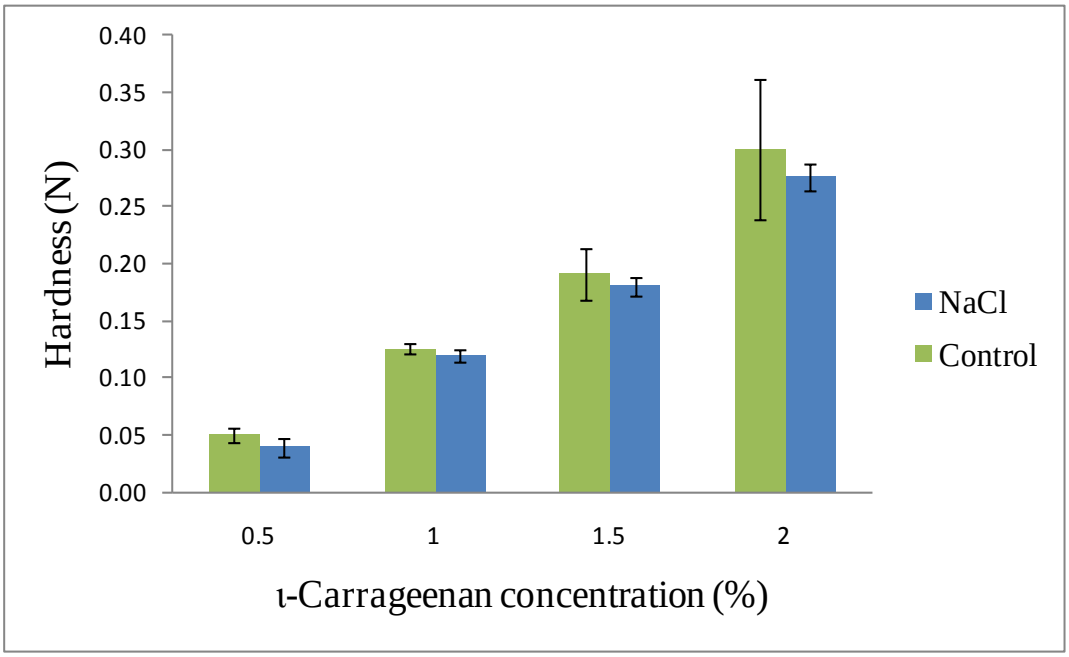

Figure 26. Hardness of ι-carrageenan gels at pH 4.5.

The difference in the hardness of ι-carrageenan gels formed at two pH values was minimal as shown in Figure 27. The advantage of using ι-carrageenan in *Perna* production would mean no syneresis, however the results obtained indicate that the gels formed are semi-solid and elastic in nature which is not the expectation of final *Perna* to be.

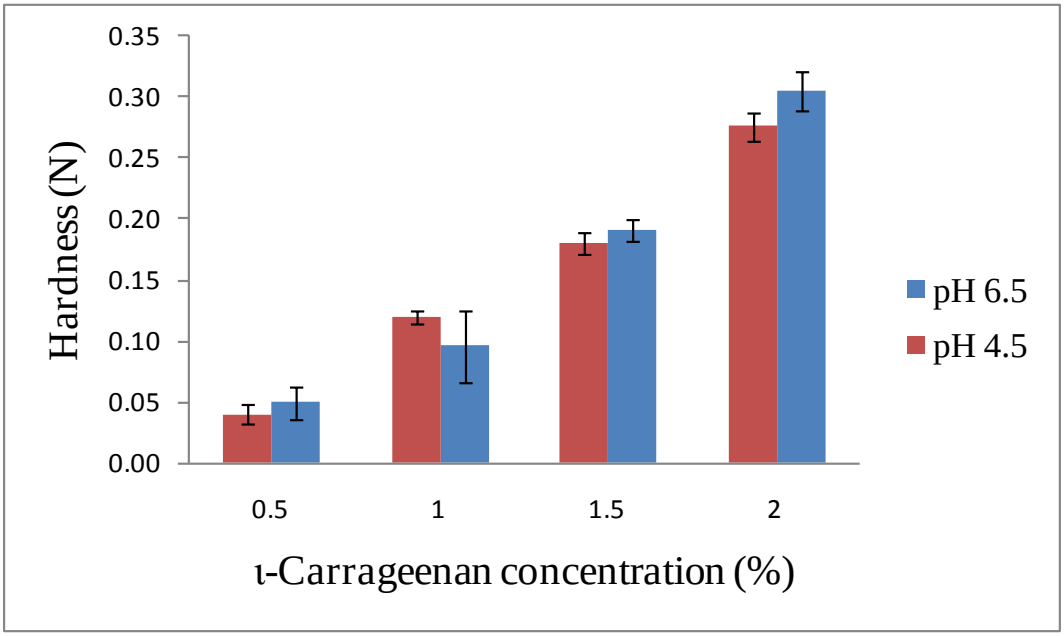

Figure 27. Hardness of ι-carrageenan gels made at pH 6.5 and 4.5.

Of more importance was the hardness comparison between κ- and ι-carrageenan gels. Figure 28 clearly shows that κ-carrageenan gels were, on certain occasions, more than three times harder than those obtained from ι-carrageenan. All these findings suggest the use of κ-carrageenan in the production of *Perna* gel. This is the topic of discussion next.

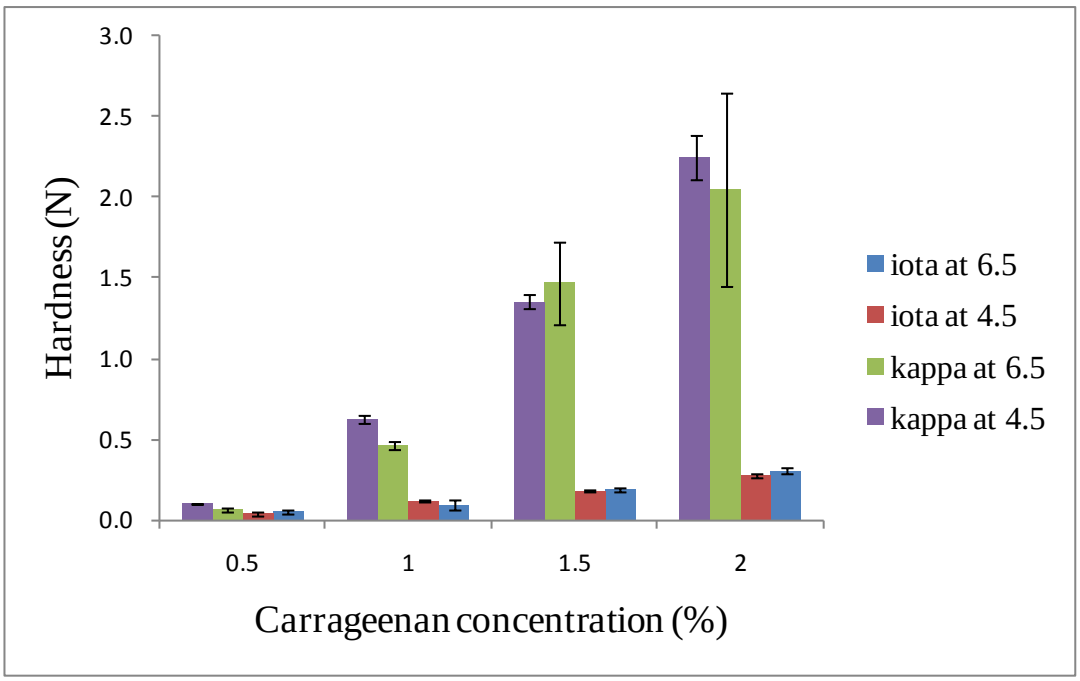

Figure 28. Comparison of κ- and ι-carrageenan gels at pH 6.5 and 4.5.

Evaluation of carrageenans gelation in *Perna*

The live mussels were cooked in batches of 1.0 kg in 1.5 L of tap water at 100 °C for 5 min and subsequently cooled, cleaned of beards and minced as described previously. This mince from the 3.0 mm plate was then blended with the ingredients shown in Table 25.

Table 25. Typical formulation of *Perna* production in the laboratory.

Treatment code	Mussel mince (g)	Carrageenan(κ or ι) (g)	NaCl (g)	KCl (g)	Glucose (g)	LAB culture (g)	Water (mL)
A	200	4.0	4.0	0.0	4.0	0.04	20.0
B	200	4.0	0.0	4.0	4.0	0.04	20.0
C	200	0.0	4.0	0.0	4.0	0.04	20.0
D	200	0.0	0.0	4.0	4.0	0.04	20.0

The idea was to get all the ingredients up to a high enough temperature of about 60 °C so that the carrageenan (mixed with the glucose and salt) would disperse. The Kenwood mixing bowl and the mixing blade were immersed in near boiling water that was discarded immediately before mixing began. The minced mussel was also heated for a minute in a microwave oven (800 W). The water component was added to avoid clumping and to aid dispersion of the ingredients during mixing. When the temperature reached 40°C, the diffused culture, mixed with minimum volume of water, was added and mixing continued for one minute. The idea was to use a high temperature to aid carrageenan dispersal, and a lower temperature when the culture was added to avoid inactivating the LAB, although the temperature stabilities of the two species of LAB were unknown.

The unfermented *Perna* was then vacuum sealed immediately in red-capped plastic containers as shown in Figure 29. Importantly, the caps were kept loose to allow air to escape during evacuation. The vacuum packer was set on 20 seconds of vacuum and 3 seconds of sealing on the medium of three possible settings. Subsequently the bags were incubated at 30 ± 2°C for 96 hours for fermentation to take its course. This was followed by refrigeration at around 4°C for a minimum of 24 hours (by then Day 5) after which the analyses were conducted once the vials had attained ambient temperature.

Figure 29. Vacuum packed *Perna* before fermentation.

Results and Discussion

The plan was to test the gelation potential of carrageenans in *Perna*. The *Perna* treatments were prepared according to a strict temperature regime described in Materials and Methods, such that the carregeenans were added to heated minced mussels (~60 °C) to ensure dissolution, but the culture was added only as the mixture cooled so that the LAB were not adversely affected by heat. Fermentation was over four days at 30°C as always, followed by overnight refrigeration. This work was done in October, 2013.

i) Properties of *Perna* in the presence of κ-carrageenan

On Day 5, *Perna* with added κ-carrageenan was removed from refrigeration to attain room temperature. All treatments appeared very dark (colour measurements were not made), and had an unpleasant strong acidic smell perhaps consistent with the measured pH values, a surprisingly low 3.2, a certain indicative of successful fermentation. (Measurement error cannot be excluded, but the result was taken at face value). Syneresis was excessive in all treatments (Table 26). There was no evidence of successful gel formation.

Table 26. Properties of *Perna* with κ-carrageenan as a potential gelling agent. Each treatment comprised 200 g.

Treatment code	κ-Carrageenan (g)	Salt used (4.0 g)	*Perna* properties				
			Visual appearance	Smell	Syneresis (per 200g)	Product recovery (per 200g)	pH
A	4.0	NaCl	Dark brown	Strong acidic	51	149	3.17
B	4.0	KCl	Dark brown	Strong acidic	63	137	3.12
C	0.0	NaCl	Dark brown	Strong acidic	52	148	3.23
D	0.0	KCl	Dark brown	Strong acidic	66	134	3.22

ii) Properties of *Perna* in the presence of ι-carrageenan

Two weeks after the experiment with κ-carrageenan, the work was replicated with ι-carrageenan using a new purchase of live mussels. The result was much better: syneresis was much reduced, with smell being more pleasant and the colour a lighter brown (Table 27). However, no gelation was evident.

Table 27. Properties of *Perna* with ι-carrageenan as a potential gelling agent. Each treatment comprised 200 g.

Treatment code	ι-Carrageenan (g)	Salt used (4.0 g)	*Perna* properties				
			Visual appearance	Smell	Syneresis (per 200 g)	Product recovery (per 200 g)	pH
A	4.0	NaCl	Light brown	Acidic	6	194	3.82
B	4.0	KCl	Light brown	Acidic	9	191	3.78
C	0.0	NaCl	Light brown	Acidic	6	194	3.95
D	0.0	KCl	Light brown	Acidic	8	192	3.69

The contrasting results between Tables 26 and 27 raised several questions. For example, were the differences in colour, smell, syneresis and pH were due to the different carrageenans, or to different mussel physiological condition, or to some other factor, or to various combinations of these factors?

First consider the carrageenans. Tables 22 and 24 show that under acid conditions, ι-carrageenan would be less likely to generate syneresis than κ-carrageenan, and this fact alone may account for some difference. At the same time the pH was much lower where κ-carrageenan was used, and the fluid-holding capacity may be reduced more around pH 3.2 (Table 26) than around pH 3.8 (Table 27). Thus there are no obvious answers.

As discussed briefly in the Introduction, physiological state of mussels varies with season and the mussels used for the κ-carrageenan treatments may have been inferior for this work. However, as for the arguments surrounding carrageenans, an explanation is confounded by differences in pH.

Another possibility to explain the difference is boiling time for the mussels. In the initial experiments to develop *Perna* by John Qiang (2012), an undergraduate project student at AUT, mussels were first used raw after tedious extraction from the shells as described in the Introduction. Figure 30 shows the first result, where although fermentation was successful, unpleasant slurry was formed probably due to the combination of raw mussel gut and other microflora, mussel gut enzymes and added LAB.

Figure 30. The unpleasant slurry created by fermenting raw mussels with LAB.

Idea being that although LAB should take over, under circumstances where microbial load is high, these bacteria and endogenous enzyme may contribute to decarboxylation to generate biogenic amines. The practice of using raw mussels was immediately discontinued and prior cooking eventually yielded useful results. The excessive syneresis and low pH of the κ-carrageenan treatments (Table 26), suggested that the poor *Perna* properties might have been caused by insufficient cooking. With the failure of carrageenans to generate a useful gel, gelatine was the next gelling agent used in the attempt to make a successful *Perna* gel. This is the topic of discussion next.

Chapter 5

Gelatine gels

After the unsuccessful attempts to make a *Perna* gel with carrageenans, gelatine was chosen for experimentation. The reasons for this choice were discussed in Chapter 1. It is however, worth repeating that the preferred source of gelatine would be fish, but this was not available in New Zealand. Davis brand gelatine (Figure 31) was chosen as a convenient source.

Before attempting *Perna* gelation, the behaviour of gelatine colloidal solutions were explored at three temperatures, ambient, 30°C and 4°C, to replicate the sorts of temperature conditions sequentially encountered in *Perna* production.

Behaviour of gelatine colloidal solutions at *Perna* production temperatures

Materials and Methods

Based on the Davis preparation recommendation, seven treatments of 10 g of domestic gelatine (Davis, Auckland) was added to 500 mL of deionised water. These mixtures was then heated to 70°C on a hot plate using a magnetic stirrer until the gelatine crystals were fully dispersed. Each was cooled to ambient, then either held there overnight or sequentially heated overnight at 30°C and then 4°C to see its gelation behaviour.

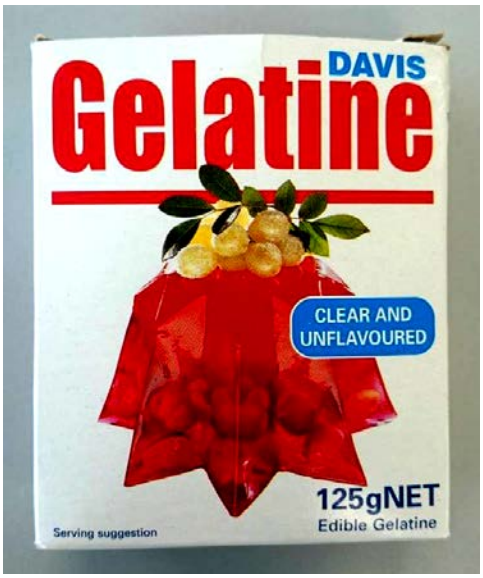

Figure 31. The Davis gelatine used in all experiments.

Results and Discussion

Firm gels were only produced when the mixtures were refrigerated to 4°C. Hence refrigeration after fermentation of *Perna* would be absolutely essential for gel formation.

Evaluation of gelatine gelation in *Perna* – Experiment 1

For gelatine experiments, commercially cooked and frozen mussels devoid of shells were used rather than live mussels bought from a supermarket. This was a convenient form and moreover, may be a better source of material for *Perna* for reasons to be discussed in the concluding Chapter 6.

Materials and Methods

Frozen cooked mussel meat (Figure 32) was thawed at ambient temperature prior to double mincing as described previously. The resulting mince was then blended with salt, glucose, water and LAB culture (Figure 33) as shown in Table 29.

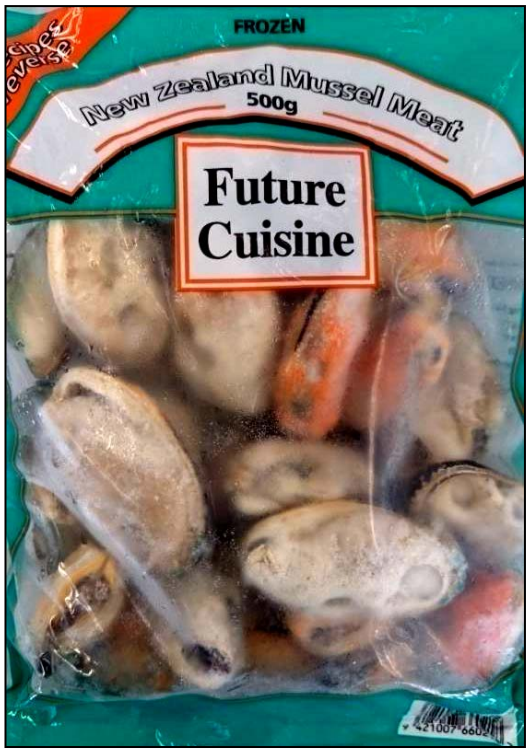

Figure 32. Frozen cooked mussel meat from Future Cuisine purchased at Countdown, Auckland.

Figure 33. Mussel dough (mince, salt, glucose, water and LAB) before the addition of gelatine.

At around the same time, gelatine, NaCl and glucose (Table 28) were added gradually to 500 mL of deionised water that was magnetically stirred (Figure 34). The mixture was held at 70°C for 10 minutes with stirring until the hydrocolloid was completely dispersed after which it was removed from the heat. When the temperature of mixture fell to 40°C, it was considered cool enough not to threaten the viability of the LAB culture. It was mixed with *Perna* for a minute, resulting in variable concentrations of gelatine, but constant proportions of salt and glucose (Table 29). The concentration of LAB culture was unavoidably variable, but as will be seen this did not affect the fermentation.

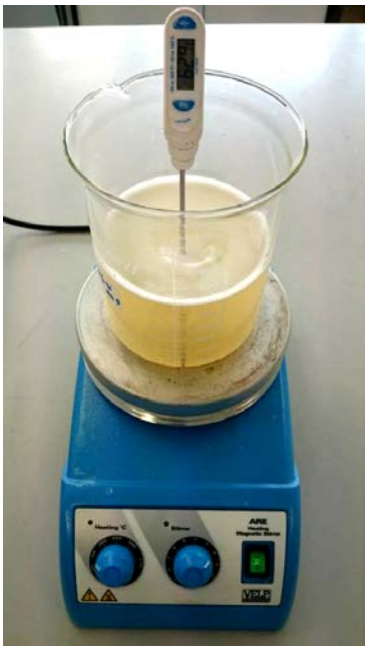

Figure 34. Gelatine preparation on a hot plate with a magnetic stirrer mixing the solution and a digital thermometer recording the temperature.

Table 28. Formulation of the gelatine mixture – Experiment 1.

Davis gelatine (g)	Water (mL)	NaCl (g)	Glucose (g)
20	500	10	10

Table 29. Formulation of *Perna* with gelatine – Experiment 1

Treatment code	Mussel mince (g)	NaCl (g)	Glucose (g)	LAB culture (g)	Water (mL)	Gelatine mixture (mL)
A	400	8.0	8.0	0.04	40.0	0
B	400	8.0	8.0	0.04	40.0	20
C	400	8.0	8.0	0.04	40.0	40
D	400	8.0	8.0	0.04	40.0	60
E	400	8.0	8.0	0.04	40.0	80

The resulting unfermented *Perna*, Treatments A to E, was then filled in to Pam's containers for vacuum packaging, the details of which have been described earlier. However, one difference was that the containers were de-aerated twice prior to final vacuum packaging. The process of de-aerating the containers twice was believed to have more significance in air escape from between the mussel mince and kept the product unsullied for a longer period. Subsequently the containers were incubated at 30 ± 2°C for 96 hours followed by refrigeration at about 4°C for a minimum of 24 hours (by then Day 5) before the analyses were conducted. These were appearance, smell, pH, texture and colour. (Colour data were unremarkable between treatments, but are presented in Appendix 8.)

Results and Discussion

On Day 5, *Perna* with variable gelatine concentrations was removed from refrigerated temperatures and analysed at ambient temperature. All treatments were significantly dark brown and had an unpleasant strong acidic odour (Table 30). Moreover the syneresis was abundant in all the treatments. The reason for this was not clear but it could be due to one or a combination of the following causes:

1. The use of cooked thawed mussels which may have a lower water-holding capacity.
2. The pH of the fermented sample was very low averaging at about 3.6. Proteins bind least water at their isoelectric point, the pH of which may (or may not) be around that pH. The isoelectric point of gelatine is 4.70 (Hudson & Sheppard, 1929), but that is irrelevant because synersis was significant even in Treatment A that contained no gelatine.

3. In these very acidic condition; gelatine is known to hydrolyse perhaps degrade causing liquid expulsion. The low pH phenomenon has also been discussed in previous chapters and will be further examined below and discussed in Chapter 6.

Table 30. Properties of *Perna* with gelatine as a potential gelling agent. Each treatment comprised 200 g.

Treatment code	Gelatine mixture (mL)	*Perna* properties			
		Colour	Smell	Syneresis	pH
A	0	Dark brown	Strong acidic	Significant	3.72
B	20	Dark brown	Strong acidic	Significant	3.66
C	40	Dark brown	Strong acidic	Significant	3.60
D	60	Dark brown	Strong acidic	Significant	3.71
E	80	Dark brown	Strong acidic	Significant	3.71

As shown in Figure 35, there was no significant difference in the hardness of *Perna* that could be attributed to gelatine addition. The slicing property of *Perna* was also measured and is shown in Figure 36. It declined with increasing gelatine concentration, and this may be attributed to the lower proportion of mussel mince in the final mixture. Thus, the proportion of mince to add fluid in Treatment A was 400:40 and in Treatment E, 400:120, making it easier to slice.

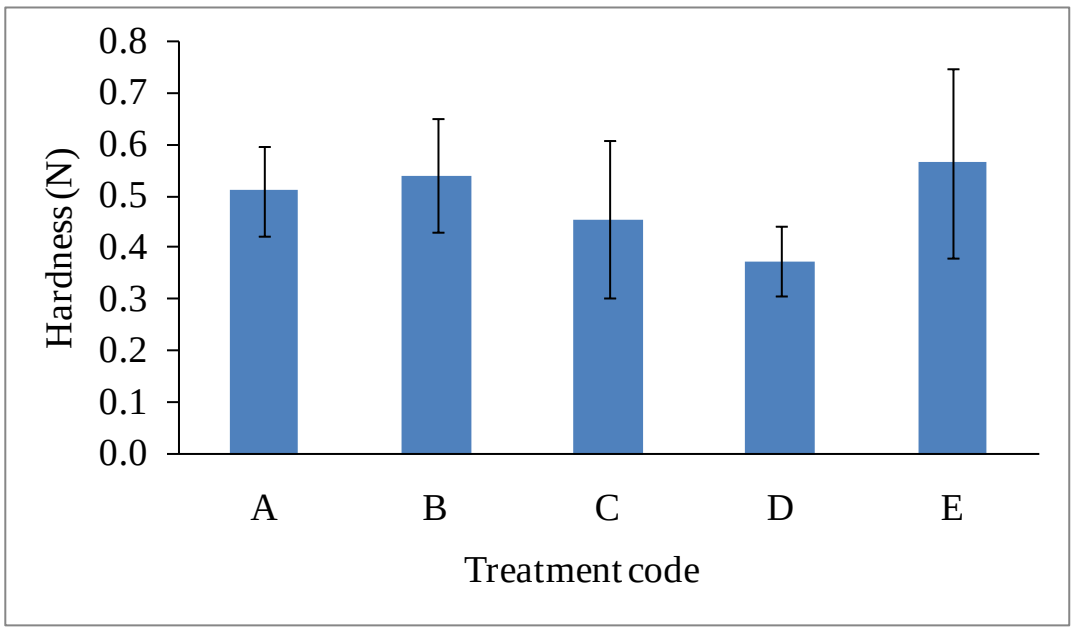

Figure 35. Hardness of *Perna* as determined with the cylindrical probe.

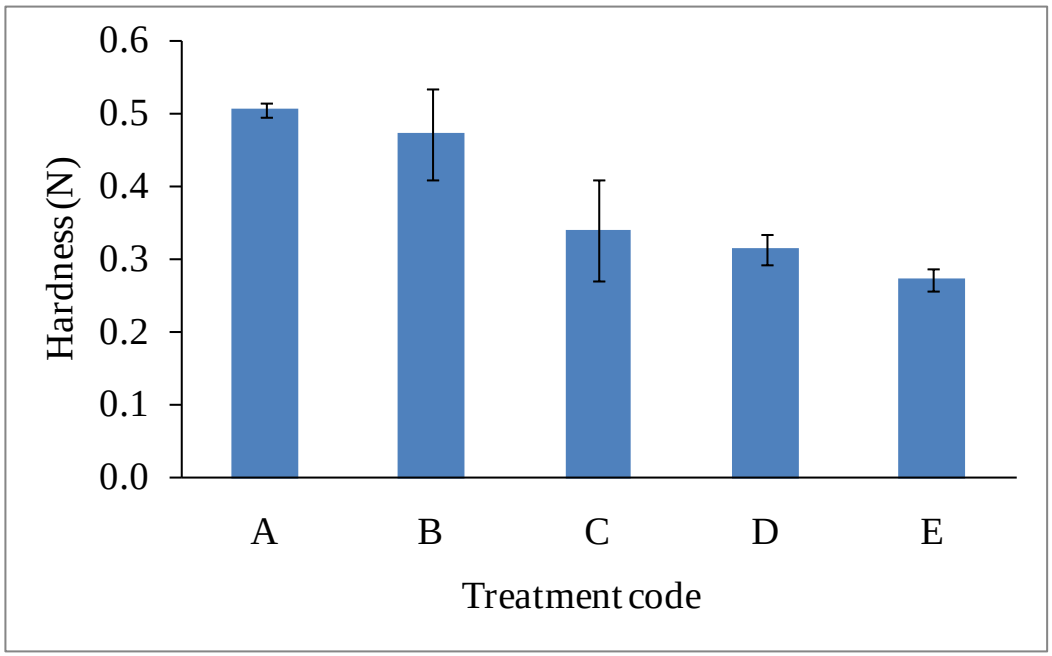

Figure 36. Slicing property of *Perna* determined with the steel blade.

What was overlooked at this time was that although the gelatine mixture was made up as recommended by Davis, the final concentrations in *Perna* were probably too low to achieve a gel. Thus in Treatment E in Table 29, the approximately original 4% (w/v) solution (20 g in 500 mL, Table 28) became diluted to about 0.6%, and for Treatment D a lower 0.48% and so on toward Treatment A. Assuming that the gelatine would equilibrate in the water intrinsic to the mussel mince, these final concentrations were probably too low to support gelation. Being unaware of this simple flaw, research proceeded in a misplaced effort to find a cause of gelation failure in pH and salt concentration. Only after these experiments were done – as now reported below – did the solution to gelation become obvious, ultimately yielding worthwhile results, as also reported below.

Believing that a low pH might have been the cause of poor gelation with gelatine, the next experiment was simply a repeat of this one except that the glucose concentration was reduced from 2% to 0.5%.

Evaluation of gelatine gelation in *Perna* – Experiment 2

The low pH reported in Experiment 1 above prompted a repeat but with the glucose concentration reduced from 2% to 0.5% in both the formulation of the gelatine mixture and in the formulation of *Perna*. The idea was that by limiting glucose a higher pH might be the result, and may overcome the syneresis problem, and at the same time may result in gelation. (As explained above, gelation would be highly unlikely.)

Materials and Methods

The experimental details were as reported in Experiment 1 except that 10 g of glucose in Table 28 became 2.5 g, and in the formulation of *Perna*, 8 g in Table 29 became 2 g.

Results and Discussion

On Day 5, after the treatments had attained room temperature the colour was measured, the results of which are in Appendix 9. The treatments were dark brown except for Treatment E which was a lighter shade of brown, possibly because it contained the least mussel flesh. Treatments were strong acidic in smell but with insignificant syneresis except for Treatment E (Table 31), the treatment with the highest addition rate of gelatine mixture. There was no sign of gel formation.

Table 31. Properties of *Perna* with gelatine as a potential gelling agent. Each treatment comprised 200 g.

Treatment code	Gelatine mixture (mL)	Colour	*Perna* properties		
			Smell	Syneresis	pH
A	0	Dark brown	Strong acidic	Insignificant	4.22
B	20	Dark brown	Strong acidic	Insignificant	4.34
C	40	Dark brown	Strong acidic	Insignificant	4.13
D	60	Dark brown	Strong acidic	Insignificant	4.12
E	80	Light brown	Strong acidic	Significant	4.21

As a matter of routine, hardness and sliceability data of *Perna* were collected (Figures 37 and 38 respectively). There was no significant difference in the hardness of Treatments A to D. Treatment E which had the highest proportion of gelatine and less mussel was the least hard.

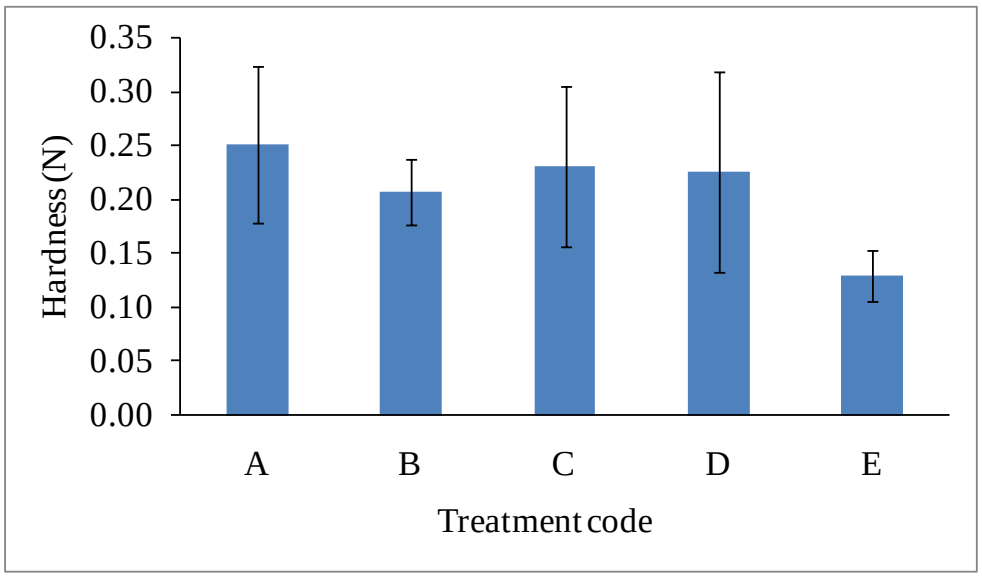

Figure 37. Hardness of *Perna* determined with the cylindrical probe.

The data collected from the knife test (Figure 38) using a blade of 9mm in length depicted that it was most difficult to slice Treatment A which contained no gelatine, presumably due to the resistance of mussel flesh. The slicing pattern was more or less the same for Treatments B to E.

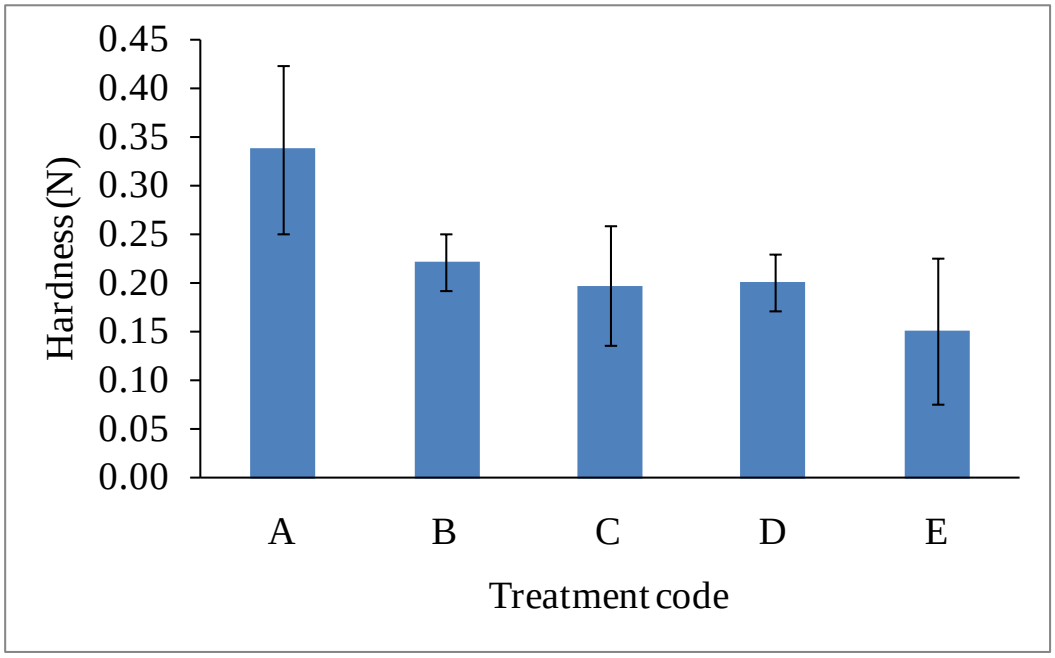

Figure 38. Slicing property of *Perna* determined with the steel blade.

At this point of the study, the true cause of failure to form a gel was still not recognised. However, the one positive outcome of this Experiment 2 was that the excessively low pH of *Perna* encountered in Experiment 1 and elsewhere in this thesis might simply be solved by lowering the glucose concentration. It must be pointed out however, that Experiments 1 and 2 were independent and a simple definitive experiment comparing the pH effects of variable glucose concentrations remains to be done.

With the continuing failure to make a gelatine gel in *Perna*, further experiments were undertaken to eliminate possible causes. These were pH and salt causes, but because these eventually proved to be irrelevant, these experiments and their results are presented only as summaries.

Evaluation of gelatine gelation under two acidic conditions

To test the gelation of gelatine under acidic conditions the formulation in Table 29 was modified such that the roughly 440 mL of mussel mince, NaCl, glucose, culture and water was replaced with the same volume of a salted lactic acid buffer at two pH values, 3.5 and 4.5. These buffers were prepared as shown in Table 32. The volume of 90% lactic acid chosen was derived from a calculation based on the assumption that of the 2% glucose, half will be fermented to lactic

acid. That is 1 g per 100 g equivalent to 10 g per kg or L. That is about 0.06 M based on 186 g/mol. That will yield 0.12 M lactic acid, because you get 2 mole of lactic acid from 1 mole of glucose. That is quite strong however *Perna* is buffered due to protein. Now 0.12 M lactic acid is 0.12 mol/L = 10 g lactic acid per kg of *Perna* (final concentration). That is the figure we needed to work out how much to add to *Perna* if we were to simulate fermentation.

Table 32. Formulation[9] for lactic acid buffer at pH 3.5 and 4.5.

90% Lactic acid (mL)	Distilled water (mL)	NaCl (g)	NaOH (g)	Distilled water (mL)
16.2	1950	40	15 or 10	200

These two acids were then used to prepare heated, approximately 4% solutions of gelatine as shown in Table 33, that were subsequently used to add to the lactic acid buffer after the manner of Experiments 1 and 2 (Table 34).

Table 33. Formulation for lactic-acidified gelatine.

Gelatine (g)	Lactic acid (mL)	NaCl (g)
12	300	6

Table 34. Treatments with gelatine in lactic acid buffer of pH 3.5 and 4.5.

Treatment code	Lactic acid buffer (mL)	Gelatine mixture (mL)
A	200	0
B	200	40
C	200	80

Treatments A, B and C were distributed among three 50 mL plastic containers, capped, and left to cool to ambient. This was followed by incubation at 30 ± 2°C for 96 hours and then overnight storage at 4°C for gelling – if any – to take its course. Before inspection and measurements were made, the containers were equilibrated to ambient temperature. Texture was measured with the stainless cylindrical probe with a diameter of 6mm.

[9] Glucose was excluded because in simulating a fermented product, glucose would be largely converted to lactic acid.

Treatment A was, as expected, only liquid because it had no added gelatine. Treatments B and C had formed thick suspension however no gel was created, with hardness values (data not shown) about 10% of the values in Experiments 1 and 2, showing that mussel was the source of hardness in those two experiments, not gelatine. At the time the acid experiment was done, I still erroneously thought that acid hydrolysis at 30°C was responsible, so two further experiments were done, focusing on salt at neutral pH.

Evaluation of gelatine gelation under neutral condition with and without salt

Using methods, volumes and masses analogous to those in Tables 32 and 33 but with water rather than lactic buffer, the gelation experiments were repeated. The outcome was that gels were not formed. Only then was it belatedly realised that final gelatine concentrations were much too low. This realisation opened the way to definitive *Perna* gelation with gelatine.ss

Development of concentrated gelatine gels

This experiment was dedicated to dissolving as much gelatine possible in water, where the volume of water remained the same and concentration of gelatine increased.

Materials and Methods

Mixtures of gelatine in deionised water were prepared according to Table 35, where the lowest ratio was 2:100, nominally 2% and highest 48:100, nominally 48%. They were prepared by heating as shown in Figure 34.

Table 35. Preparation of Davis gelatine colloids at different concentrations. Froth formation is scaled where 0 = none and 3 = abundant.

Treatment code	Gelatine (g)	Deionised water (mL)	Froth formation
A	2	100	0
B	6	100	0
C	12	100	0
D	24	100	1
E	48	100	3

These treatments were distributed amongst two 50 mL red-capped plastic containers, capped, then left to cool to ambient, followed by overnight incubation at 4°C. Before inspection and texture measurement, the containers were equilibrated to ambient temperature.

Results and Discussion

As mentioned earlier this experiment was solely dedicated to dissolving as much gelatine as possible in water. The maximum concentration achieved was 48 g in 100 mL of water (Treatment E) and as expected it took longer, roughly 25 min for it to disperse as a colloidal solution. Moreover excessive froth was formed during and after its dissolution (Figure 39).

Figure 39. Froth formation after the dissolution of gelatine with Treatment A being the least in gelatine and Treatment E having the most.

On inspection, Treatments B, C, D and E had formed a gel (Figure 40) with likely increasing hardness as gelatine concentration increased. Treatment A with 2% gelatine had formed no gel. It immediately became obvious that the cause of non-gelation in prior experiments in this chapter, was almost certainly because the concentration was much too low. (Thus in previous Treatments E – those highest gelatine concentrations in Experiments 1 and 2 above – the final gelatine concentration was around 0.7%. The hardness measured there was solely due to mussel tissue.)

Figure 40. Obvious firmness of the gelatine gels. Treatment A is not included because it would flow on vial inversion.

Figure 41 shows that Treatment E required 6 N of peak force for penetration with the stainless cylindrical probe compared to Treatment A which required only 0.1 N.

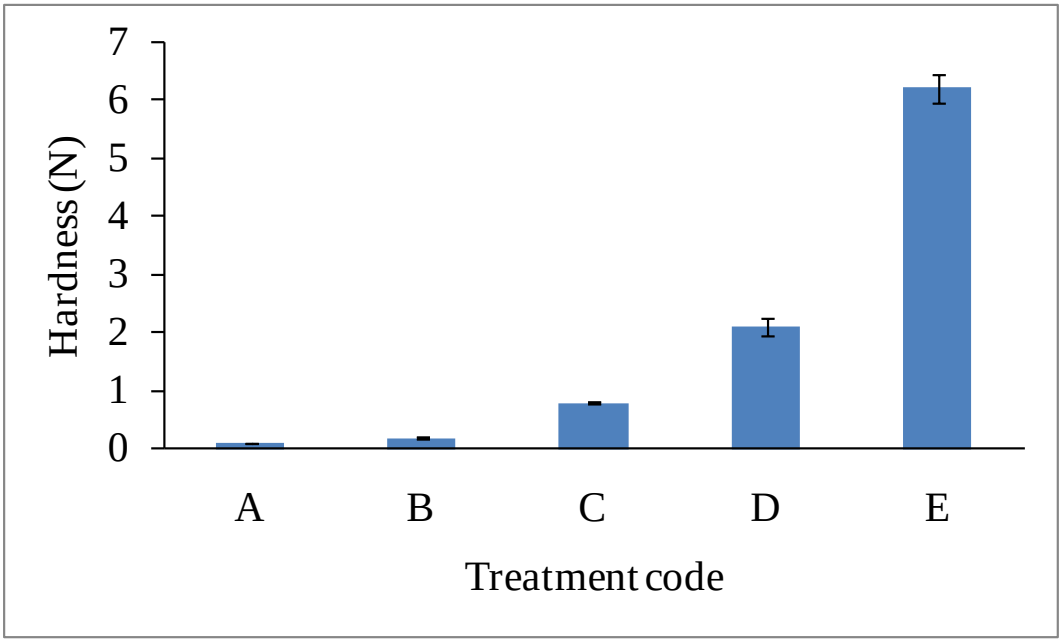

Figure 41. Hardness of the more concentrated gelatine gels determined with the stainless cylindrical probe.

Incorporating concentrated gelatine colloidal solutions into *Perna*

Knowing that concentrated gelatine colloidal solutions could be formed, the aim now was to incorporate these into the *Perna* process.

Materials and Methods

A nominally 40% gelatine colloid was prepared by dissolving Davis gelatine in deionised water at 70°C (Table 36) using the technique described earlier.

Table 36. Formulation of a concentrated gelatine hydrocolloid.

Gelatine (g)	Water (mL)	NaCl (g)	Glucose (g)
100	250	5	5

One kilogram of cooked frozen mussels (Future Cuisine, Auckland) were thawed, and then minced twice as described previously. The mussel mince were then blended with salt, glucose and LAB culture as shown in Table 37.

Table 37. Formulation of *Perna* with concentrated gelatine colloids.

Treatment code	Mussel mince (g)	NaCl (g)	Glucose (g)	LAB culture (g)	Water (mL)	Gelatine colloid (mL)
A	400	8.0	8.0	0.04	40	80
B	400	8.0	8.0	0.04	40	120

After the temperature of gelatine colloid dropped to 40°C, it was mixed with the unfermented *Perna* at different concentration for a minute (Table 37). The resultant mixtures were immediately stuffed into Homebrand containers holding about 300 g. These were deaerated twice followed by final vacuum packaging. The treatments were incubated at 30 ± 2°C for 96 hours followed by refrigeration at around 4°C for a minimum of 24 hours (by then Day 5) before the routine analyses were conducted however colour examination was exempted.

Results and Discussion

The two treatments were removed from refrigeration and allowed to attain room temperature. The vacuum packaging of treatments was intact strongly suggesting that fermentation had been successful (Figure 42). Upon opening, the *Perna* appeared to be liquid, but that was only an illusion. The mixture had clearly gelled, and had a pleasant smell typical of *Perna*. As is perhaps obvious from Figure 43, the gelled *Perna* was yellower than those made with carrageenans. Suggesting a cause that either gelatine or the ratio of male to female mussel meat or a combination of the two was responsible for the bright yellow colour of *Perna*. This was well outside the scope of the thesis and was not explored further.

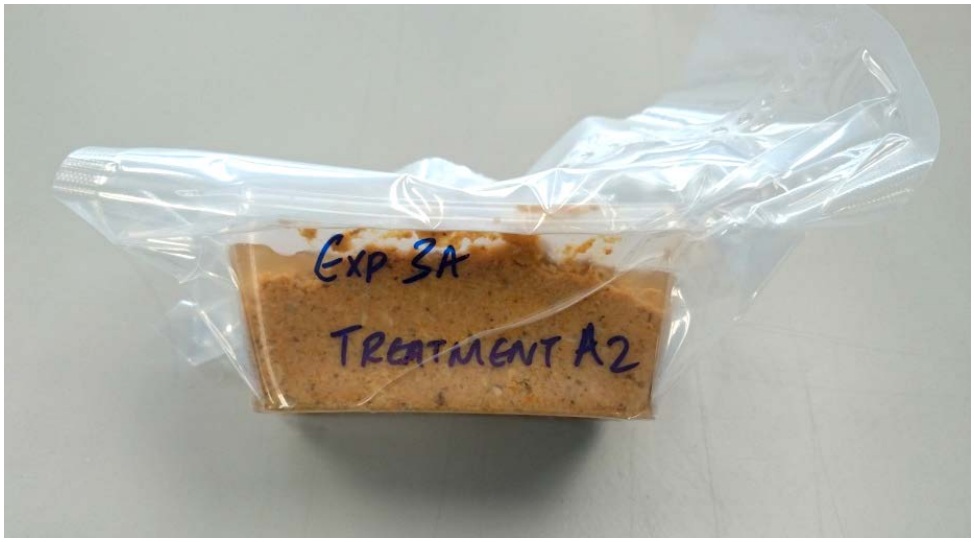

Figure 42. Intact vacuum packaging of *Perna* after fermentation.

Figure 43. *Perna* after the removal of vacuum packaging and the lid. It appeared to be a sloppy liquid but that was an illusion.

Treatments A and B were then analysed for texture using the blade and the cylindrical probe. Treatment B, with a higher concentration of gelatine, required the most force to penetrate with either object. As is evident from the histograms in Figures 44 and 45 that the force required to penetrate and slice the gelled product was much higher (values of above 3 N) than in Figures 35, 36, 37, and 38 where values obtained was less than 0.5 N.

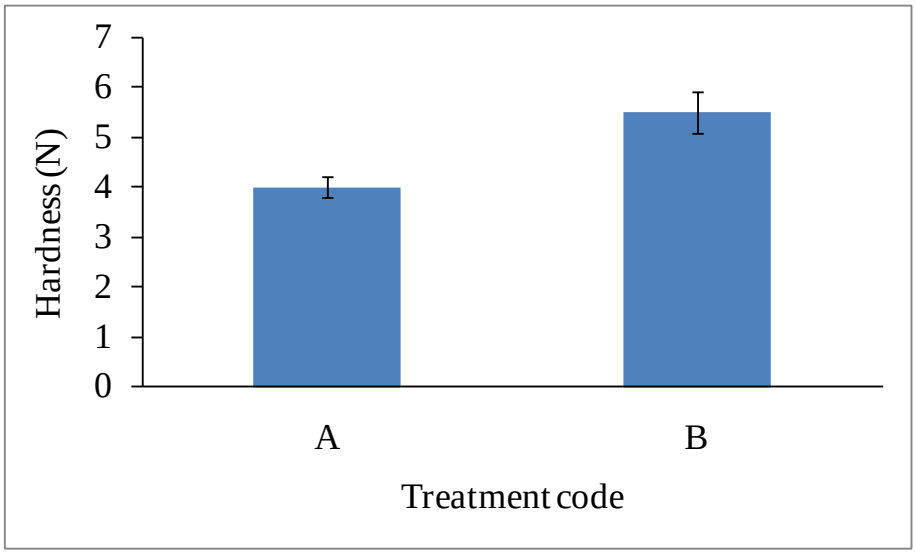

Figure 44. Hardness of gelled *Perna* using the cylindrical probe.

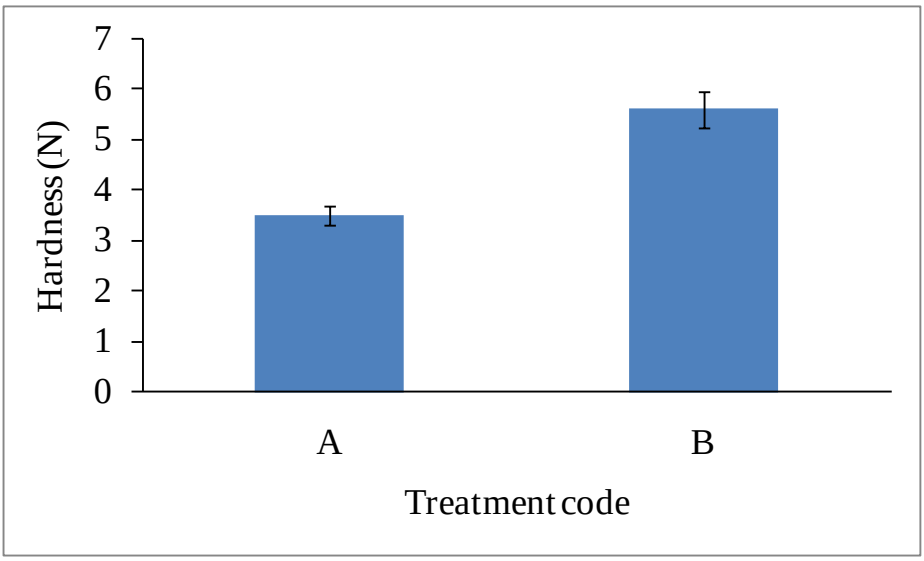

Figure 45. Slicing property of gelled *Perna* as determined with the steel blade.

The mussel gels were then sliced on a chopping board using a domestically[10] sharp knife shown in Figure 46. The cut was not clean, as say for cheeses, indicating that the mussel particles were the hardest component of the gelled *Perna*. Table 38 shows other properties of the gelled *Perna*. The pH of both treatments was a desirable 4.35.

Figure 46. Gelled *Perna* sliced on a chopping board using a domestically sharp steel knife.

[10] The knife was sharp but not the point of fine honing, and would be typical of a sharpness of a preparation knife used in a domestic kitchen.

Table 38. Properties of *Perna* with gelatine as a potential gelling agent. Each treatment comprised 300 g.

Treatment code	Gelatine colloid (mL)	*Perna* properties			
		Appearance	Smell	Syneresis	pH
A	80	Light brown	Pleasant	None	4.35
B	120	Light brown	Pleasant	None	4.35

Thus, gelatine concentrations in *Perna* of this order – nominally about 8% final at most in Treatment B – were capable of creating a potentially useful gel.

Dispersing solid gelatine in tap water

Materials and Methods

The previous experiment was encouraging enough to prompt an experiment where the need to prepare a concentrated colloidal solution was rejected in favour of adding solid gelatine to tap water and relying on the 30°C incubation over 4 days to disperse the gelatine. Two treatments were carried out in such a manner in which 5 and 10 g of gelatine was added to 50 mL of water at room temperature and incubated. The Treatments were examined on Day 2 and at the end of Day 4.

Results and Discussion

Surprisingly, the gelatine started dispersing in the water slowly initially but completely toward the end of 4 days of incubation for both treatments. This confirmed that 96 hours of incubation at 30°C was enough for gelatine to completely disperse in water. However the likelihood of complete dispersion of gelatine crystals into the mussel mince could only be tested by experiment.

Incorporating solid gelatine in *Perna*

Materials and Methods

One kilogram of cooked frozen mussels (Future Cuisine, Auckland) were thawed, and then minced twice as described previously. The mussel mince were then blended with salt, glucose, LAB culture and solid gelatine as shown in Table 39.

Table 39. Typical formulation of *Perna* with solid gelatine.

Treatment code	Mussel mince (g)	NaCl (g)	Glucose (g)	LAB culture (g)	Water (mL)	Solid gelatine (g)
A	400	8	8	0.04	55	22
B	400	8	8	0.04	70	28

The final concentrations of solid gelatine in Treatment A and B were 4.7% and 5.7% respectively. The resultant mixtures were immediately stuffed into Homebrand containers holding about 300 g. These were deaerated twice followed by final vacuum packaging. The treatments were incubated at 30 ± 2°C for 96 hours followed by refrigeration at around 4°C for a minimum of 24 hours (by then Day 5) before the routine analyses were conducted but exempting colour measurement.

Results and Discussion

The initial vacuum was no sustained. There was clear gas accumulation in all vacuum bags (Figure 47) together with mould development (Figure 48). All the treatments had an intensive pungent odour. The *Perna* was declared inedible.

Figure 47. Visible gas formation in *Perna* with solid gelatine indicating unsuccessful fermentation and production of odours that were obnoxious.

Figure 48. Discoloration and growth of mould in *Perna* with solid gelatine.

The outcome of this experiment was unexpected because gelatine is made from heated collagen. However, it appears likely that spore-forming bacteria had survived gelatine processing, and were reactivated by incubation at 30°C before the culture microbes could generate enough lactic acid to inhibit the growth of contaminating species. These observations suggest a future study in which a microbiologically certified gelatine is used, and as a second factor, the concentration of added culture is increased.

Chapter 6

Conclusions and Future Outlook

This thesis was a response to the limited product forms that have been developed for the New Zealand green shell mussel in export markets. The dominant form is individually quick frozen mussels on a half shell accounting to about 83% of the export trade. This work follows the pioneering work of AUT undergraduate student John Qiang (2012) and postgraduate student Gerald Dsa (2013), who developed a lactic-fermented spreadable product called *Perna*. Mission of this project was to create a gelled version of what they made. The idea was and remains to create a product that could be sliced and presented as a component of an antipasto/entrée. As such it would be suited to domestic and food service applications. I was partly successful in creating such a product but was slowed by having to explore a mussel preparation issue that was deemed too important to ignore.

The boiling time experiments in Chapter 3 suggested, but never proved, that the buffering capacity of mussel proteins increased with boiling time to 10 minutes. It was found that if mussels were cooked between 7 and 10 min, a desirable pH of about 4.5 could be achieved for *Perna* using the particular culture chosen, BFL-F02. pH of 4.5 is desirable because it is acidic enough that *Perna* can be classed as an acidic food for which hygiene requirements are much less stringent than for higher pH foods. *Perna* preparations with pH values below 4, although almost certainly hygienic, had intense acidic smells that were unpleasant. These results reinforce the view that pH must be well controlled to achieve a desirable sensory quality in the final product. Different starter cultures vary in their ability to generate lactic acid, so if BFL-F02 were replaced with other cultures to achieve different flavour profiles, preparation conditions would have to be explored. In respect of final pH, one strategy that could be employed to limit pH decline might be to reduce the concentration of added glucose, the precursor of lactic acid. The 2% concentration chosen was arbitrary. In this respect, Chr. Hansen, a major supplier of fermentation cultures reports that 0.3% to 0.8% glucose is adequate in fermented meat products but states that up to 2% is also used.

Turning now to the aim of creating a sliceable fermented *Perna*, the results with carrageenans were disappointing, with κ-carrageenan resulting in extreme syneresis at times and ι-carrageenan forming more of a flaccid product. The mussel particles were much harder than the carrageenan gels that were prepared, so any attempt at slicing would result in a crushing action. Carrageenans was chosen not only because gels can be prepared with it, but because it is a product of the sea. From a marketing perspective the choice and source of ingredients is important. As

many as possible should be derived from the sea, the products of which have a perceived healthy image in contemporary New Zealand society ("Omnibus Recreational Fishing Survey," 2007).

Another gelling hydrocolloid derived from the sea is alginate, which when exposed to calcium ions forms gels of varying strengths that depend largely on the proportion of the sugar acid guluronate (G) to mannuronate (M). The more G in adjacent residues, the stronger the gel to the point that some alginates are sufficiently strong to serve as dental impressions. Another valuable property of alginates is that they are soluble in cold water and do not need a heating and cooling cycle to form gels, which is the case with most other hydrocolloids ("Alginates", n.d.). Time did not allow the study of alginates in *Perna*. Alginate gelation in foods can present challenges because gelation can start the moment calcium ions are added. For *Perna* it would be useful to delay gelation to a time towards the end of fermentation. Certainly it would not be useful to have gelation occurring as the ingredients were being mixed. How might gelation be delayed? There are many strategies employed in alginate gelation, including using a solid calcium salt that is slow to dissolve, and the use of a sequestrant that temporarily competes with alginate for calcium ions (Dsa, 2013). Another strategy that may be useful in *Perna* would be to use a calcium salt that is minimally soluble at the pH of unfermented *Perna*, but fully soluble at pH 4.5. Calcium carbonate and di calcium hydrogen phosphate are two such salts (Imeson, 2009).

Much more success was achieved with gelatine. Initial failures were due to low concentrations being inadvertently being used, but once that mistake was realised a gelled *Perna* was successfully made. It was light brown with a soft crumbly texture when sliced. This confirms that the mussel tissue (particles) were the harder component and gelatine the softer. The solution to this problem – if it is indeed a problem – would be to comminute the mussel tissue more finely so generating a more homogeneous *Perna*. The current degree of comminution is through a 3-mm mincing plate which produces recognisable mussel particles with a coarse mouthfeel and this would be lost on further comminution, perhaps to the detriment of the product. (Fine comminution also has implications on fat oxidation as discussed below.)

This work stopped at the point of adding the concentrated gelatine colloidal suspension. After this it occurred to the supervisor that it may be possible to add solid gelatine and water to the *Perna* mix equivalent to adding a concentrated gelatine colloidal suspension. A necessary requirement would be that gelatine would have to disperse in water at 30°C within four days. This was easy to test and showed that it dispersed within four days. A fermentation trial was then conducted using solid gelatine and water. Whereas the mixture created a gel, it was found that the gelatine was sufficiently contaminated with spoilage organisms to result in a putrid mess. This result reinforces the importance of heat treating ingredients other than culture, salt, glucose and carrageenan, which each have very low microbial contamination profiles. (Other fermentation trials

with other ‘recipe’ ingredients have confirmed the importance of heat treating all suspect ingredients like dried capsicum for example.) How dry gelatine could be heat-treated is unknown at this time.

The choice of gelatine is also important. Porcine gelatine would be the least acceptable to many consumers on the basis of culture/religion. Bovine gelatine was chosen because it was freely available locally and fish gelatine was unavailable in New Zealand, but is available in overseas markets. Importantly however, the chosen fish gelatine must have a high Bloom value, from a test named after its inventor, which rates gelatines on the basis of their hardness at a defined concentration and temperature. Scientifically speaking, bloom strength is the weight in grams that is required for a specified plunger to depress the surface of a standard, thermostated gel to a defined depth under standard conditions (Karim & Bhat, 2009).

Fat oxidation was mentioned above, and its importance is discussed here. Mussels are rich in highly unsaturated fatty acids that while nutritionally useful are also prone to oxidation particularly after cooking. The use of cooked-then-frozen mussels – as used partly in the current project – was convenient, but unless the mussels were packed under a nominally inert atmosphere, some fat oxidation can be guaranteed. Even if mussels are cooked immediately before mincing, exposure to air during mincing will always accelerate fat oxidation. And the more finely it is minced the greater the exposure to air. Fat oxidation can be minimised by addition of antioxidants but the choice of antioxidant would be important. Just as for the choice of gelatine where fish gelatine might be better accepted, the antioxidant should be perceived as ‘natural’. Another method of minimising fat oxidation would be to perform comminution in a vacuum-capable bowl chopper. Such equipment is widely used in the German Wurst (sausage) industry. Not only does it eliminate air to minimise oxidation but would also aid evacuation of *Perna* mixtures before fermentation.

Findings from this research suggests that

- Bovine gelatine can be successfully used to make gelled form of *Perna* and is likely to have commercial potential. The product appears to have no direct equivalents in any market, and for this and other reasons, the technology developed to date may be protectable by patent.

- Iota and kappa carrageenans cannot be used in making a *Perna* gel with former producing a soft product and latter responsible for extreme syneresis with little to no gel.

- A pH of 4.5 is deemed suitable for *Perna* to attain desirable organoleptic properties as mentioned earlier. This can be achieved by cooking live mussels in boiling water for a minimum of 7 minutes but no more than 10 minutes. In addition, using the right lactic acid

starter culture and reducing the amount of added glucose will prevent the pH from plunging too low or too high.

Finally, during the course of this research, number of rather interesting phenomena's were encountered which remained unexplored mainly due to time constraints. Here are some of them and also proposals for further research:

1. Low glucose concentration to eliminate drastic decline in pH which is very important for sensory and textural quality of fermented *Perna* requires further work.

2. Combination of two or more lactic acid starter cultures should be explored to study the various flavour and textural profiles that can be imparted to *Perna*.

3. Hydrocolloids such as fish gelatine and alginates require a definite research for *Perna* to be able to go to the wider market. Optimistically speaking, if fish gelatine and/or alginates were successful in producing gelled *Perna*, the product can go for a Halal certification which could expand the potential customer base.

4. If concentrated gelatine colloidal solution were to be replaced by solid gelatine then that will require the use of a more hygienic gelatine preparation to minimise its microbial load. All of this is in the land of the unknown, and requiring further experientation.

5. To better understand the process of fat oxidation during *Perna* production warrants more research.

6. The colour of the final product would most likely depend on the ratio of male to female mussel meat, former being cream fleshed and latter apricot orange and would require further study to better understand this effect if the final product requires to be of a consistent colour.

7. The *Perna* produced in this work was with base ingredients such as glucose, salt, starter culture and water. Further work would be required to explore the addition of natural flavours/ingredients and antioxidants to the product which would have a positive effect on the flavour, minimise fat oxidation, and make the product more consumer friendly.

8. Finally, sensory trials and focus groups needs to be done to understand consumer perception and how gelled *Perna* might be used when sold as a commercial product.

With the past progress and present developments, the future looks bright for fermented mussel products and its bivalve counterparts.

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Appendix

Appendix 1. Colour data related to Table 11 in Chapter 3

The boiling time had minimal effect on the colour of fermented *Perna*. The only significant difference was the increase in the redness/greenness of *Perna* with an increase in boiling time.

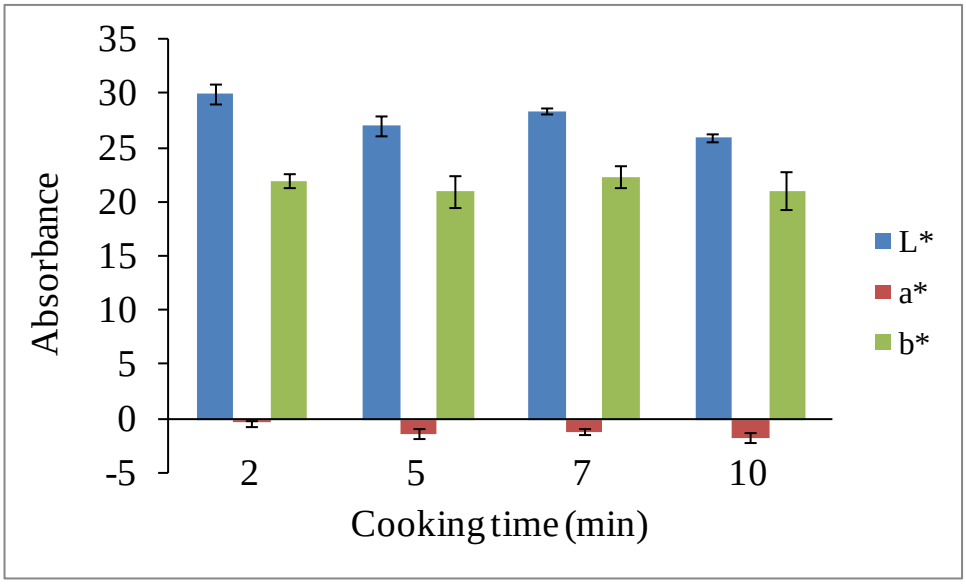

Figure 49. Colour of *Perna* when cooked for different time.

Appendix 2. Colour data related to Table 13 in Chapter 3

The colour data obtained was very similar in pattern to the histogram in Appendix 1. Nothing spectacular can be mentioned about this and was done as part of routine analysis.

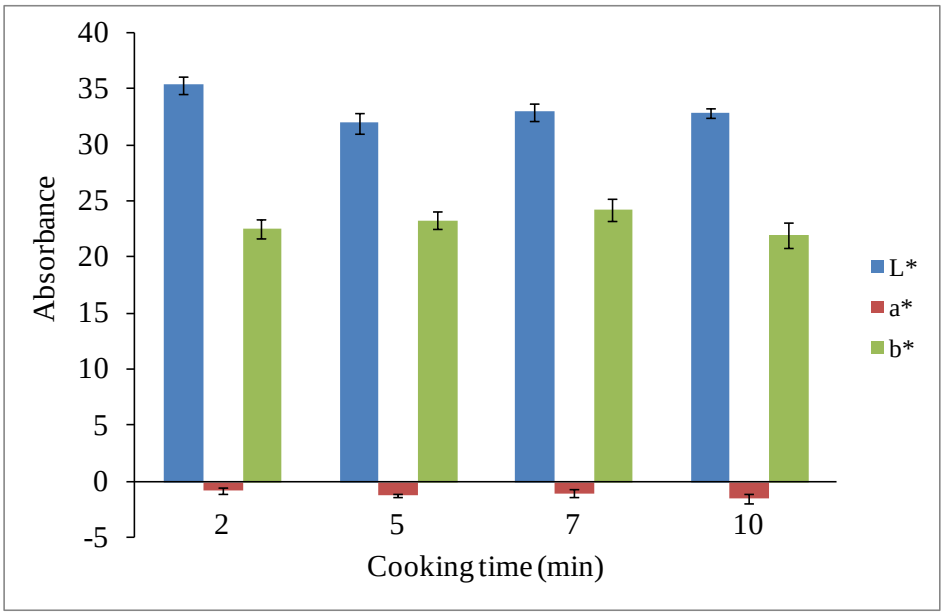

Figure 50. Colour of *Perna* when cooked for different time (Replicate 1).

Appendix 3. Colour data related to Table 14 in Chapter 3.

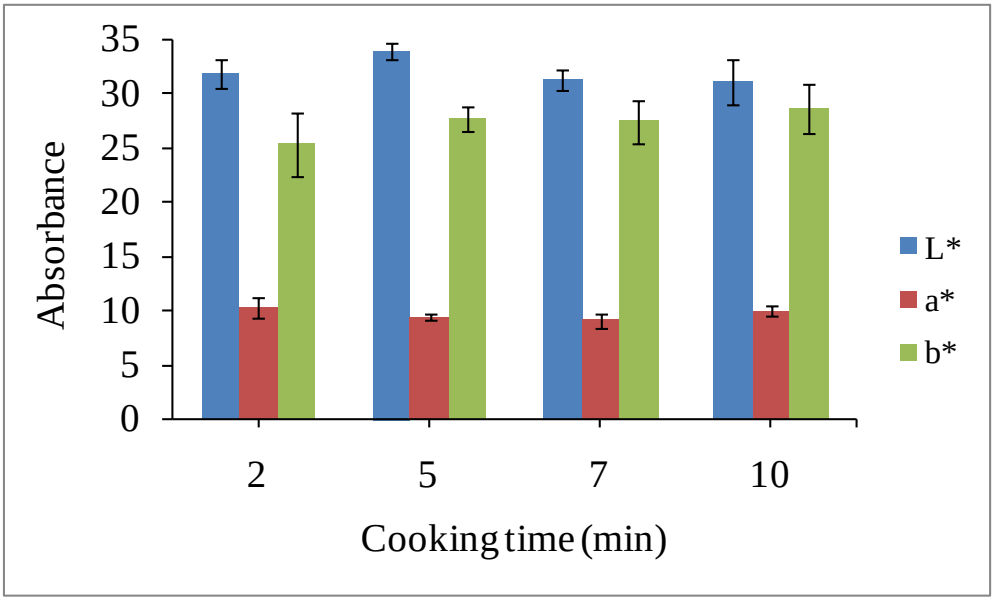

Figure 51. Colour of *Perna* when cooked for different time (Replicate 2).

Appendix 4. Colour data related to Table 16 in Chapter 3.

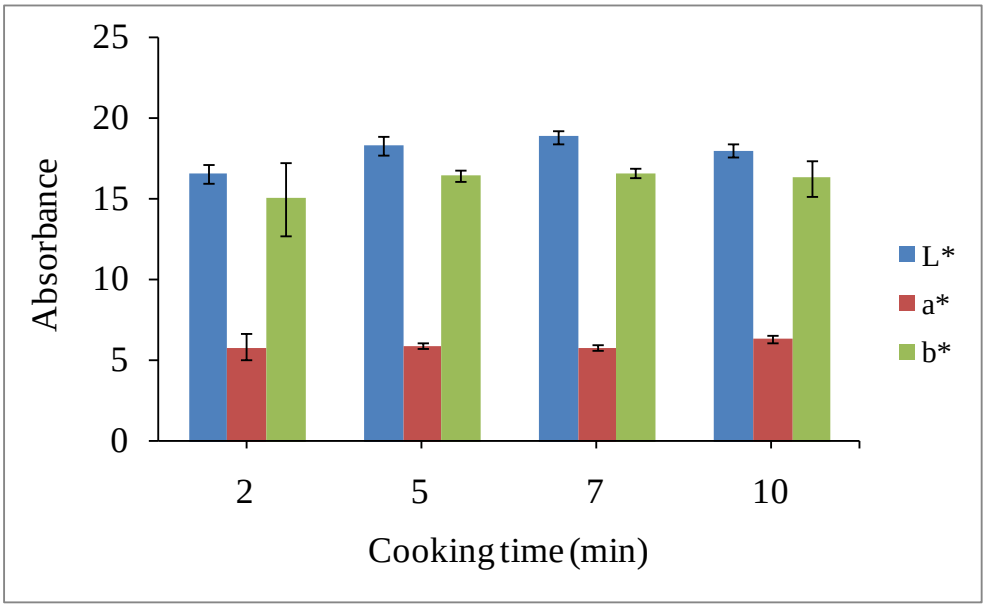

Figure 52. Colour of *Perna* when cooked for different time (Replicate 3).

Appendix 5. Colour data related to Table 17 in Chapter 3.

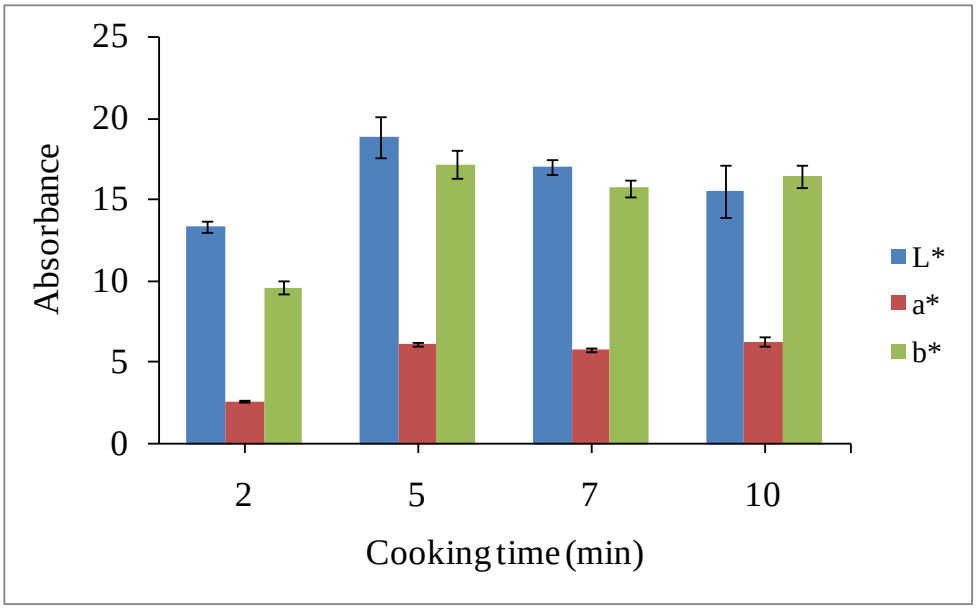

Figure 53. Colour of *Perna* when cooked for different time (Replicate 4).

Appendix 6. Colour data related to Table 21 in Chapter 4

Generally, higher concentrations of κ-carrageenan reflected more light, and were greener and yellower. Colour differences between salt treatments were minimal.

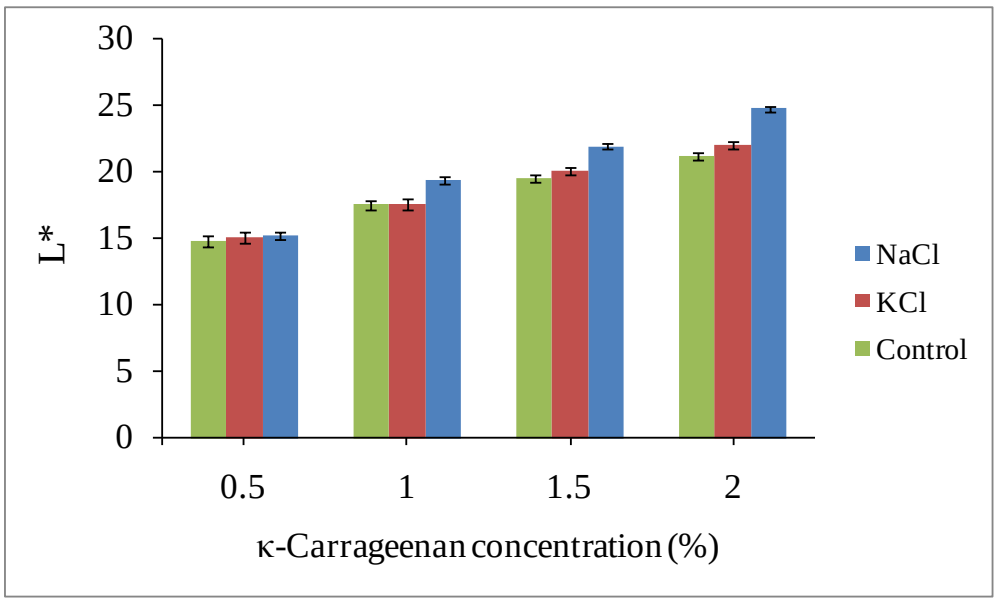

Figure 54. Lightness of κ-carrageenan gels at pH 6.5.

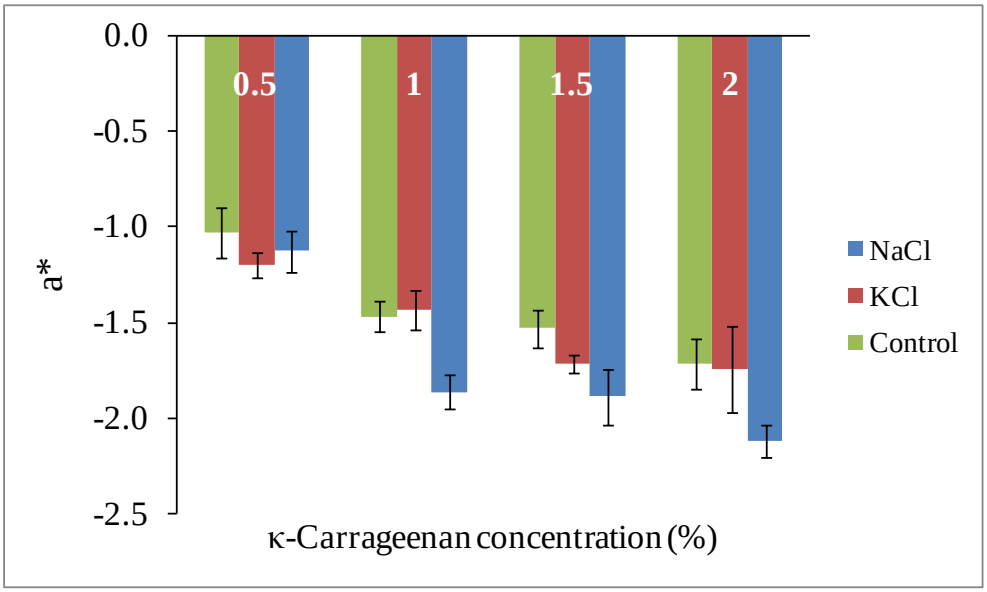

Figure 55. Redness/greenness of κ-carrageenan gels at pH 6.5.

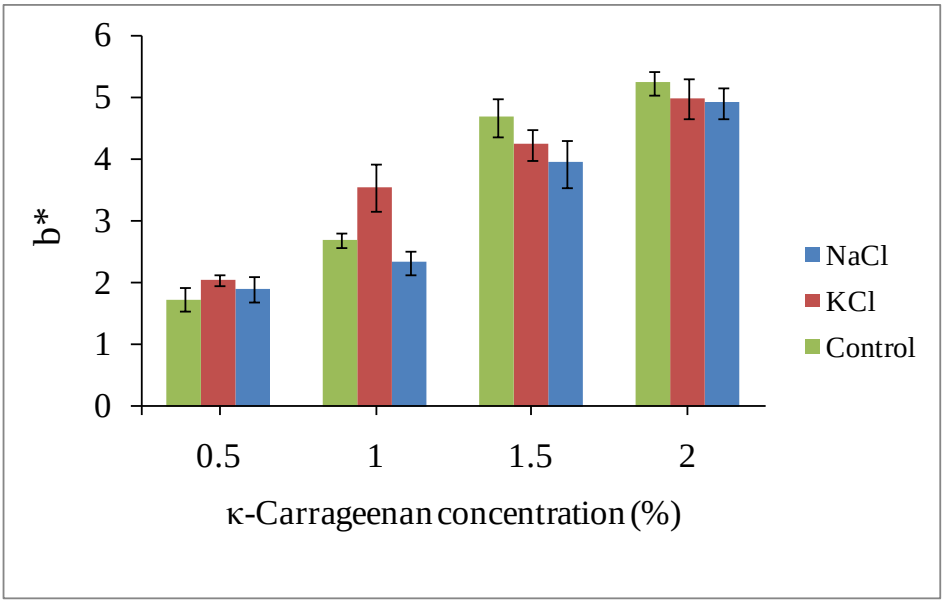

Figure 56. Yellowness/blueness of κ-carrageenan gels at pH 6.5.

Appendix 7. Colour data related to Table 22 in Chapter 4

Generally, higher concentrations of κ-carrageenan reflected more light, and were greener and yellower which was very similar to the gels obtained at pH 6.5 in Appendix 6.

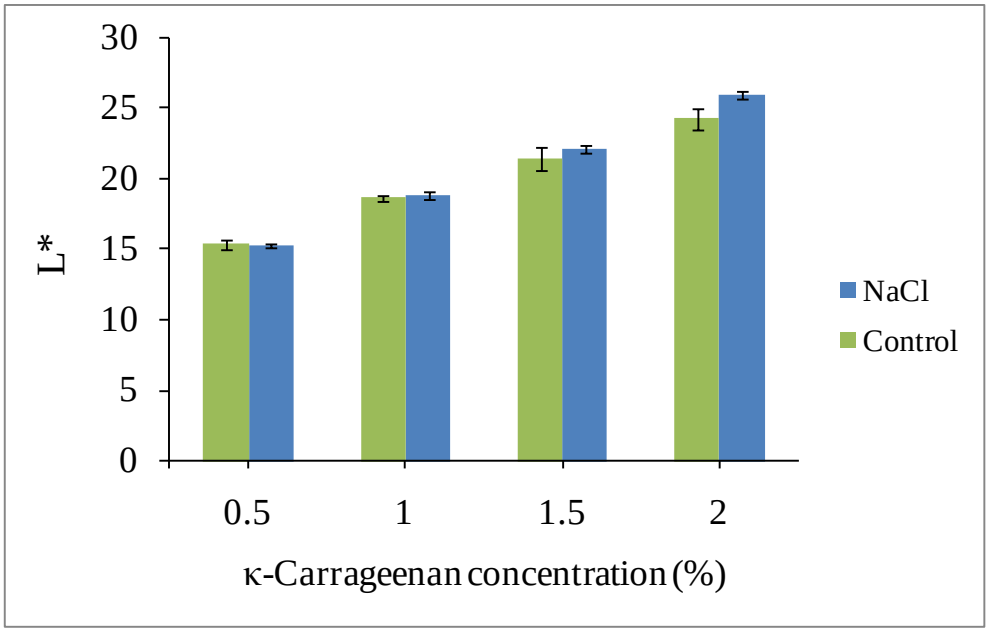

Figure 57. Lightness of κ-carrageenan gels at pH 4.5.

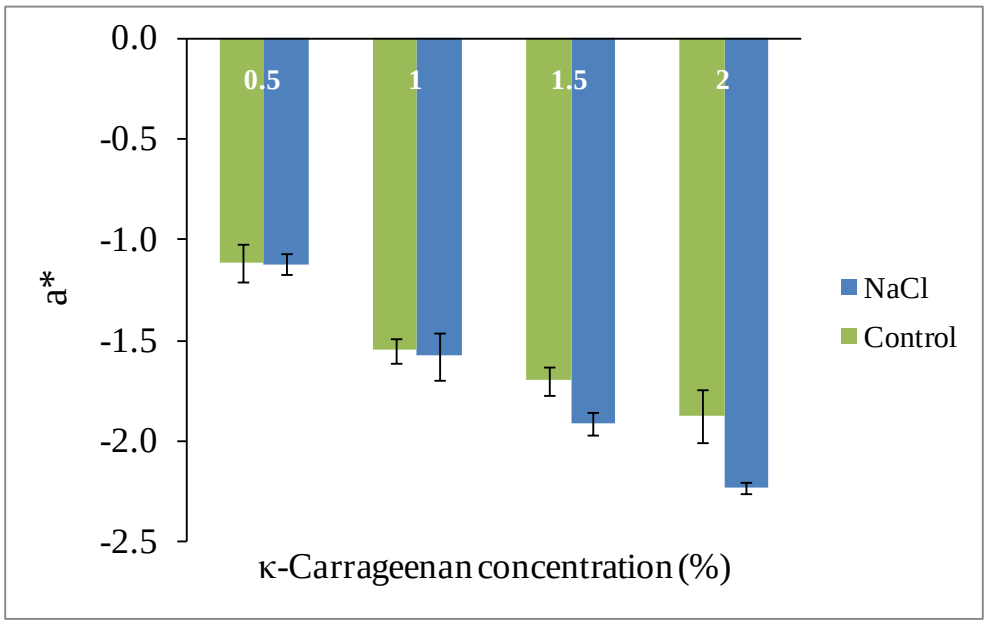

Figure 58. Redness/greenness of κ-carrageenan gels at pH 4.5.

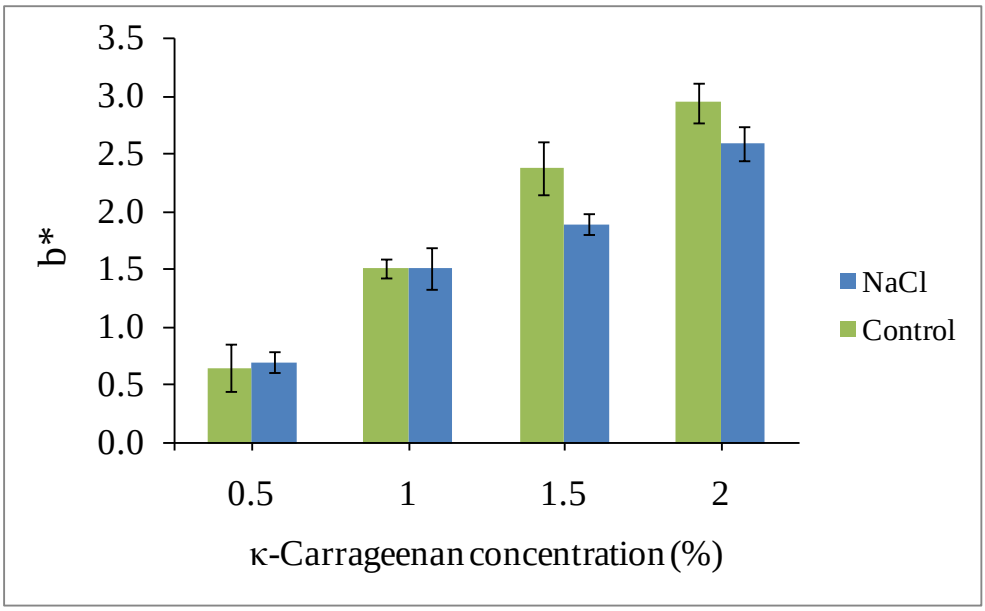

Figure 59. Yellowness/blueness of κ-carrageenan gels at pH 4.5.

Appendix 8. Colour data related to Table 30 in Chapter 5.

The colour data obtained was unremarkable between treatments.

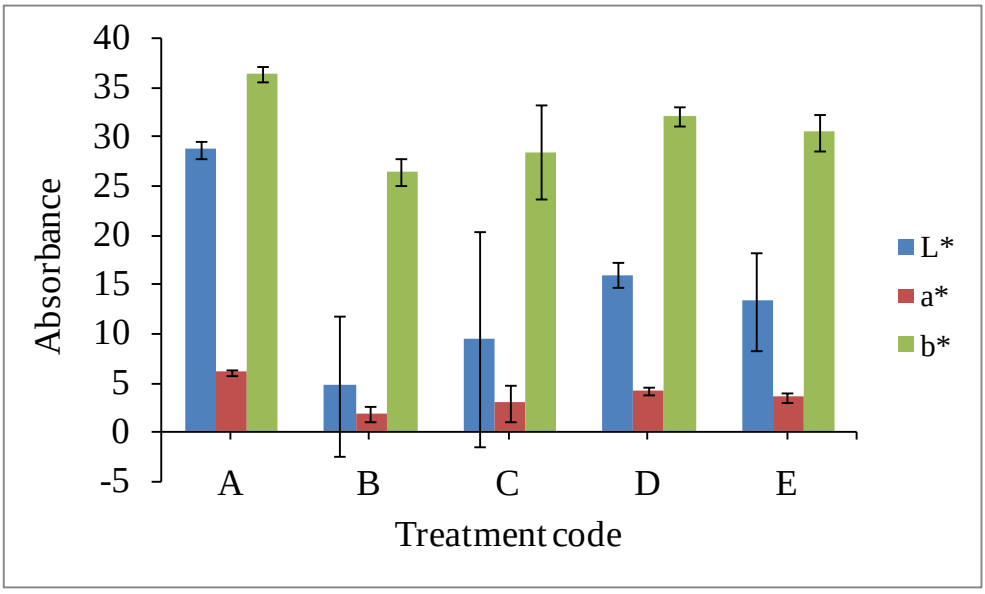

Figure 60. Colour of *Perna* made with gelatine (Experiment 1).

Appendix 9. Colour data related to Table 31 in Chapter 5.

The colour data obtained from this replicated experiment was not any different from the first experiment.

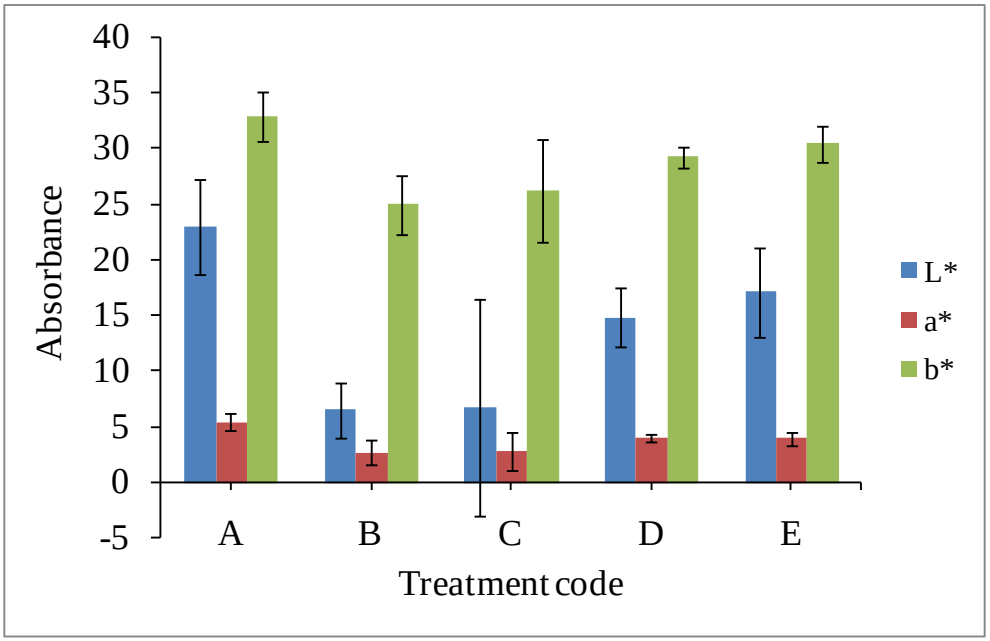

Figure 61. Colour of *Perna* made with gelatine (Experiment 2).