Department of Agriculture and Environment
School of Science

Effects of pre and post freezing treatments on barramundi (*Lates calcarifer*, Bloch) fillet quality

Sona Younus Zakhariya

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DECLARATION

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgment has been made.

This thesis contains no material, which has been accepted for the award of any other degree or diploma in any university.

Sona Younus Zakhariya **December 2014**

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PREAMBLE

The proposed research aims to evaluate the shelf life and quality of barramundi *(Lates calcarifer,* Bloch*)* fillets after being subjected to various pre and post freezing treatments employed by industry. The impacts of pre and post treatments were assessed, by using tools from biochemistry and microbiology. The fish fillets after exposure to various pre-treatment procedures were frozen in a blast freezer prior to cold storage.

This thesis comprises of 9 chapters, followed by references and appendices:

Chapter 1 is an introduction, which briefly highlights the current issues of the barramundi industry and an overview about the various freezing techniques, most common pre and post freezing treatments, assessment techniques to evaluate the shelf life and quality of perishable products including seafood. This chapter also substantiates and underlines the justification and the significance, aim and objectives of the current research.

Chapter 2 reviews the existing literature on the significance of Australian barramundi industry, issues related to transport, the current marketability of barramundi. This chapter also explains about the frozen storage of fish and loss of quality, the effect of pre and post treatments such as, freeze-thaw cycles, thawing techniques, glazing, packaging, and use of phosphates on the quality and shelf life of different species. Significance of microbiological and physiochemical analyses were also reviewed and discussed.

Chapter 3 details the general methodology of the current research. The common materials and methods, which are used in all experiments are described here and specific materials and methods constitute the part of relevant chapters. The common microbiological and physiochemical procedures used in this research to analyse the quality and shelf life of barramundi fillets are also described in this chapter too.

Chapter 4 to *Chapter 8* form core experiments of the research. These experiments investigates the effects of different ice forms, different pre-freezing temperatures, such as time-temperature abuse (TTA), effects of refreezing cycles, different concentration of sodium tripolyphosphate, refreezing with sodium trio polyphosphate on the shelf life of barramundi fillets. These chapters are written in such a way that they can be published in separate peer-reviewed journals. Please see in appendix A for the details of the publications. The readers

may find a bit of duplication in the introduction section in these chapters as these chapters were written in a format to match the format of the various journals. However, in order to avoid the repetition in 'Materials and Methods' in these chapters and to reduce the length of the thesis, common methodology sections were deleted from these core chapters which formed the basis of Chapter 3 (General Methodology).

Chapter 4 reports the effect of time-temperature abuse on microbiological and physiochemical properties of barramundi fillets and is accepted for publication in Journal of food processing and preservation.

Chapter 5 summarizes the effect of two forms of ice on biological and physiochemical properties of barramundi fillets and was submitted for publication in Journal of Food Processing and Preservation.

Chapter 6 researches the effect of repeated freeze thawing on barramundi fillets in order to prevent freezing-thaw related deterioration in shelf life of barramundi fillets and was published in the Journal of Food Processing and Preservation.

Chapter 7 investigates treatment with STPP on the quality and shelf life of barramundi fillets when exposed to up to three freeze-thaw cycles and is under-review for publication in Plos One Journal.

Chapter 8 explains the effect of glazing, packaging, and use of sodium tripolyphosphate (STPP) on the properties of barramundi fillets and is currently under preparation for publication.

Chapter 9 highlights and discusses briefly the entire research in order to achieve a more thorough understanding of the underlying factors, which influence the quality of barramundi fillets. This chapter synthesises the main results to establish a protocol for all pre and post freezing treatments of barramundi fillets. This chapter ends with the conclusions and the recommendations for future research.

ABSTRACT

The effects of pre-freezing treatments such as time-temperature abuse (TTA), use of ice forms, freeze–thaw cycles, use of polyphosphate and post freezing treatments such as glazing and packaging were assessed on the microbiological and physiochemical properties of barramundi (*Lates calcarifer*) fillets. The effects of different pre-blast freezing (PBF) temperature periods and exposure time (time-temperature index) investigations indicated that there was minimal change to the microbiological and physiochemical properties of fillets stored at -20°C from 0h to 16 days. TVC, TVBN, pH, protein, colour and rheological parameters of fillets that underwent PBF temperature at 0°C and 5°C for 16 days changed significantly compared to those treated at -20°C. The shelf life of PBF barramundi fillets at 0°C and 5°C was 8 days. During transportation and subsequent storage, fish are likely to be exposed to inconsistent storage conditions (temperature abuse) for a certain period and such temperature abuse may accelerate quality and shelf life changes in fillets. In another pre-freezing (flake ice and slurry ice) study, results demonstrate that the post-freezing quality of fillets was better preserved following up to 16-days chilling in slurry ice than in flake ice. Therefore longer shelf life can be obtained by chilling barramundi fillets in slurry ice. The repeated freezing and thawing (3 cycles) had a detrimental effect on the physical, chemical and microbiological quality of barramundi fillets. The quality of freeze- thawed fillets lessened with number of freezing cycles. Therefore, it is important to prevent temperature variations or abuse during freezing and transport to avoid the negative effect of freezing and thawing.

The effects of 0.05% sodium tripolyphosphate (STPP) subjected to 3 cycles of freeze-thaw were also investigated. STPP treatment maintained the quality of barramundi and reduced protein denaturation and TVBN compared to non-treated fillets showing STPP can maintain the quality and shelf life of fillets exposed to three freeze-thaw cycles. Five percent STPP treatment and packaging resulted in the lowest increase in TVC and TVBN. STPP treatments were also effective for maintaining rheological parameters such as hardness, cohesiveness and stiffness of barramundi fillets.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
a*	Redness
BF	Before Freezing
b*	Yellowness
CARL	Curtin Aquatic Research Laboratory
CFU g^{-1}	Colony forming unit per gram
Cm	Centimetre
C	Celsius
D	Day/s
d.b.	Dry basis
g/100 mL	Gram per 100 milli litre
H	Hour/s
Kg	Kilogram
Kgf	kilogram force
kgf.mm	kilogram force millimetre
kgf/mm	kilogram force per millimetre
L*	Lightness
L	Litre
LSD	Least Significant Difference
mg/L	Milligram per litre
mL	Millilitre
mg 100 g^{-1}	Milligram per 100 gram

N	Newtons
PBF	Pre-blast freezing
WHC	Water holding capacity
w.b.	Wet basis
STPP	Sodium tripolyphosphate
S	Second
S.E.	Standard error
TVBN	Total volatile base nitrogen
TVC	Total viable count

THE LIST OF PUBLISHED ARTICLES FROM THIS THESIS

1. Zakhariya, S. Y., Fotedar, R. & Prangnell, D. (2014). Effects of Refreezing on Microbiological and Physiochemical Properties of Barramundi (*Lates calcarifer*, Bloch) Fillets. *Journal of Food Processing and Preservation*, **38**, 2183-2191.

2. Zakhariya, S. Y., Fotedar, R. & Prangnell, D. (2015). Effects Of Time-Temperature Abuse On Microbiological And Physiochemical Properties Of Barramundi *(Lates calcarifer,* Bloch*)* Fillets. *Journal of Food Processing and Preservation*, (In Press)

CHAPTER 1

INTRODUCTION

CHAPTER 1: INTRODUCTION

1.1 BACKGROUND

The barramundi (*Lates calcarifer*, Bloch) is a large predatory fish found in tropical regions of Australia and throughout the Indo-West Pacific (Love & Langenkamp, 2003). Barramundi can be found in diverse natural habitats such as open sea, brackish, estuarine, and freshwater environments (Russell & Garrett, 1985). The gross value of Western Australian fisheries production in 2011–12, was $385 million; the total value of fisheries production for Western Australia included $276 million of wild-catch production, 72 per cent of the state's total fisheries production value, and $109 million of aquaculture production (the remaining 28 per cent) (Skirtun *et al.*, 2013).

Barramundi has a firm, succulent white flesh, fine-grained texture and mild flavour (Phillips, 2010). The fish is low in fat and cholesterol, high in protein, and contains the Omega 3 and Omega 6 fatty acids (Phillips, 2010). Consumers consider Australian Barramundi to be a premium; great tasting, appealing, authentic and classy fish (Lawley, 2010). Barramundi have gained a reputation as one of Australia's finest eating fish and as a result are the most important freshwater-estuarine commercial fish in Australia (Department of fisheries, 2011). In Australia, its natural distribution extends from the Ashburton River in Western Australia, throughout the Northern Territory, to the Maryborough River in Queensland (DPI, 2014). Barramundi are grown in a variety of culture systems across Australia (Queensland Government, 2014). As per Skirtun *et al.* (2013), the total value of fisheries production for Western Australia included of wild-catch production, (72 per cent) and aquaculture production (the 28 per cent). According to the Australian farmed barramundi industry, barramundi is farmed in all states of Australia except Tasmania (Australian Barramundi Farmers Association, 2014). It has an estimated value of production at around $45 million at farm gate and produces 5,000 tonnes of product (Australian Barramundi Farmers Association, 2014). Western Australia's barramundi production is small compared with other states (Department of fisheries, 2011). However, one enterprise at Cone Bay in the Kimberley, Western Australia has received approval to increase their production of barramundi to 1,000 tonnes per annum (Department of fisheries, 2011).

Marine Farms recognises the need for extensive research into many aspects of aquaculture due to the fledgling status of the industry in Australia. There is a market perception that farmed

seafood is inferior in quality to wild-caught stock, reducing the value and market-share of farmed seafood. This is particularly the case with barramundi. The expansion of barramundi markets is also presently limited by potential quality loss during the freezing process. Fresh product is generally of superior quality to frozen product. If the barramundi freezing process can be refined, then drip loss could be reduced and shelf life significantly extended (David Prangnell, personal communication, 25th February 2010).

Marine species deteriorate rapidly after death due to the effect of a wide variety of biochemical and microbial degradation mechanisms (Rodríguez *et al*., 2006). For perishable products with short shelf life, time-temperature history is of great importance during transportation, storage and distribution throughout the supply chain (Browne & Allen, 1998; Dubellar *et al.*, 2001; Simpson *et al.*, 2012). Temperature abuse during any phase of the distribution chain generally affects quality. However, the loss of quality depends directly on the nature of fish species and on the handling and storage conditions (Whittle *et al.*, 1990; Olafsdottir *et al.*, 1997). It is difficult to control and monitor the temperature of perishable food products throughout the distribution and storage in a cost-effective way (Simpson *et al.*, 2012). Fish shelf life is influenced by a number of factors, such as feeding, initial microbiological quality, season, geographical origin and handling (Koutsoumanis, 2001).

Among the different onboard handling systems that efficiently cool fish, the most common one employed is chilling (Whittle *et al.*, 1990; Olafsdottir *et al.*, 1997). The prolonged chilled storage has proved to have a negative effect if further processing is to be carried out on the fish material (Slabyj & True, 1978; Aubourg & Medina, 1997). Icing is one of the important pre-processing procedures in the processing of fish. The preservation of fresh aquatic food products has traditionally involved the use of flake ice (Mendes *et al*., 2001). Slurry ice, also known as fluid ice, slush ice or liquid ice, has been reported to be a promising technique for the preservation of aquatic food products in an ice-water suspension at subzero temperature (Chapman, 1990; Harada, 1991). Many studies have described the advantages of the application of slurry ice systems to farmed sea bream (Huidobro *et al*., 2001), turbot (Rodríguez *et al*., 2006) and to wild albacore (Price *et al*., 1991), and hake (Losada *et al*., 2004). Even though autolytic process onset the degradation process in fish as a result of endogenous enzymatic changes, the spoilage of fish held in ice is predominantly a bacterial growth as they are considered to play a dominant role in fish spoilage (Alfred, 1998). Gram & Huss (1996) reported that there are several important specific intrinsic factors in fish which

greatly influence the microbiology and spoilage such as the poikilothermic nature of the fish and their aquatic environment; a high post-mortem pH in the flesh (usually >6.0); the presence of large amounts of non-protein-nitrogen (NPN); the presence of trimethylamine oxide (TMAO) as part of the NPN fraction.

The extension of the shelf-life by means of refrigeration is due to reductions in the growth rate and metabolic activity of the microorganisms that cause the deterioration (Cakli *et al.*, 2007). Even though frozen storage is the most utilized preservation method in seafood industry, freezing and frozen storage of fish cause undesired textural changes in the fillet (Beyrer & Klaas, 2007). During the freezing process and frozen storage, fish muscle can undergo a number of changes, such as denaturation and aggregation of the myofibrillar proteins (Barroso *et al.*, 1998) that are shown to be a reason for textural changes of frozen stored fish (Beyrer & Klaas, 2007). This results in alteration of the functional properties of muscle proteins, loss of water-holding capacity and juiciness and unwanted changes in texture, which produces a hard, dry and fibrous product (Barroso *et al.*, 1998).

The quality and shelf life of fresh refrigerated seafood has been the focus of many studies world-wide. Although temperature reduction decreases the rate of enzymatic activity, there is constantly need for better chilling and freezing procedures. Temperature seems to be the most important factor that influences the spoilage as we all as the safety of meat (Koutsoumanis & Taoukis, 2005). The quality of fish depends primarily on processing conditions and handling. Temperature abuses can occur throughout the storage and transportation. Enzymatic and chemical reactions are usually responsible for the initial loss of freshness whereas microbial activity is responsible for the overt spoilage and thereby establishes product shelf life (Gram & Huss, 1996).

A wide variety of methods for the measurement of chemical and quality changes in frozen stored fish can be found in the literature, but due to differences in fish from different species and changing characteristics of fishing grounds (Love, 1964), and partly due to seasonal variations in the composition of fish muscle (MacCallum *et al.*, 1968; Castell & Bishop, 1973), a universally accepted method for measuring changes, in even one attribute, has so far proved elusive (Mills, 1975).

1.2 SIGNIFICANCE

The outcome of the proposed research would

- assist in improving the shelf life of barramundi fillet.
- assist in better utilisation of barramundi fillet.
- add value to barramundi fillet.
- assist in better understanding of the underlying factors, which influence the quality of barramundi fillet.
- assist in assessing the better understanding of freezing method.
- assist industry to optimise time temperature index that assures food quality and shelf-life.
- assist in determining the suitable post freezing treatments without the use of chemicals in order to prevent (or reduce) the drip loss.

This study investigates the microbiological and physiochemical changes that occur in the barramundi meat under pre and post freezing treatments to serve as a basis for further comparison of barramundi meat quality under commercial conditions. All these factors are closely related and aimed to increase the shelf life of the barramundi fillets.

1.3 AIM

To investigate the effects of pre and post freezing treatments on the quality and shelf life of barramundi fillets

1.3.1 Objectives

- To evaluate the texture of barramundi fillet using tools from microbiology, and biochemistry.
- To assess the quality and shelf life of fillet from freshly killed barramundi.
- To investigate the time-temperature index of barramundi fillet before freezing.
    - To investigate the effect of 7 pre-blast freezing chilling times on the quality and shelf life of barramundi fillet.
    - To investigate the effect of 3 different pre-blast freezing temperatures (5°C, 0°C, ─20°C).
- To investigate the effect of 2 types of ice on the quality and shelf life of barramundi fillet.

- To determine the effect of phosphate treatment glazing and packaging on quality and shelf life of barramundi fillet.
- To investigate the effect of refreezing on barramundi fillet quality and shelf life.

CHAPTER 2

LITERATURE REVIEW

CHAPTER 2: LITERATURE REVIEW

2.1 BACKGROUND

2.1.1 Barramundi

Barramundi *(Lates calcarifer)* is a catadromous teleost, which inhabits shallow waters in estuaries, rivers and bays from east Africa through the South-East Asian archipelago to Australia's east-coast (Chenoweth *et al.*, 1998a). Individuals spend much of their lives in freshwater regions of rivers but undergo annual spawning migration into bays and estuaries (Chenoweth *et al.*, 1998b). Barramundi are tropical euryhaline fish, which more commonly known as Asian seabass or giant seaperch in countries outside of Australia. Barramundi were originally classified as belonging to the family Centropomidae, subfamily Latinae, but have since been re-classified as belonging to the family Latidae (Nelson, 1994) (Figure 2.1). Barramundi can tolerate a wide range of salinity levels from fresh to seawater, and inhabit a wide range of habitats, from freshwater to brackish and marine systems (Queensland government, 2014). In addition, they are fast growing, with a growth rate of approximately 1 kg/year, can reach a marketable size (350 g–5 kg) in 6–24 months (depending on conditions), have a desirable white flesh, and command a relatively high market price (Boonyaratpalin, 1997; Yue *et al.*, 2009). Aquaculture production of barramundi began in Thailand in the 1970s (Yue *et al.*, 2009), and is also established other countries in South East Asia mainly in Indonesia, Malaysia, Philippines and Taiwan. They are now an important aquaculture species in much of Southeast Asia and Australia (Tucker *et al.*, 2002).

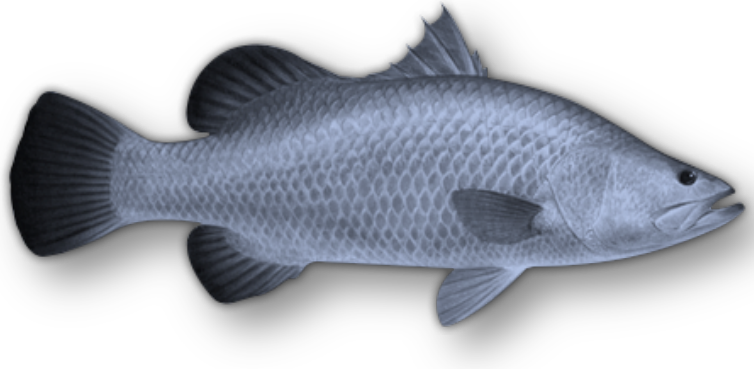

Figure 2.1: Australian barramundi (*Lates calcarifer*)

2.1.2 Australian Barramundi Industry

In 2011–12, the gross annual value of Australian aquaculture production increased by $100 million to $1.1 billion, and accounted for 46 per cent of the gross value of Australian fisheries production (Skirtun *et al.*, 2013). In the same period, the volume of Australian aquaculture production increased by 10 per cent to 84,605 tonnes, accounting for 36 per cent of total Australian fisheries production (Skirtun *et al.*, 2013). Barramundi aquaculture is presently one of the fastest growing aquaculture sectors in Australia and specifically in Western Australia (Glencross, 2007). In Australia, barramundi aquaculture began in the mid-1980s (Palmer *et al.*, 2007) and has since grown rapidly, increasing from 980 tons in 2000/2001 to 2,075 tons in 2005/2006 and 2,996 tons in 2007/2008 and the value of production increased from $9.2 million to about $34 million over that time period (ABARE, 2009). The market demand and the production rate of barramundi has increased prominently due to its high quality. In 2013, Skirtun *et al.* reported that the gross value of barramundi production in Western Australia increased significantly in 2011–12 as compared to 2010-11. The distribution of commercial catch of barramundi in Australian waters, 2010 (FAO, 2014) is presented in Figure 2.2. The product composition of the gross value of production of Australian fisheries has not changed substantially since 2006–07 (Skirtun *et al.*, 2013). The total commercial catch of Barramundi across Australia in 2010 was 1676 t, comprising 635 t in the Northern Territory, 254 t in the East Coast Inshore Fin Fish Fishery (Queensland), 730 t in the Gulf of Carpentaria Inshore Fin Fish Fishery (Queensland) and 57 t in the Kimberley Gillnet and Barramundi Fishery (Western Australia) (Fisheries Research and Development Corporation, 2012). In 2001-02 the farm gate price per fish (whole fish basis) is estimated to have averaged $7.95 a kilogram, giving the Queensland farmed barramundi industry an estimated gross value of production of $6.7 million in 2001-02 (Love & Langenkamp, 2003). The total value of fisheries production for Western Australia included of wild-catch production, (72 per cent) and aquaculture production (the 28 per cent) (Skirtun *et al.*, 2013). Barramundi aquaculture has established into a valuable business. Since its establishment in the mid-1980s, the Australian barramundi farming industry has been increasing production by around 20 per cent a year (Love &Langenkamp, 2003). Since consumers prefer larger fillets some growth in production has been delayed by some farmers moving to the production of large fish for fillets rather than smaller, plate size fish (Maguire, 2002).

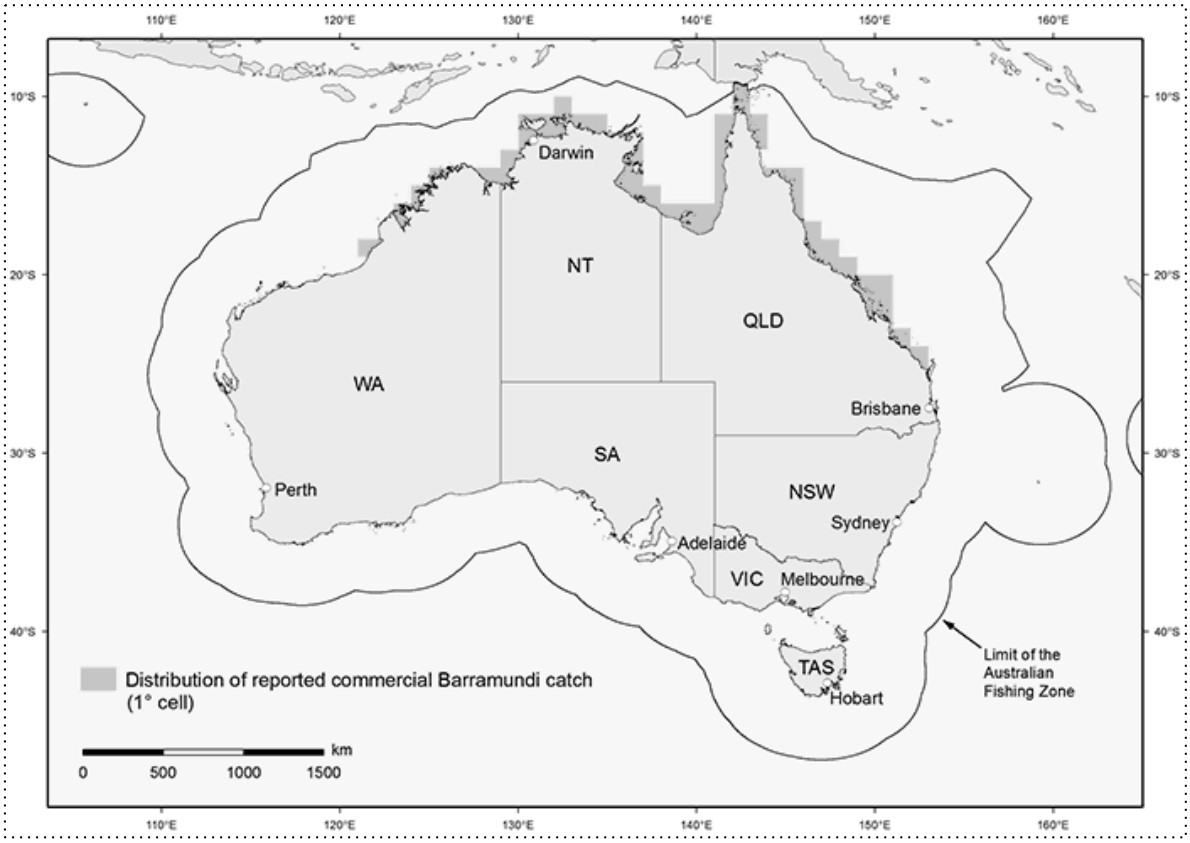

Figure 2.2: Distribution of reported commercial catch of barramundi (*Lates calcarifer*) in Australian waters, 2010

Source: (FAO Fishery Statistics 2006)
Although introduced for aquaculture into a number of other countries, the only countries that have reported production to FAO so far are shown in this map.

2.1.3 Marketability/ Imports to Australia

Some Australian seafood which is harvested and processed far from retail outlets, e.g. barramundi fillets, are only marketed frozen (CSIRO, 2014). FAO (2014) reported that annual barramundi production has been relatively static since 1998, at ~20,000–27,000 tonnes. Indonesia, Malaysia and Taiwan Province of China are also major producers and Thailand is the major producer, with about 8,000 tonnes/yr since 2001 (FAO, 2014). The major producer of barramundi is shown in Figure 2.3. Although supply of barramundi farmed in Australia into the Australian domestic market is expected to increase substantially over the next decade, cheap imports from Asia may also affect market prices for NSW farmers, as well as continued or increased imports of white flesh fillets, particularly Nile perch which are very similar to barramundi, but cheaper (DPI, 2014). The global average value of farmed

barramundi was USD 3.80/kg in 1994 and rose to USD 4.59/kg in 1995 but had fallen to USD 3.92 by 1997; since then it has been around USD 3.7/kg except for 2002, when it fell markedly to below USD 3.0/kg (FAO, 2014). Thus DPI (2014) reported that export market opportunities are likely to be limited, due to the higher cost of producing fish in Australia compared to Asia. CSIRO (2014) stated that large quantities of frozen seafood are imported into Australia because local supplies, particularly of boneless fish fillets, do not meet the demand. Generally, imported frozen seafood is cheaper than fresh local varieties.

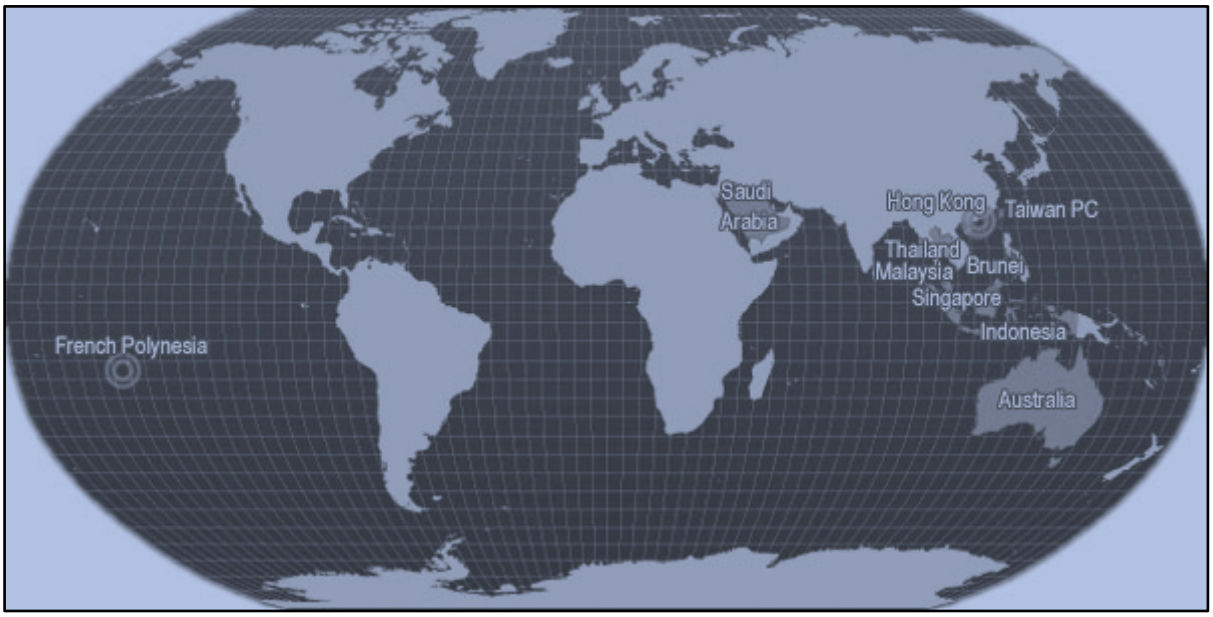

Figure 2.3: Main producer countries of barramundi (*Lates calcarifer*)

Source: (Fisheries Research and Development Corporation 2012)

2.1.4 Transport

Seafood safety is strictly dependent on the different phases of the seafood production, transportation and sales chain (Cai *et al.*, 2014). The risk potential, shelf life and final quality of chilled products processed and packed under good manufacturing practices and good hygiene practices (GMPs and GHPs) are determined by the applied temperature conditions in the chilled distribution chain (Nychas *et al.*, 2008). Since in practice significant deviations from specified conditions often occur, temperature monitoring and recording should be a pre-requisite for food chain control (Wells & Singh, 1989). Time and temperature control are significant factors when distributing fresh and frozen seafood products from farms to

commercial firms. Several studies have been published modeling microbial growth of a number of spoilage bacteria in fish (Dalgaard, 1995; Dalgaard *et al.*, 1997), but only few focus on the effect of fluctuating temperature conditions, that could potentially reflect the actual distribution chain (Dalgaard *et al.*, 2002). The storage temperatures should also be as constant as possible during storage, especially at temperatures around 0°C (IIR, 2000). However, when addressing the quality issue of chilled meat, from production to final consumption, in an integrated and structured way, such a period should be included in the evaluation of quality losses and safety risks in the chill chain (Nychas *et al*., 2008). Hence, it is essential to enhance the freshness of fish when exposed to inconsistent fluctuating temperature during transportation and storage.

2.2 EFFECT OF CHILLING METHODS ON QUALITY AND SHELF LIFE

During processing and storage, fish quality may decline as a result of several factors, which are directly related to production of off-flavours and odours in foods (Aubourg & Medina, 1999). Icing is an important pre-processing technique when handling fish and it preserves quality, if carried out properly. With the introduction of mechanical refrigeration, ice could be produced in different forms such as block, cube, tube or flake ice (Kauffeld *et al*., 2010). Most of these forms of ice require a certain degree (Kauffeld *et al*., 2010). Microbial and autolytic breakdown mechanisms were significantly slowed down in the slurry ice batches, this type of ice proving to be a better storage system for lobster than traditional flake ice (Aubourg *et al*., 2007). During iced storage of raw fish, the quality of the fish muscle deteriorates. Endogenous proteolytic enzymes, able to hydrolyze different muscle proteins, are important early in this deterioration process (Cepeda *et al.*, 1990). It was reported that during chilled storage of fish, significant changes such as deterioration of sensory quality and loss of nutritional value have been detected as a result of changes in the protein and lipid fractions, formation of amines (volatile and biogenic) and hypoxanthine, and changes in the physical properties of the muscle (Bennour, 1991; Olafsdóttir *et al.*, 1997) are known to occur. During chilled storage of fish, significant deterioration of sensory quality and loss of nutritional value have been detected as a result of changes in chemical constituents, that have a major impact on the commercial value (Ashie *et al.*, 1996; Olafsdóttir *et al*., 1997; Losada *et al.*, 2005). Indeed, prolonged chilled storage has been shown to have a negative effect if further processing is to be carried out on the fish material (Slabyj & True 1978; Aubourg & Medina, 1997; Aubourg, 2001). To increase shelf life and reduce the rate of microbial and

biochemical degradation, different preservative methods, mainly based on low temperature, have been employed (Yin *et al.*, 2014). It includes refrigerated ice storage between 0 and 4°C, super-chilled storage in the range of -1 to -4 °C, by means of slurry ice or in super-chilled chambers without ice and frozen storage at -18 to -40 °C (Gallart-Jornet *et al.*, 2007).

Ice slurry is increasingly used for chilling, storage and transportation of fish on board fishing vessels and barges, at farms, and inside processing plants (Kauffeld *et al*., 2010). Ice slurry has a high energy storage density because of the latent heat of fusion of its ice crystals (Kauffeld *et al*., 2010). Rodrı´guez *et al.* (2005) showed that storage of horse mackerel in a brine-based ice slurry led to a substantial enhancement of shelf life, from 5 days with flake ice to 15 days; and also involved a significantly slower formation of total volatile base nitrogen (TVBN) and trimethylamine nitrogen (TMAN) after 8 days of storage. Similar inhibitory effects on quality loss mechanisms were also reported for sardine, with an increased shelf life of 15 days in ice slurry as compared to 8 days in flake ice (Carmen *et al.*, 2005). Using ice slurry to cool and preserve produce is a preferred method for the modern produce packing operation (Kauffeld *et al*., 2010). The Norwegian Institute of Fisheries and Aquaculture discovered that cod placed in ice slurry for three days (the maximum allowable time for such storage) became on average 4% heavier without any change in quality (Joensen *et al.*, 2001). The slurry maintains a constant low temperature level during the cooling process, and provides a higher heat transfer coefficient than water or other single- phase liquids (Kauffeld *et al*., 2010).

Furthermore, for some markets further away from the main fish producers, the relatively short shelf life necessitates either expensive air freight, frozen distribution or inclusion of more preservative factors (Yin *et al.*, 2014). Once the fish are caught, on-board storage conditions exert a strong effect on the quality of manufactured fish products and, accordingly, on their commercial value (Piggot & Tucker, 1987; Ashie *et al.*, 1996).

2.3 FREEZING

Fish is highly nutritious, because it has high protein content, vitamins, low saturated fat and also contains omega fatty acids known to support good health (Erkan & Ozden, 2007); yet it is one of the most rapidly perishing foods because of its short shelf life (Gandotra *et al.*, 2012b). Therefore frozen storage is an important preservation method for fish and fish products. Frozen storage has been widely employed to retain fish properties before it is

consumed or employed in other technological processes (Pigott & Tucker, 1987; Erickson, 1997; Aubourg *et al.*, 2002). Freezing involves lowering the product temperature generally to −18 °C or below (Fennema *et al*., 1975; Espinoza Rodezno *et al.*, 2013). The concept of frozen storage relies on lowering the product's temperature to slow down spoilage so that the thawed fish can retain its freshness (Kolbe *et al.*, 2004; Tan & Fok, 2009). Freezing is the only large-scale method that bridges the seasons, as well as variations in supply and demand of seafood while maintaining quality close to that of fresh products (Espinoza Rodezno *et al.*, 2013). Extending the shelf life of fish may also be a way to increase profitability, since product prices in the fresh market are higher than in the frozen market (Duun & Rustad, 2007). There is a growing market segment for frozen seafood (Espinoza Rodezno *et al.*, 2013). Although freezing is known as an effective way of preserving whole fish and its product forms including fish fillets, some quality alterations do occur during freezing. It is important to control the freezing process, including the pre-freezing preparation and post-freezing storage of the product, in order to achieve high quality products (George, 1993; Espinoza Rodezno *et al.*, 2013). Seafood is also susceptible to freezer burn (Gashti, 2002). In a cold and dry atmosphere, ice sublimes (Gashti, 2002). The degree of denaturation varies, depending on species (Fukuda *et al.,* 1981; Seo *et al.*, 1997; Benjakul *et al.*, 2003). The surface of frozen product is easily dehydrated and rancidity occurs from such adverse influence (Gashti, 2002).

Quality deterioration is seen during freezing and frozen storage due to the osmotic removal of water, denaturation of protein and mechanical damage (Thyholt & Isaksson, 1997). Enzymes and other components are released (Nilsson & Ekstrand, 1993, 1995; Benjakul & Bauer, 2001). Endogenous proteolytic enzymes, able to hydrolyze different muscle proteins, are important early in this deterioration process (Cepeda *et al.*, 1990). Freezing slows enzyme activity and inhibits microorganism growth (Benjakul & Sutthipan 2009). However, lipid hydrolysis and oxidation still occur (Benjakul & Sutthipan 2009). During the frozen storage of fish lipid hydrolysis and oxidation have been shown to occur and become an important factor of fish acceptance as influencing rancidity development, protein denaturation and texture changes (Mackie, 1993; Aubourg *et al.*, 2002). The oxidation of highly unsaturated lipids is directly related to the production of off flavors and odors in foods (Pearson *et al.*, 1977; Pigott & Tucker, 1987; Aubourg *et al.*, 1998). Though lipid oxidation is the major form of deterioration in stored muscle foods, fish meat contains high contents of proteins, which can also be influenced by oxidative reactions (Cai *et al.*, 2014); and results show that

decreasing the freezing temperature would reduce the oxidation of proteins during frozen storage (Cai *et al.*, 2014). The extent of quality loss of marine frozen food is dependent upon many factors, which include storage temperature and time, packaging, rate of freezing-thawing, and temperature fluctuations and freeze-thaw abuse (Srinivasan *et al.*, 1997; Benjakul & Sutthipan, 2009).

One of the most important quality characteristics of fresh fish is muscle texture, and excessively soft fillets may cause problems for the industry (Hallett & Bremner, 1988; Haard, 1992; Andersen *et al.*, 1997; Sigholt *et al.*, 1997). Texture softening of tilapia (genetically improved farmed tilapia strain of *Oreochromis niloticus*) was mainly influenced by the autolysis and denaturation of muscle protein during chilled and frozen storage (Tsuchiya *et al.*, 1992; Benjakul *et al*., 1997). Although microbial spoilage can be terminated effectively by frozen storage, a number of changes in proteins shorten the shelf-life of frozen fish (Benjakul *et al.*, 2003). These changes are caused by an essentially irreversible phenomenon known as denaturation of the muscle proteins and are principally attributed to the denaturation/degradation of the structural muscle proteins, mainly the myofibrillar proteins (Jasra *et al.*, 2001). During storage of frozen foods, the most important changes such as protein denaturation (Garthwite, 1992), drip loss (Garthwite, 1992), discoloration (Chandrasekaran, 1994), lipid oxidation, and sublimation and recrystallization of ice (Londahl, 1997) can result in off-flavors, rancidity, dehydration, weight loss, loss of juiciness, drip loss, and textural changes (Londahl, 1997) and loss of water holding capacity (Barroso *et al.*, 1998) which produces a hard, dry and fibrous product (Barroso *et al.*, 1998). The texture of the fish muscle changes from soft, moist and succulent to unacceptably firm, hard, fibrous, and dry during frozen storage (Jasra *et al.*, 2001).

Prolonged frozen storage of Egyptian shore crabs (*Carcinus maenas*) at -10°C affected the chemical characteristics of crab meat (Aman *et al.*, 1983). Muscle proteins of freshwater prawn tails are susceptible to freezing-thawing processes, particularly during the first month of frozen storage (Srinivasan *et al.*, 1997; Benjakul & Sutthipan, 2009)**.** Aubourg *et al.* (2002) reported that satisfactory quality was maintained for up to 7 months of frozen storage of horse mackerel (*Trachurus trachurus*) provided that a short chilling time (not longer than 3 days) was employed. Benjakul *et al.* (2003) observed that extended frozen storage caused the denaturation of protein as well as cell disruption in threadfin bream (*Nemipterus bleekeri*),

bigeye snapper (*Priacanthus tayenus*), lizardfish (*Sauruda micropectoralis*) and croaker (*Pennahai macrophthalmus*) but the degree of changes was dependent upon species.

The correct preservation temperature for various fish species may be different. The optimal preservation of tuna was achieved at temperatures between -60°C and -70°C (Chow *et al*., 2004; Chow, 1988). For cod it was -40°C, and for salmon fillets it was in the range between -45°C and -60°C (Magnussen & Johansen, 1995; Mørkøre & Lilleholt, 2007). Whereas, preferred storage temperature for a high quality frozen product is far above that for other fish species (Indergård *et al*., 2013). Nicholson (1973) has reported that the type of freezer, operating temperature (difference between product and contact medium), air speed, initial product temperature, product thickness, and shape, density, and species affect freezing rates. It has also been reported that the ice crystals formed in white muscle are twice as large as those formed in red muscle (Kaale & Eikevik, 2013b). The surface of the food experiences a rapid change in temperature compared to the core part of the product (Kaale *et al.* 2013c). This behavior is due to both the formation of ice crystals just a few millimeters from the surface during the superchilling process and the lack of ice crystals formed at the center of the food product (Kaale *et al.* 2013c). The chemical, microbiological, and sensory parameters of farmed halibut hardly deteriorate at all during the 1st 21 d of ice storage (Akse & Midling, 2001). Oehlenschlaeger (1995) showed that plaice (*Pleuronectes platessa*) stored on ice for up to 25 d, could be marketed up to day 18 and was edible up to day 20 of ice storage (Guillerm-Regost *et al.*, 2006). Endogenous collagenases may break down the connective tissue during iced storage in the fish muscle and thereby lead to undesirable textural changes and gaping (Bremner & Hallett, 1985; Cepeda *et al.*, 1990; Ando *et al.*, 1995; Ashie *et al.*, 1996). During chilled storage, the collagenolytic activities from skeletal muscle of fish were dependent on fish species, and were most potent at pH values close to neutrality or higher (Bracho & Haard, 1995; Teruel & Simpson, 1995; Hernandez-Herrero *et al.*, 2003; Hultmann & Rustad, 2007). Thus, a great variety of physical and chemical changes occur during freezing. Recrystallization and surface drying are accelerated by temperature fluctuations during freezing, although the importance of these physical changes decreases at lower storage temperatures (Canet, 1989; Alvarez & Canet, 1998; Sousa *et al*., 2005). It is well known that the more time the product spends in the freezer, the more ice crystals will be formed within the product (Kaale *et al.* 2013c). Kolbe & Kramer (2007) have reported that slow freezing results in a small number of large crystals, most of which are extracellular. Fast freezing, produces a large number of small ice crystals both intracellular and extracellular (Espinoza

Rodezno *et al.*, 2013). Ice crystals are formed in the tissue during freezing, and their size, shape and extra- or intracellular location, depend on the freezing conditions (Howgate, 1979). Smaller ice crystals are associated with, higher quality with lower drip loss and good texture (Espinoza Rodezno *et al.*, 2013).

In general, freezing and subsequent thawing treatments cause fish muscle proteins to denature (Srinivasan *et al*., 1997). Freezing followed by immediate thawing had little effect on the characteristic thermal transitions of cod muscle; however, after two weeks at -10°C it became apparent that myosin had undergone some partial denaturation (Schubring, 1999). Frozen foods tend to lose weight during frozen storage due to sublimation of water, which produces a porous and dehydrated layer at surface of the food product, and alters physical and organoleptic characteristics of the frozen food (Campañone *et al*., 2001). Freezing does not improve product quality; it only increases shelf life with minimal quality changes, when done correctly (Espinoza Rodezno *et al.*, 2013). New frozen product formulations and new freezing technology benefits should be assessed taking into account the frequent temperature abuses occurring in commercial and domestic storage (Estrada-Flores, 2002). An overall analysis of fish quality showed an increase in the shelf life with a decrease of temperature (Tolstorebrov *et al.*, 2014). Temperature of -15°C is permitted for short periods during transportation or local distribution (Flair flow Europe, 2000). Moreover, food retail display cabinets should be at -18°C with good storage practice, but not warmer than -12°C (Flair flow Europe, 2000). The rancid odor of herring fillets stored at -20°C for 12 months was strong, while fillets stored at -80°C maintained a high quality for 18 months (Hyldig *et al*., 2012). Large amounts of salmon are normally transported and stored as frozen products, due to long shipping distances in the global market (Einen *et al*., 2002). However, such a low temperature is unacceptable for industrial use, due to the high investment and running costs (Tolstorebrov *et al.*, 2014). Freshness is an essential factor that determines the quality, consumer acceptability and ultimately commercial value of fish and fishery products (Liu *et al*., 2013). Freezing is the only large-scale method that bridges the seasons, as well as variations in supply and demand of seafood while maintaining quality close to that of fresh products (Espinoza Rodezno *et al.*, 2013). Further to this, there is an increasing demand for frozen seafood. A variety of studies on freezing and frozen storage and the tools used to assess the quality and shelf life of various species are shown in Table 2.1.

Table 2.1: Shows different studies on freezing and frozen storage and the tools used to assess the quality and shelf life of various species

Species	Tools used (◆) & Brief (✓) Summary	References
Sea bream (*Sparus aurata*)	◆ Chemical, physical and sensory assessment ✓ The limit for acceptability of cultured sea bream stored in ice was about 17-18 days	(Alasalvar *et al.*, 2001)
Galda (*Macrobrachium rosenbergii*) and Bagda (*Penaeus monodon*)	◆ Biochemical changes (TVBN and TMAN aasessment) ✓ No significant variation in TVBN contents between Galda and Bagda.Significant variation in TMAN between Galda and Bagda. In all cases, Bagda showed higher amounts of TMAN than the Galda	(Ali *et al*., 2010)
Blue Whiting (*Micromesistius poutassou*)	◆ Lipid damage indices, TVBN ✓ Results indicate that this fluorescence detection is sensitive enough for assessing freshness loss during chilling of a lean fish species and appears to be the equal if not the superior of a recognized method such as TVBN to assess fish spoilage	(Aubourg *et al*., 1998)
Horse mackerel (*Trachurus trachurus*)	◆ Chemical and sensory lipid damage indices ✓ Satisfactory quality was maintained up to 7 months of frozen storage of horse mackerel provided that a short chilling time (not longer than 3 days) was employed	(Aubourg *et al*., 2002)
Sea Bass (*Dicentrarchus labrax*)	◆ Proximate composition and amino acid content ✓ Changes in proximate composition	(Beklevük *et al*., 2005)

	and amino acid content of frozen (-18°C) wild sea bass (Dicentrarchus labrax, L. 1758) fillets were investigated and reduction was noticed during 9 months of storage. Methionine, tyrosine and histidine were in lower concentrations than the other amino acids during the frozen storage	
Threadfin bream (*Nemipterus bleekeri*), bigeye snapper (*Priacanthus tayenus*), lizardfish (*Sauruda micropectoralis*) and croaker (*Pennahai macrophthalmus*)	◆ Physicochemical changes of muscle protein as well as the tissue integrity ✓ Extended frozen storage caused the denaturation of protein as well as the cell disruption in all species, but the degree of changes was dependent upon species	(Benjakul *et al.*, 2003)
Cat fish (*Silurus glanis* Linne)	◆ Biochemical and physicochemical changes ✓ The freeze-thaw process therefore has a detrimental effect on the quality of catfish fillets, particularly after chilled storage	(Benjakul & Bauer, 2001)
Mud crabs (*Scylla serrata*)	◆ Biochemical and physicochemical changes ✓ The faster freezing process together with the lower frozen storage temperature is suggested for soft shell crab to obtain the prime quality, in comparison with that employed for hard shell crab	(Benjakul & Sutthipan, 2009)
Tiger shrimp (*Penaeus*	◆ Physical and chemical changes ✓ It is important to prevent	(Boonsumrej *et al.*, 2007)

monodon)	temperature fluctuations during transportation and storage to avoid the freezing and thawing effect and to maintain the quality of the frozen shrimps.	
Hake (*Merluccius merluccius*)	◆ Raman spectroscopy of the structural changes in hake muscle proteins and their relation with functionality and texture. ✓ Changes in protein secondary structure were observed due to storage temperature, accompanied by changes in apparent viscosity and shear resistance	(Careche *et al.*, 1999)
Rohu meat (*Labeo rohita*)	◆ Biochemical, microbiological and organoleptic properties. ✓ The product treated with cryoprotective agents had a better acceptability compared to control group	(Dutta, 2009)
Cod (*Gadus morhua*)	◆ Microbiological and Biochemical analyses ✓ Superchilling extended the microbial shelf life of vacuum packed cod fillets by several weeks compared to traditional ice chilling. It was reported in this study in order to maintain good quality of the cod fillets during superchilling, the process has to be optimized with regard to freezing rate and degree of superchilling to minimize protein denaturation and LL.	(Duun & Rustad, 2007)

Atlantic salmon (*Salmo salar*)	◆ Texture, fillet gaping, colour, drip loss and yield ✓ Less gaping in frozen pre-rigor fillets than in frozen post-rigor fillets, and the colour and firmness tended to be higher in frozen pre-rigor than in frozen post-rigor fillets. Unfrozen pre-rigor was documented to have the best colour score, less fillet gaping, firmest texture and lowest drip loss	(Einen *et al.*, 2002)
Meat (*Longisimus dorsi* muscle)	◆ Cook losses and textural properties ✓ The results showed that STP concentration, STP dipping and cooking times had significant effects on the changes of textural properties and cook losses of meats. Changes in cook losses were really affected by cooking time, STP concentration and dipping time, showing the improvements can be easily obtained by dipping in STP solutions.	(Erdogdu *et al.*, 2007)
Channel catfish (*Ictalurus punctatus*)	◆ Weight loss, relative moisture loss, color, and lipid oxidation (Thiobarbituric acid; TBA) ✓ Demonstrated that catfish fillets cryogenically frozen had better quality characteristics than blast frozen catfish fillets after 6-months of storage.	(Espinoza Rodezno *et al*., 2013)
Kutum (*Rutilus frisii kutum*)	◆ Chemical, microbiological, textural and sensory characteristics ✓Control samples were found to have a shelf life of about 6 days, whereas	(Etemadian *et al.*, 2011)

	samples treated with polyphosphate were found to be acceptable up to 9 days	
Kutum (*Rutilus frisii kutum*)	◆ Microbiological, chemical and sensory changes ✓ Best result in the use of polyphosphates, can be expressed that using 2% solution of sodium tripolyphosphate with vacuum packaging showed lower bacterial load, TBA index, total sulfhydryl groups oxidation, and higherwater holding capacity, sensory quality and increased shelf life compared with other treatments	(Etemadian *et al.*, 2012)
Kutum (*Rutilus frisii kutum*)	◆ Chemical, physical, and textural analyses ✓ Results indicated that dipping kutum fillets in polyphosphates solution was the most effective method to improve the quality properties of fillets during ice storage when compared with other phosphate-treated groups	(Etemadian *et al.*, 2013)
Mystus seenghala	◆ Biochemical and microbiological changes ✓ Freezing of fish decreased the bacterial growth and biochemical decomposition of fish muscle, thereby increasing the shelf life; while chilling at 4±1°C allows the comparatively rapid proliferation of bacteria, protein denaturation, lipid hydrolysis and oxidation; thereby reducing the shelf	(Gandotra *et al.*, 2012b)

	life	
Labeo rohita	◆ Biochemical and microbiological changes ✓ Significant quality loss was observed in fish during storage. However, the present frozen conditions retained the fish under acceptable microbial conditions for human consumption up to 14th day beyond which it became unfit for human consumption	(Gandotra *et al.*, 2012a)
Sardines (*Clupea pilchardus*)	◆ Proximate and amino acid compositions, protein solubility in sodium dodecyl sulphate/β-mercaptoethanol (SDS/β-ME), total —SH group content and amino acid chemical score were determined. ✓ Study suggests that a slow defrosting process (refrigerator at 4°C) is preferable to a much quicker process (microwave oven) for thawing frozen sardine fillets	(Garcıa-Arias *et al.*, 2003b)
Sardine (*Sardina pilchardus*)	◆ Proximate and fatty acid compositions ✓ Freezing–reheating significantly affected (P<0.001) the fatty acid composition with the content of oleic acid increasing and those of the o-3 fatty acids, decreasing more in MR than in OR	(Garcıa-Arias et al., 2003a)
Atlantic Pollock (*Pollachius vireos* L).	◆ Microbiological and chemical variation ✓ Storage conditions of -30 degree	(Gashti, 2002)

	seem to be good for minced fish to maintain high quality. The quality evaluation (microbiological and chemical variation) for mince fish after frozen stored showed after 50 days was acceptable	
Pink cuskeel (*Genypterus brasiliensis*), Searobin (*Prionotus punctatus*), Mussel (*Perna perna*) and Red shrimp (*Pleoticus muelleri*),	◆ Drip losses, sensorial attributes ✓ Dipping: searobin and pink cuskeel fillets, shrimp, mussel in 2% and 5% STPP and Phosphate Blend solutions, respectively, can be used to prevent the large thawing and cooking-related yield losses	(Gonçalves *et al.*, 2008)
Sea-bob shrimps (*Xiphopenaeus kroyeri*)	◆ Physical and chemical changes ✓ Demonstrated the effectiveness of the glazing process as a protecting agent for frozen shrimp. A reasonable range of water uptake could be between 15% and 20% to guarantee the final quality	(Gonçalves *et al.*, 2009)
Atlantic halibut (*Hippoglossus hippoglossus* L.)	◆ Instrumental, chemical, sensory, and bacteriological analysis ✓ The texture, liquid-holding capacity, and color did not change significantly from approximately day 8 of storage until the end of the experiment at day 26	(Guillerm-Regost, 2006)
Common carp (*Cyprinus carpio*)	◆ Physicochemical and protein structural changes ✓ It is imperative to minimize temperature fluctuations during storage and in transportation of frozen carp to reduce the change of physicochemical	(Guo *et al.*, 2013)

	and protein structural properties and avoid the decrease of carp muscle quality	
Weakfish (*Cynoscion regalis*)	◆ Psychrotrophic bacterial populations, chemical and sensory qualities ✓ Results showed that the vacuum skin packaging significantly reduced lipid rancidity development and slowed lipid hydrolysis of fish as compared to traditional overwrapping	(Huang *et al.*, 1993)
Farmed Atlantic cod (*Gadus morhua*)	◆ Textural properties and proteolytic activities ✓ The temperature abused fillets were less resilient and had lower values of gumminess than those subjected to iced storage throughout the storage period. From the proteolytic profiles, it is suggested that the textural changes observed after temperature abuse may have been caused by collagenase-like enzymes	(Hultmann & Rustad, 2007)
Atlantic salmon (*Salmo salar*)	◆ Muscle proteins and texture ✓ Differences in quality characteristics such as textural properties and amounts of free amino acids and extractable proteins between the irradiated and control fillets, were evident only late in the storage period (after 14 days of iced storage). Even with the increased numbers of microorganisms seen in the control group, no severe deterioration of fish	(Hultmann & Rustad, 2004)

	texture or increase in proteolytic activity was observed during the storage period	
Atlantic cod (*Gadus morhua*)	◆ Proteolytic enzyme activities and muscle quality ✓ After 5 days of iced storage, the stressed fish had significantly lower water holding capacity, reduced hardness and yellowish colour compared to the control group, and no differences in the other parameters investigated. Collagenase-like enzymes were most important in the early post mortem period, whereas the contribution of cathepsins increased as the muscle pH was reduced.	(Hultmann *et al.*, 2012)
Carp (*Labeo rohita* (Hamilton))	◆ Histochemical, biochemical and electrophoretic ✓ Storage of muscle fillets of L rohita seems to be quite safe up to 6 months at -20°C as far as the degradation of myofibrillar proteins is concerned.	(Jasra *et al*., 2001)
Atlantic salmon (*Salmo salar*)	◆ Microscopic analysis, ✓ The size of ice crystals formed in pre-rigor muscle was significant smaller than that formed in post-rigor muscle. It was also observed that the size of intracellular ice crystals formed in pre-rigor red muscles was significant smaller than that in white muscle	(Kaale & Eikevik, 2013a)

Atlantic salmon (*Salmo salar*)	◆ Microscopic analysis ✓ The formation of ice crystals within salmon muscle regardless of the superchilling rates was an important factor in reducing cell structure damage	(Kaale *et al.,* 2013e)
Shrimp (*Pandalus borealis*)	◆ Dielectric measurements ✓ The performance of the prototype instrument at temperatures above freezing is entirely satisfactory giving results that are within ±2% of the glaze as measured by the weight gain	(Kent & Stroud, 1999)
Sea bass (*Dicentrarchus labrax*) and saithe (*Pollachius virens*)	◆ Microbiological Analyses, chemical analyses, instrumental analyses and sensory analyses ✓ It can be concluded that the treatment of 5% sodium tripolyphosphate solution is the most effective to reduce the bacterial loads when compared with other phosphate groups. Phosphate treatment can be an alternative way to improve the quality of frozen-thawed fish	(Kilinc *et al*., 2007)
Sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*)	◆ Microbiological, color and sensory analyse ✓ Using slurry ice pretreatment for 2 h before the storage period presumably caused the deleterious effect on appearance as well as salt and water uptake	(Kılınc *et al.*, 2007)
Anchovies (*Engraulis encrasicolus*, Linneaus, 1758)	◆ Microbiological, chemical and sensory analyses	(Kose *et al.*, 2001)

	✓The results showed that the product was edible after three months of frozen storage at -18±1°C. Therefore, it was concluded that anchovies can be marketed frozen out of season either cooked or uncooked if processed into 'Hamsikusu'	
Lizardfish (*Saurida micropectoralis*)	◆ Physicochemical and biochemical changes ✓ Packaging of minced flesh of lizardfish, under conditions lacking in oxygen, might induce a quality loss during frozen storage	(Leelapongwattana *et al*., 2005)
Channel Catfish (*Ictalurus punctatus*)	◆ Color evaluation ✓ Results suggest that while the yellow discoloration of fresh catfish fillets is related to carotenoid contents, the intensification of the discoloration during storage appears to be related to another chemical phenomenon	(Li *et al.*, 2013)
Yellow grouper (*Epinephelus awoara*)	◆ Physicochemical analyses, sensory evaluation, microbiological analyses ✓ The results of these analyses showed optimal quality for yellow grouper fillets stored under these conditions, and established a microbiological shelf-life of 26 days	(Li *et al.*, 2011)
Grass carp (*Ctenopharyngodon idella*)	◆ Biochemical and physical properties ✓ Superchilling of grass carp fillets is still a potentially promising technique, however, the degree of superchilling has to be optimized to minimize the	(Liu *et al.*, 2013)

	tissue structure damages and protein denaturation as well as the concurrent quality deterioration	
White shrimp (*Penaeus vannamei*)	◆ Texture, Determination of yield, freezing loss and moisture content, cutting force, appearance, color and histological study, TVB ✓ The toughening of shrimp was observed while sodium bicarbonate containing traces of citric acid treatment with sodium chloride could reduce the texture change occurred during the freezing	(Lopkulkiaert *et al.*, 2009)
Horse mackerel (*Trachurus trachurus*)	◆ Chemical analyses, sensory evaluation ✓ Results agreed with sensory assessment, which showed a marked increase in shelf life and good quality period when the slurry ice treatment was employed.	(Losada *et al*., 2005)
Silver carp (*Hypophthalmichthys molitrix*)	◆ Proximate anlyses and quality analyses ✓ It may be concluded that the gelforming ability of silver carp mince is retained for a minimum of 135 days when frozen stored at -20°C after prewashing treatment and addition of cryoprotectants	(Majumdar *et al.*, 2012)
Salmon (*Salmo salar*)	◆ Physicochemical, textural and sensorial properties ✓ Freezing the salmon for 24 h before smoking (BFS) did not increase its shelf life (30 days) over that of	(Martinez *et al.*, 2010)

	refrigerated smoked salmon (RFS). However, maintaining the fish frozen at -18°C (AFS) increased its shelf life (>45 days) and invested the flesh with greater firmness, cohesiveness and colour intensity	
Seabass (*Lates calcalifer*)	◆ Microbiological, chemical and sensory changes ✓ The effective retardation of microbiological, chemical, and sensory deterioration of seabass slices stored under MAP could be achieved by pretreatment with pyrophosphate	(Masniyom *et al*., 2005b)
Cod (*Gadus morhua*)	◆ Ion chromatography (IC) and spectrophotometric methods ✓ The advantage of the IC method was the ability to separate and determine different soluble phosphate species in the fish muscle. The IC results showed that pyrophosphate (P_2O_7) and triphosphate (P_3O_{10}) were partly degraded into orthophosphate (PO_4) during the storage period	(Nguyen *et al*., 2012)
Sardine (*Sardina pilchardus*)	◆ Organoleptic assessments, chemical analyses (total volatile nitrogen (TVBN), trimethylamine (TMA-N), trimethylamine oxide (OTMA-N) and hypoxanthine) and physical measurements (GR Torrymeter readings and pH) ✓ Results obtained indicate that TVBN and TMA-N parameters are not good freshness indicators for this species,	(Nunes *et al.*, 1992)

	but Torrymeter readings and hypoxanthine values can be used as indicators of freshness	
Catfish (*Chrysichthys nigrodigitatus*)	◆ Proximate analyses, Drip Loss Measurements and Carcass Quality, Organoleptic Assessment ✓ Drip loss decreases with decrease in storage temperature, hence, fish stored at 29C had the least drip loss of 5.02%, hence the best carcass quality/ filleting characteristics	(Oyelese, 2007)
Atlantic salmon (*Salmo salar*)	◆ Yield and liquid-holding capacity, chemical analysis, color analysis and texture analysis ✓ Freezing only after smoking led to fewer changes in quality than freezing before smoking, whereas refreezing the finished products had little additional effect on quality	(Rørå & Einen, 2003)
Atlantic salmon (*Salmo salar*)	◆ Viewing and image processing by light microscope,Textural measurements, Salt content ✓ It can be concluded that freezing does affect the muscle structure of the smoked fillets, the fibers shrank and extracellular space increased which can on the other hand lead to liquid leakage from the smoked fillets. Although freezing leads to fiber shrinkage, the yield during the smoking process was not affected	(Sigurgisladottir *et al.*, 2000)
Horse mackerel (*Trachurus trachurus*)	◆ Physical, biochemical and sensory properties	(Simeonidou *et al.*, 1997)

and mediterranean hake (*Merluccius mediterraneus*)	✓ The pH, expressible water (EXW), quantities of trimethylamine (TMA), dimethylamine (DMA), formaldehyde (FA), the total volatile base nitrogen (TVBN) the thiobarbituric acid number (TBA), peroxide value (PV) and amount of free fatty acids (FFA) increased, while sensory attributes (odour, taste, texture) decreased during the frozen storage period	
Black tiger shrimp (*Penaeus monodon*) and white shrimp (*Penaeus vannamei*)	◆ Physicochemical properties and microstructures ✓ The freeze–thawing process caused denaturation of proteins, cell disruption, as well as structural damage of muscle in both shrimps. White shrimp generally underwent physicochemical changes induced by the freeze–thawing process to a greater extent than did black tiger shrimp	(Sriket *et al.*, 2007)

2.3.1 Freezer Types and their Uses

Studies reported that (Kaale & Eikevik, 2013a; Kaale *et al.*, 2013b; Kaale *et al.*, 2013d; Kaale *et al.*, 2013e) partially freezing food products using an impingement freezer (Impingement Advantec Lab Freezer) results in suitable properties of ice crystals with regard to size, distribution and shape, provided that the partial freezing occurs at a high rate. Kaale and Eikevik (2013a) reported that the samples were super-chilled (partially frozen) at -20°C, 153 W/m2 K (pressure differences of the fan at the impingement freezer) for 4.2 min, which is referred to as process S (slow super-chilling), and at -30°C and 227 W/ m2 K for 2.1 min, which is referred to as process F (fast super-chilling), to achieve an ice content of 20%. This approach removes heat from the fish by circulating cold air at -30 to -40°C with velocity ranging from 1.5 to 6 m s^{-1} over the product (Tan & Fok, 2009). The air speed and

temperature selected depend on the size and shape of the product (Tan & Fok, 2009). A number of freezing techniques are used in the seafood industry including air blast freezing, plate freezing, immersion freezing, cryogenic freezing and cryo-mechanical freezing (Espinoza Rodezno *et al.*, 2013). The most regularly operated techniques are air blast freezing and cryogenic freezing. The freezing technique affects not only energy consumption but also frozen food quality (Espinoza Rodezno *et al.*, 2013). The major advantage of the air blast freezer is its versatility (Dempsey & Bansal, 2012). Since air is a low viscosity fluid it has the ability to easily follow around irregular surface geometries, thus providing a more uniform freezing rate over the whole product (Dempsey & Bansal, 2012). Seafood is frozen in plate freezers or individually quick frozen (IQF) by contact freezing (Gashti, 2002). Other freezing methods such as plate freezing (contact freezing) offer faster cooling times (Hessami, 2004) but can only be used with products of a suitable geometry, i.e. a flat surface to match the plate bed (Dempsey &Bansal, 2012). The quality of the frozen seafood is related to freezing rate (Goswami, 2010). It is important to control the freezing process, including the pre-freezing preparation and post-freezing storage of the product, in order to achieve high quality products (George, 1993). Many marine products are frozen for longer shelf life and can be distributed in frozen condition with the development of freezer chains (Gashti, 2002).

2.3.1.1 Air Blast Freezer

One of the most flexible and regularly used techniques in the seafood industry is air blast freezing. Tan & Fok (2009) stated that to produce a good product, freezing must be accomplished quickly. The blast freezers can achieve this within 6 to 8 hours, which is still considered quick. Fast freezing, produces a large number of small ice crystals both intracellular and extracellular; smaller ice crystals are associated with, higher quality with lower drip loss and good texture (Espinoza Rodezno *et al.*, 2013). In another study, Benjakul & Sutthipan (2009) observed that hard and soft shell mud crab muscles underwent physicochemical changes, especially protein denaturation during frozen storage at -20°C for 12 weeks; thus, soft shell mud crab muscle was more susceptible to denaturation during frozen storage than the muscle of hard shell mud crab muscle. Therefore, the faster freezing process together with the lower frozen storage temperature is suggested for soft shell crabs to maintain prime quality, in comparison with that employed for hard shell crabs (Benjakul & Sutthipan, 2009).

Air blast freezing is classified as a forced convection phenomenon where the use of fans increases the products surface heat transfer coefficient and produces a more uniform air temperature throughout the freezer (Dempsey & Bansal, 2012). In a study, freezing the shrimps under the air-blast freezer at the air velocity of 6 m/s gave the least %freezing loss and same cutting force as the fresh shrimp (Boonsumrej *et al.*, 2007). Air blast freezers are designed to supply cool air over the food product with a uniform air velocity throughout the freezer (Johnson *et al.*, 1994; Valentas *et al.*, 1997; Da-Wen Sun, 2001; Pearson & Lamb, 2001; Maroulis & Saravacos, 2003; Becker & Fricke, 2005). Air temperature must be at least -35°C, and in some cases -45 °C (Mallett, 1994). Changes in product packaging have to be taken into account when sizing air blast freezer throughout (Dempsey & Bansal, 2012). In a study, it was found the surface heat transfer coefficient of whole, unpackaged chickens reduced by an order of magnitude when the chickens were wrapped in plastic and stored in vented boxes (Mannapperuma *et al*., 1994). Kemp & Chadderton (1992) surveyed a plant, which changed the type of cardboard packaging used, as and the study reported that freezing time was increased by 8 hours since the change of packaging was determined as the major cause of the plant's freezing problems.

2.4 FREEZE-THAW CYCLES AND EFFECTS

The extent of quality loss is dependent upon many factors, which may include storage temperature, rate of freezing-thawing, temperature fluctuations, freeze–thaw abuse during storage, transportation, retail display and consumption (Srinivasan *et al.*, 1997; Boonsumrej *et al.*, 2007). Fluctuating temperature may cause melting and refreezing of water resulting in larger ice crystals, which can be disruptive to the microstructure of fillets (Mackie, 1993; Duun & Rustad, 2007). Freeze-thaw cycles directly affect the biochemical and physicochemical properties of catfish fillets (Benjakul & Bauer, 2001). Boonsumrej *et al.* (2007) reported that shrimp samples could be freeze–thawed up to 2 cycles. In addition to this, the authors stated that it is important to prevent temperature fluctuations during transportation and storage to avoid the freezing and thawing effect and to maintain the quality of the frozen shrimp (Boonsumrej *et al.*, 2007). Changes in physicochemical properties of protein induced by freeze-thawing were different between white shrimp and black tiger shrimp and the degree of change was more pronounced with increasing number of freeze-thawing cycles (0, 1, 3 and 5) (Sriket *et al.*, 2007), and this freeze-thawing caused denaturation of proteins, cell disruption, as well as structural damage of muscle in both

shrimp species; however, in the same study white shrimp generally underwent physicochemical changes induced by the freeze-thawing process to a greater extent than did black tiger shrimp. The spacing between the muscle fiber increased and the muscle fibers were torn as the number of freeze-thaw cycles increased (Boonsumrej *et al.*, 2007). The freeze–thaw process may cause protein and lipid oxidation, which would affect texture of muscle (Xia *et al*., 2009). In meat, protein oxidation may lead to decreased eating quality such as reduced tenderness and juiciness, flavor deterioration, and discoloration (Xiong, 2000).

It is known that quick thawing normally gives a better quality of thawed fish than slow thawing (Ohmori *et al*., 1981). Despite the widespread use of running water in various forms of plant for thawing frozen fish blocks, the basic factors underlying the design of such equipment have not been established (Ohmori *et al*., 1981).

During transportation and storage of seafood, temperature fluctuation is generally common and these directly influence microbiological and physiochemical changes in the muscle. The freeze-thaw process is detrimental to overall physicochemical and textural quality and affects the thermal properties of freshwater prawns (Soottawat & Friedrich, 2001; Xia *et al.*, 2009). Hamm (1979) showed that freezing and thawing disrupt muscle cells and cause the release of enzymes from mitochondria into the sarcoplasm. Hale & Waters (1981) reported that fiber shrinkage and exudation of fluid (drip loss) are deleterious changes in muscle tissue following freezing and thawing. Jiang *et al.* (1991), using actomyosin ATPase as an index, showed that grass prawn (*Panaeus monodon*) myofibrillar proteins were destabilised by freeze–thaw treatments. Wagner & Anon (1985) investigated the denaturation effect on the myofibrillar proteins of bovine muscle and found that thaw resulted in muscle tissue having higher water loss and lower thermal stability.

2.5 EFFECT OF SODIUM TRIPOLYPHOSPHATES, GLAZING, AND PACKAGING

There are also freezing supplementary methods to increase fish shelf life in the refrigerator and freezer, such as the use of additives (Vogel *et al.*, 2006; Sallam, 2007), and the use of various materials for packaging and methods of packaging (Masniyom *et al.*, 2005b; Manju *et al.*, 2007). Phosphates are legally permitted additives that are widely used to aid processing or to improve the eating quality of many foods, particularly meat and fish products (FAO, 2006). Phosphate compounds have been used in fishery products to improve the functionality,

and to increase the water holding capacity (FAO, 2006). Phosphates are normally present in all living things and are an essential component of the human diet (FAO, 2006). Phosphates have been used widely to stabilise the quality of food products (Nguyen *et al*., 2012). Addition of phosphates to seafood products inhibited the growth of bacteria in fish stored in ice (Kim *et al.*, 1995) and retarded the oxidation of unsaturated fatty acid in seafood products (Masniyom *et al.*, 2005b). Although phosphates have been shown to improve meat quality, several countries have banned their use in raw meat production (Smith &Young, 2007). A phosphate is a salt of phosphoric acid; when a number of simple phosphate units are linked to form a more complex structure, this is known as a polyphosphate (FAO, 2006). The phosphates used in foods may be simple phosphates, diphosphates containing two phosphate units, tripolyphosphates containing three units, or polyphosphates containing more than three phosphate units (FAO, 2006). The increased water holding capacity produced by phosphates is achieved through muscle fiber expansion caused by electrostatic repulsions, which allows more water to be immobilized for the myofibril lattices (Offer &Trinick, 1983). Inhibition of oxidative changes may be through the chelation of pro-oxidative metal ions by phosphates (Matlock *et al.,* 1984). The effect of phosphates on the functional properties of meat products depends on the type of phosphate, the amount used, and the specific food products (Lindsay, 1996). The addition of polyphosphates to seafood products inhibited the growth of bacteria in fish stored in ice (Kim *et al.*, 1995; Zaika *et al.,* 1997), retarded the oxidation of unsaturated fatty acids in seafood products (Dziezak, 1990), and reduced the drip loss in fish under storage (Alvarez *et al.*, 1996; Masniyom *et al.*, 2005b).

Polyphosphates are a common cryoprotectant and legally permitted additives that are widely used to aid processing or to improve the eating quality of many foods, particularly meat and fish products (Aitken, 2001). Polyphosphates, which are polymers of phosphoric acid, have the distinction of being able to act as buffers, emulsifiers, dispersants, antioxidants, and sequestrates (Etemadian *et al.*, 2013). Use of polyphosphates in seafood can increase water-holding capacity of muscle, decrease thaw drip and cooking loss (Turan *et al.*, 2003), improve textural properties of comminuted meat products (Zhuang *et al.*, 2008), inhibit the growth of bacteria in fish stored in ice and retard the oxidation of unsaturated fatty acids in seafood products (Masniyom *et al.*, 2005b). Therefore the use of such materials in refrigeration can play an effective role in maintaining fish quality (Etemadian *et al.*, 2011). The surface film could act as a barrier to oxygen resulting in a reduction in oxidation during prolonged frozen storage (Tenhet *et al.*, 1981). Etemadian *et al.* (2011) reported that

treatment of *Rutilus frisii kutum* fillets with STPP and tetrasodium pyrophosphate (TSPP) and a mixture of these compounds was effective in inhibiting microbial growth and that treating with polyphosphates is an alternative method for improving the shelf life of fish and fish products. Etemadian *et al.* (2012) also reported that using a 2% solution of STPP with vacuum packaging resulted in lower bacterial load, TBA index, total sulfhydryl groups oxidation, and higher water holding capacity, sensory quality and increased shelf life.

2.5.1 Glazing

Glazing is the application of a layer of ice to the surface of frozen products by spraying, brushing on water or by dipping, and is widely used to protect products from the effects of dehydration and oxidation during cold storage (Žoldoš *et al.*, 2011). The ice layer sublimes instead of the fish below and it also excludes air from the surface of the fish, thereby reducing the rate of oxidation (Bogh-Sorensen, 2002). Good glazing practice can be beneficial, particularly when other aspects of storage and transport are far from ideal, but poor glazing involving partial thawing of the fish and slow refreezing in cold storage may do more harm than good (Johnston *et al.*, 1994). Kilinc *et al*. (2009b) reported that dipping frozen-thawed fish species (sea bass and saithe) in 5% phosphate solution improved their quality when compared with the control group (no STPP) and also concluded that phosphate treatment can be an alternative method for improving the quality of frozen-thawed fish. However, Turan *et al.* (2003) stated that glazing + packaging may be more important than phosphate treatment in frozen rainbow trout as it prevents drip loss and protects the moisture content of the inner and surface layers of the product. Turan *et al.* (2003) reported that use of sodium polyphosphate and sodium metaphosphate with NaCl was not effective at preventing drip loss in rainbow trout. Dehydration is one of the issues faced by seafood industry. Generally, two protective methods are used, usually in combination: glazing and packaging in order to protect the product from dehydration (Turan *et al.*, 2003). During frozen or cold storage, seafood products may develop surface drying and dehydration, which may lead to freezer burn, and may suffer from quality loss owing to oxidation or rancidity (Vanhaecke *et al*. 2010). Glazing of seafood products typically prevents the incidence of these processes during frozen storage (Jacobsen & Fossan, 2001).

2.5.2 Packaging

The protective effect of correctly chosen packaging methods and materials seems to improve the shelf life of frozen fish and meat products (Ahvenainen & Malkki, 1985). Vacuum packaging, can prevent the growth of some food-borne pathogens and spoilage bacteria commonly present on meat (Venter *et al*., 2006), and so, is widely used for packaging primal cuts for distribution to retailers (Pennacchia *et al.*, 2011). A plastic vacuumed packaging also resulted in a significant reduction of lipid oxidation in Atlantic salmon during frozen storage (Tolstorebrov *et al.*, 2014). The combination of packaging material and storage at −25 °C had a similar effect on the PV (Peroxide value, meq of O_2 kg^{-1}) of fat and TBARS (Thiobarbituric acid reactive substances, mg of malondialdehyde kg^{-1} of fish) formation as storage at −45 °C with lower quality packaging material (Indergard *et al*., 2013). In addition, Mathew & Shamsundar (2002) observed that there was a marginal decrease in non-protein nitrogen (NPN) content and the reduction in urea was reflected in the increase in total volatile base nitrogen (TVBN) and pH values during ice storage of shark (*Scoliodon laticaudus*) fillets, when fillets were packed in polythene bags and ice did not have direct contact with meat. It was reported that packaging is important in air blast freezing as it prevents dehydration, freezer burn and adherence by freezing and oxidation (Dempsy & Bansal, 2012). Therefore, packaging is an important common practice to freeze meat or fish products during transport and distribution. The detriment of packaging is a decrease in heat transfer and hence an increase in the freezing time due to the insulating properties of the packing material and excess enclosed air (Dempsy & Bansal, 2012).

2.6 SPOILAGE INDICATORS

There are many factors that influence the quality of fish, both during cultivation, handling and slaughter, and during processing and storage (Haard, 1992; Sigholt *et al.*, 1997; Hultmann *et al.*, 2004). The rate of spoilage increases shortly after capture. Depending on intrinsic factors such as species and season and technological factors such as handling practices prior to freezing, freezing rate, temperature of storage, or presence of protective barriers against oxidation, the practical storage life of frozen fish may vary substantially (Careche *et al.*, 1999). In addition, handling procedures pre-slaughter are important in terms of fish welfare (Damsgård, 2008; Brown *et al.*, 2010; Digre *et al.*, 2010; Hultmann *et al.,* 2012). The most common way a food loses its acceptable quality is from the growth of microorganisms or from non-microbial causes like lipid oxidation (Gashti, 2002). Food that

seems acceptable can be unsafe for consumption because of the growth or presence of pathogenic microorganisms or because of toxic chemicals (Gashti, 2002). Loss of freshness is due to a complex combination of biochemical, chemical, and physical processes, and is followed by muscle spoilage due to microbiological contamination (Delbarre-Ladrat *et al.*, 2006). Autolytic modifications include protease action on proteins and connective tissue, and also fat hydrolysis (Delbarre-Ladrat *et al.*, 2006).

TVBN analysis is an important parameter for determining the freshness of seafood products (Erkan, 2005). TVBN, including trimethylamine, dimethylamine, ammonia and other volatile basic nitrogen compounds, is produced mainly by bacterial decomposition of fish flesh (Liu *et al.,* 2010) i.e. associated with seafood spoilage, which increases as spoilage progresses. The content of TVBN in freshly caught fish is typically 5-20 mg 100 g^{-1} fish. 30- 35 mg 100 g^{-1} of muscle is generally regarded as the limit of acceptability for ice-stored cold water fish (Huss, 1995). In many studies, the TVBN level was very close to the rejection level when bacterial counts reached 7 log cfu g^{-1} (Koutsoumanis & Nychas, 2000). TVBN has been widely proposed as fish spoilage indicator since it showed a close relationship with the sensory score (Pons-Sanchez-Cascado *et al.*, 2006).

Changes in pH have also been used or proposed as indices of the freshness of iced aquatic species (Cheuk *et al.*, 1979; Fatima & Qadri, 1985; Erkan, 2005). Variations among the initial pH values may be due to the species, season, diet, and level of activity or stress during the catch as well as type of muscle (Ocano-Higuera *et al*., 2011). Post-mortem pH values can vary from 6.0 to 7.1 depending on species, season of catching, diet, level of activity and other factors (Li *et al.*, 2011). The drop in pH during rigor of fresh pre-rigor fillet is caused by conversion of glycogen to lactate during post-mortem glycolysis (Love, 1988). However, a higher lactate level can hardly explain the lower ultimate pH of the frozen pre-rigor fillets (Einen *et al.*, 2002). On the contrary, a slightly higher pH in frozen pre-rigor fish should be expected because the glycolytic enzymes tend to be denatured due to freezing and thawing. Freezing and thawing may cause muscle fibre shrinkage and increase the extra cellular space (Sigurgisladottir *et al*., 2000), and other cell damage (Ma & Yamanaka, 1991; Sikorski & Sun Pan 1992). This may change the osmotic equilibrium and the ionic strength, which could affect the muscle pH (Einen *et al*., 2002)**.** The increase in product pH during storage might be due to accumulation of metabolites of bacterial action on meat and meat products and deamination of meat proteins (Bachhil, 1982; Jay, 1986). The increase in pH value indicates

that alkaline compounds were accumulated through autolytic activities or microbial metabolism (Pons-Sanchez-Cascado *et al.*, 2006).

The high levels of moisture, nutrient content and high pH render fish an easily perishable product, often going bad within a short period of time after mortem (Li *et al.*, 2011). Loss of water binding capacity can often occur in fish fillets due to fiber shrinkage, cell damage, lower protein solubility, protein denaturation and aggregation taking place during the freezing process and after thawing (Mackie, 1993; Espinoza Rodezno *et al.*, 2013). Flores & Bermell (1984) reported that this decrease in the water holding capacity (WHC) can be due to the loss of the functionality of the myofibrillar proteins, which are principally responsible for water retention in the muscle (Hamm, 1986).

2.7 QUALITY ANALYSIS

No matter how effective a storage technique may be theoretically, a heavily contaminated or poor-quality product, eventually reduce the shelf life under that condition of storage (Ashie *et al*., 1996). Good initial handling techniques are therefore critical to reduced microbial loads (Ashie *et al.*, 1996). The freezing of fish is an effective method for long term preservation and it has been shown that fish stored for up to three months under ideal conditions cannot be distinguished from fresh fish regarding color, taste and texture (Cappeln *et al.*, 1999; Nielsen & Jessen, 2007). The most important changes occurring during storage of frozen foods are discoloration (Chandrasekaran, 1994), lipid oxidation, denaturation of protein (Bhobe and Pai 1986) and sublimation and recrystallization of ice (Londahl, 1997). These can result in off-flavors, rancidity, dehydration, weight loss, loss of juiciness, drip loss, and textural changes (Londahl, 1997). However, the susceptibility of different fish species to changes induced by frozen storage is significantly different (Badii & Howell, 2002).

Fish spoilage is a complex process in which physical, chemical and microbiological mechanisms are implicated (Davis, 1993). It is known that loss of freshness and spoilage pattern in fish varies markedly from species to species (Ocano-Higuera *et al*., 2011). Several post-mortem changes take place, immediately the fish dies, which are due to the breakdown of the cellular structure and biochemistry as well as to the growth of microorganisms that are either naturally associated with the fish, or with contamination during handling (Ehira & Uchiyama, 1987). There are many external and internal influencing factors that affect the freshness quality of fish, including processing parameters and different stages of post-mortem

transformation such as the early stage of rigor, rigor mortis, and end of the rigor, the autolysis process, and microbiological spoilage after death (Cheng *et al*., 2014). Such transformation stages consist of physical, chemical, physicochemical, and biochemical processes, followed by bacterial spoilage, protein degradation, and ATP decomposition, which accelerate the loss of freshness, destroy the structure of muscle, and degrade the quality of fish (Ayala *et al*., 2010). Among these factors influencing the freshness quality, textural and structural measurements play a critical role in the evaluation of fish quality (Cheng *et al*., 2014).

A wide variety of methods for measuring chemical and quality changes in frozen stored fish can be found in the literature, but due to a variety of complicating factors, such as seasonal changes, pH, pre-freezing history, size and fishing ground characteristics, it is difficult to find one method that gives good results for commercial or research application (Quaranta & Perez, 1983). Nowadays, methods for evaluating freshness and quality of different marine species are based on measurements of post-mortem changes associated with sensory, chemical and physical changes and microbiological growth (Gökodlu *et al.*, 1998).

2.7.1 Microbiological Quality Assessment

The microbiological quality of meat depends on the physiological status of the animal at slaughter, the spread of contamination during slaughter and processing, the temperature and other conditions of storage and distribution (Nychas *et al.,* 2008). The time to spoilage depends mainly on storage temperature and fish species (Gram & Huss, 1996).

The proliferation of microorganisms in foods depends on the properties of the product as well as its processing and storage conditions (Huis in't Veld, 1996). In most processed food products, the working assumption is that ‘‘zero time’’ parameters, including a target range of initial microbial population, can be set by proper design and control of the processing conditions (Koutsoumanis *et al.*, 2002). On the other hand, initial microflora in fresh fish can vary significantly, depending on a number of environmental factors such as sea water temperature, handling and processing after catch (Huss, 1995). Scientific attention on meat microbiology increased when large amounts of meat started being shipped long distances (e.g., from Australia to the UK) and continued in the 1950s with the growth of supermarkets (Anbalagan *et al*., 2014). The shelf life of fresh fish is greatly influenced by autolysis and growth of microbial populations (Jeyasekaran *et al.*, 2005). Autolysis or ‘self digestion’ is responsible for early spoilage in fish and begins with the autolytic endogenous enzymes

found throughout the fish muscle (Huss, 1995). Enzymatic and chemical reactions are usually responsible for the initial loss of freshness whereas microbial activity is responsible for the overt spoilage and thereby establishes product shelf life (Gram, 1995; Gram & Huss, 1996). Post-mortem biochemical changes in fish muscle are strongly influenced by the post-catch handling practices (Ocaño-Higuera *et al.*, 2011). These post-mortem changes that directly and strongly affect its quality and shelf life are associated with protein and ATP degradation, drop of pH, lipid oxidation, undesirable compounds production as trimethylamine (TMAN) and the molecular low weight volatile bases (TVBN), which are produced by bacterial action (Ocaño-Higuera *et* al., 2011). Likewise, the muscle undergoes changes in texture, water-holding capacity and colour (Shahidi *et al.*, 1994; Alasalvar *et al*., 2002). The meat chill chain starts with two main steps; the primary and secondary chilling. Both steps are important for microbiological stability, eating quality and production yield (Koutsoumanis & Taoukis, 2005). Although the European Union legislation requires a maximum final meat temperature of 7°C before transport or cutting there is not any published information related to limits on chilling time (Nychas *et al.*, 2008).

Many bacteria are unable to grow at temperatures below 10°C and even psychrotrophic organisms grow very slowly, and sometimes with extended lag phases, when temperatures approach 0°C (Huss, 1995). At 0°C the growth rate is less than one-tenth of the rate at the optimum growth temperature (Huss, 1995). However, in the temperature range from 0 to 25°C, microbiological activity is relatively more important, and temperature changes have greater impact on microbiological growth than on enzymatic activity (Huss, 1995).

Fish quality deteriorates rapidly with higher ambient temperature; therefore low temperature storage is the method of preservation recommended to retard microbial spoilage of fish (FAO, 1993). Although spoilage of seafoods is invariably attributed to the activity of contaminating microorganisms, loss of freshness, which often precedes microbial spoilage, primarily involves autolytic reactions controlled by endogenous enzymes present in the muscle tissue as well as those leaking from the gut (Ashie *et al*., 1996). Adenosine triphosphate (ATP) is the main source of energy for metabolic activity (Ashie *et al.*, 1996). In most postharvest fish, the initial catabolism of ATP normally results in accumulation of inosine monophosphate (IMP), which contributes to the pleasant flavor of meat (Ashie *et al.*, 1996). However, individual concentrations of each enzyme differ depending on the function of each muscle tissue (Mukundan *et al.*, 1986). In fact, fresh or lightly preserved fish

products are particularly susceptible to contamination (Erkan, 2005). For this reason, mesophilic bacteria or total viable counts (TVC), has been used in mandatory seafood standards in many countries (Cai *et al.*, 2014). The ICMSF (1986) has established a microbiological acceptability limit of 7 log CFU g^{-1} for fresh water and marine species that is fit for human consumption (Li *et al.*, 2011). The initial TVC values in the range of 3.35-4.58 log_{10} CFU g^{-1} are indicative of good fish meat quality (ICMSF, 1998).

2.7.2 Chemical Quality Assessment

Microorganism activities are also related to chemical compounds (TVBN, TMA, etc.), which are suggested as indicators of fish quality (Hobbs & Hodgkiss, 1982; Jørgensen *et al.*, 1988; Liston, 1992; Huss *et al*., 1997; Gram & Dalgaard, 2002). Because the changes during decomposition are known to be very complex, a single chemical index may not be a reliable indicator for a particular sample of fish (Castillo-Yáñez *et al.,* 2007). It is well established that glucose, lactic acid, and certain amino acids followed by nucleotides, urea and water-soluble proteins (Nychas *et al*., 2008) are catabolized by almost all the bacteria of the meat microflora involved in spoilage (McMeekin, 1982; Gill, 1986; Nychas *et al*., 2007). The former compounds are the essential energy sources for growth of microorganisms on the meat despite their negligible quantity in comparison to proteins (Anbalagan *et al.*, 2014). The concentration of these compounds can affect the type (e.g., saccharolytic, proteolytic), the rate of spoilage and, moreover, seems to be the principal precursor(s) of those microbial metabolite(s) that we perceive as spoilage (Tsigarida & Nychas, 2001; Skandamis & Nychas, 2002).

The amount of TMA produced is, therefore, a measure of the activity of spoilage bacteria in the fish and so is an indicator of the degree of spoilage (Anderson *et al*., 2008). TMA is the most commonly used volatile amine in the fish industry for evaluating freshness and spoilage in marine fish, since it is produced during chilled storage of fish from bacterial utilization of trimethylamine oxide (TMAO), a naturally occurring osmoregulatory substance found in most marine fish species (Koutsoumanis *et al*., 1999). The absence (or extremely low levels) of produc- tion of TMA in some fish species during storage has been reported Koutsoumanis *et al*., 1999).

2.7.3 Proteins

The infuence of freezing on the thermal stability of fish muscle proteins was subject of numerous studies (Srinivasan *et al*., 1997; Schubring, 1999). Changes in the stability of fish muscle proteins during frozen storage have been shown to depend on species and their habitat temperatures (Davies *et al*., 1994). Freezing and frozen storage is an excellent method of preserving the organoleptic attributes and protein functionality of fish flesh over prolonged periods of time (Careche *et al.*, 1999). Myofibrillar proteins, which are the main contributors imparting textural attributes and functional properties to muscle foods are the proteins most affected during cold storage (Careche *et al.*, 1999). They suffer denaturation and or aggregation due to factors such as partial dehydration due to freezing of water and the associated concentration of solutes in the tissue, formation of formaldehyde in certain species such as hake, or interactions of proteins with lipids or their oxidation products (Sikorski *et al.*, 1976; Matsumoto, 1979; Matsumoto, 1980; Shenouda, 1980; Haard, 1990; Sikorski & Kolakowska, 1994). Proteins most involved in the aggregation are myosin and actin, the relative proportion of each being dependent on species and storage conditions (Tejada *et al.*, 1996; Careche *et al.*, 1998; Del Mazo *et al.*, 1999; Careche *et al.*, 1999). The denaturation of myofibrillar proteins reported to be related to the post-mortem storage temperature (Fukuda *et al.*, 1982; Ogata *et al.,* 1998; Jasra *et al.*, 2001). However, these factors lead to structural changes of proteins causing denaturation and aggregation remains to be established (Careche *et al.*, 1999). The type of interactions responsible for aggregation of myofibrillar proteins requires further, investigation and attention has been drawn to the importance of hydrophobic interactions and disulfide bridges (Connell, 1975; Dingle *et al.,* 1977; Gill *et al*., 1979; Laird *et al.*, 1980; Lim & Haard, 1984; Rehbein & Karl, 1985; Owusu-Ansah & Hultin, 1986; Tejada *et al*., 1996; Careche *et al.*, 1998). Muscle proteins become gradually inextractable in salt solutions and, depending on species, conditions, and time of storage, these proteins will be extracted to more or less extent in sodium dodecyl sulfate (SDS) or SDS plus mercaptoethanol, and eventually, a residue nonextractable in these solutions can be obtained (Tejada *et al.*, 1996; Careche *et al.*, 1998). This unextracted residue has a structure resembling the sarcomere, which tends to become more pronounced the longer the storage time (Tejada *et al.*, 1996). Proteins most involved in aggregation are myosin and actin, the relative proportion of them being dependent on species and storage conditions (Tejada *et al*., 1996; Careche *et al*., 1998; Del Mazo *et al.*, 1999). Although it is accepted that protein denaturation of myofibrillar proteins occurs during frozen storage, little direct evidence on

the structural changes occurring in muscle proteins during freezing and frozen storage is found other than loss of protein function or functionality (Careche *et al.*, 1999).

The reason for changes in fish muscle proteins causing insolubility and formation of aggregates during frozen storage is not clear (Badii & Howell, 2002). However, it is widely acknowledged that the formation of ice crystals, presence of formaldehyde as well as lipid oxidation products may be involved (Shenouda 1980; German & Kinsella, 1985; Howell *et al*., 1996; Howell 2000; Saeed & Howell, 2002). Some cell disruption and destruction of muscle fiber occurs due to the formation of ice crystals (Burgaard & Jorgensen, 2011). Ice crystal size and distribution were influenced by the freezing rate, freezing temperature and frozen storage temperature (Hansen *et al*., 2003). It was also noted that in frozen fatty fish, oxidative changes in lipids (Saeed & Howell, 1999) and pigments affect the odour and colour as well as proteins (Saeed & Howell, 2002), while in lean fish, the main changes are reported to involve aggregation of proteins, which alter muscle texture (Sikorski *et al.,* 1976; Badii & Howell, 2002). Protein aggregation in frozen fish depends on the fish species, storage temperature, temperature fluctuation, storage time, and enzymatic degradation (Badii & Howell, 2002). The pH of the frozen/thawed Atlantic salmon (*Salmo salar)* fillets tended to increase during cold storage starting at a pH of 6.66 and reaching a pH of 6.22 after storage for 90 h (Einen *et al.*, 2002). Long-term frozen storage normally results in a marked hardening of the flesh, considered to be due to crossing-over of the fibrillary proteins (Sikorski *et al*., 1978). However, in species characterised by a low level of protein denaturation, it has not been possible to detect any marked increase in hardness during storage (Sikorski *et al*., 1978).

2.7.4 Physical Analysis

To date, sensory and microbiological analyses are most often used to evaluate the freshness, spoilage or safety of meat and meat products (European Commission, 2005). The disadvantages of sensory analysis, which is probably the most acceptable and appropriate method, is its reliance on highly trained panellists, which makes it costly and unattractive for routine analysis (Koutsoumanis & Nychas, 1999). Compared with sensory evaluation, textural measurements using instrumental analysis methods are better and more precise for reasons of reducing the variations during measurements arising from human factors (Cheng *et al.*, 2014). The texture of fish is a main feature used to appraise the freshness quality (Chéret

et al., 2006). The muscle of fish is very prone to become soft during the post-mortem condition, which further affects the textural quality of fish muscle (Cheng *et al.*, 2014)

2.7.4.1 Texture

Although fish texture can be measured and evaluated through sensory and instrumental methods, it is difficult to come to an agreement on which is the best method and there is no single method universally accepted and applied in fish and fish product industry (Cheng *et al.,* 2014). Texture includes a variety of characteristics, such as hardness (some authors call it toughness), springiness, chewiness, and some authors also include juiciness (Szczesniak, 1963), and even greasiness (Brandt *et al.*, 1963). In addition, species with a firmer texture have relatively smaller fibers than species with a softer texture (Ayala *et al.*, 2005). Among texture attributes, hardness is the most important to the consumer, as it decides the commercial value of a meat (Chambers & Bowers, 1993). The texture of fish is an important quality characteristic, and soft fillets are a problem for the fish industry (Hallett & Bremner 1988 Haard 1992 Andersen *et al.* 1997a; Sigholt *et al.*,1997). Soft flesh leads to reduced acceptability by the consumers (Ando1999). The texture of fish meat is influenced by several factors, such as fish species, age and size of the fish within the species, fat content and distribution of muscle fat, amount and properties of the proteins, and handling stress before slaughter (Hultmann & Rustad, 2004). The most widespread method normally used as an indicator of meat sensory hardness (tenderness) is the Warner–Braztler (WB) shear test, almost the sole methodology used in raw meat (Warner, 1928; Bratzler, 1932), and which is referred to in most papers (Culioli, 1995), even as a technique used for commercial application (Shackelford *et al.*, 1995; Shackelford *et al.*, 1999; Wheeler *et al.* 1997). Safari *et al.* (2001) found a very significant negative correlation between shear force and sensory tenderness in sheep meat; nevertheless, other authors have not found a good correlation between WB shear force and overall consumer acceptance (Platter *et al.*, 2003). There is another method – the texture profile analysis (TPA) – that, although it is widely used for texture assessment in other food (Guerrero & Gu'ardia, 1999), meat researchers rarely use. Nevertheless it has been successfully used for texture assessment in fish muscle (Veland & Torrissen, 1999). The main advantage of TPA is that one can assess many variates with a double compression cycle (de Huidobro *et al.*, 2005). Variates that can be assessed with this analysis are: hardness, springiness, cohesiveness, adhesiveness, resiliency, fracturability, gumminess, chewiness, etc. In meat the variates assessed are hardness, springiness, and

cohesiveness; the three altogether permit the calculation of chewiness (Ruiz de Huidobro *et al.*, 2001; de Huidobro *et al.*, 2005). Several authors report that reproducibility of texture measurements is affected by mixing samples from different fillet locations (Botta, 1991; Einen & Thomassen, 1998; Sigurgisladottir *et al.*, 1999; Casas *et al.*, 2006). The TPA of various foodstuffs including fruits, vegetables, bakery and meat products have been reported (Penfield & Campbell, 1990; Caine *et al.*, 2003). Instrumental texture assessment on meat is made by means of a texturometer, a device that allows tissue resistance both to shearing and to compression to be measured (de Huidobro *et al.*, 2005).

Different handling methods play a significant role in fish texture measurements and dramatically influence the freshness of fish (Cheng *et al*., 2014). Fish muscle texture depends on many intrinsic biological factors that are related to muscle fibre density, and involving both fat and collagen content (Li *et al*., 2011). The fish death triggers autolytic and microbiological processes that make the muscle softer and less elastic (Ólafsdóttir *et al*., 2004). The most common texture defects are muscle softening and gaping formation caused by pre- and post-mortem treatment. The existing problems are mostly associated with the changes of chemical compositions and the degradation of muscle proteins (Aussanasuwannakul *et al.*, 2010). Storage temperature during handling and operating processes generally has a distinctive effect on fish texture measurements (Pearce *et al.*, 2011; Cheng *et al.*, 2014). Among texture attributes, firmness also termed as hardness, an essential evaluating parameter of fish freshness is closely associated with the human visible acceptability of fish products (Cheng *et al.*, 2014). The firmness of the raw muscle is a critical parameter that determines the acceptability of the seafood products (Veland & Torrissen, 1999; Casas *et al.*, 2006). This property depends on the connective tissue, consisting of mainly collagen (responsible for tensile strength) and the myofibrils, consisting of myosin and actin (Erdogdu & Balaban, 2000; Casas *et al.*, 2006).

In general, fish meat is softened rapidly during chilled storage (Montero & Borderias, 1990). Degradation in the Z-line of myofibrils was reported as a cause of meat softening in fish meat (Masniyom *et al.*, 2005a). Furthermore, the changes in connective tissue are associated with textural properties of fish meat (Masniyom *et al.*, 2005a). The connective tissue binds the muscle cells together (Masniyom *et al.*, 2005a). Weakening of the endomysium, a part of the connective tissue, was observed with the progression of the postmortem softening (Ando *et*

al. 1995); therefore, it is important to delay or prevent the progression of this phenomenon for maintaining the fish freshness (Masniyom *et al.*, 2005a).

Textural differences occur between and within species due to differences in protein structure, muscle fiber diameter and chemical contents, and measurement methods (Jonsson *et al.*, 2001). Whilst several researchers have associated low muscle pH with tough texture and high drip loss (De Vido *et al.*, 2001), others suggest the involvement of several enzymes in texture deterioration during storage (Sato *et al.,* 1991). Haard (1992) suggested that texture of fish flesh was influenced by many factors including post-mortem, pH decline, proteolysis, fat content, composition and its distribution in the fish muscle. Several publications have indicated that the muscle tissue progressively loses its firmness after rigor mortis (Azam *et al.,* 1989; Montero & Borderias, 1990; Anderson *et al.*, 1997), a process mainly related with enzymatic degradation of muscle proteins (Papa *et al*., 1997). However, hardness reduction tested by instrument correlated well with texture softening of raw fillets judged by sensory evaluation (Liu *et al.*, 2010). Meat spoilage is not always evident and consumers would agree that gross discoloration, strong off-odors, and the development of slime would constitute the main qualitative criteria for meat rejection (Nychas *et al*., 2008). Effective method texture profile analysis (TPA) is capable of giving a meaningful interpretation to a series of textural parameters (Rahman & Al-Farsi, 2005; Herrero *et al.*, 2007). Currently, TPA for fish texture measurement as a standard method is still generally referred to in the literature for textural description and analysis (Sigurgisladottir *et al.*, 1999; Martinez *et al.*, 2004; de Huidobro *et al.*, 2005; Chen, 2009; Chen & Stokes, 2011). Orban *et al*. (1997), reported that this study results which are limited to the experimental conditions examined, show how two different farming system can influence the chemical composition of fish which probably leads to other differences, mainly in texture.

2.7.4.2 Colour

The color is one of the most important parameters used to analyse the quality of seafood products. Several authors have attributed colour loss in fish muscle during storage to the oxidation of proteins with haemo groups, such as haemoglobin and myoglobin (Chaijan *et al.*, 2005). Mantilla *et al.* (2008) reported that the color change in tilapia fillets was related to a change in lightness (L∗ value), whereas no significant changes were found in redness (a∗ value) or yellowness (b∗ value). Espinoza Rodezno *et al.* (2013) stated that freezing changes

muscle contraction and texture, and it is therefore likely that the light reflectance properties of the muscle are changed during freezing. This would affect the visual impression of fillet color (Einen *et al.*, 2002). It was also noted that the higher initial color score in pre-rigor fillets is consistent with the earlier findings of Skjervold *et al.* (2001). They showed that the color change due to pre-rigor filleting was related to a change in lightness (L* value, CIE, 1976), whereas no significant changes were found in redness (a* value) or yellowness (b* value) (Einen *et al.*, 2002). Freezing and thawing may promote oxidation of carotenoids in Atlantic salmon fillets (Andersen & Steinsholt, 1992; Refsgaard *et al.*, 1998; Sheehan *et al.*, 1998), and may explain lower color scores of frozen pre-rigor fillets. The intensity of redness (a∗) increases with increasing carotenoid contents in raw flesh of Atlantic salmon, while lightness (L∗) decreases and yellowness (b∗) is not affected (Skrede & Storebakken, 1986). Higher a∗ values are observed with increasing Roche Color Card scores (Christiansen *et al.*, 1995). Increasing fat content in raw fillets has been shown to coincide with increasing L* and b* values (Mørkøre *et al.*, 2001). Furthermore, the thickness of the flesh is another factor affecting color values (Stien *et al.*, 2006; Erikson & Misimi, 2008). Erikson & Misimi (2008) suggested the computer vision method was considered suitable for automated (online) quality control and grading of salmonoid fillets according to color. The changes in skin and fillet color of anesthetized and exhausted Atlantic salmon (*Salmo salar*) were determined immediately after killing, during rigor mortis, and after ice storage for 7 d (Erikson & Mismi, 2008).

2.8 CONCLUSIONS

The current review clearly concludes that quality loss during the freezing process of fillets is a major concern for seafood industry. Seafood is processed and frozen on vessel immediately after catch or frozen on land at the factory after cleaning, filleting and cutting (Gashti, 2002). Post-mortem cold storage of fish muscle results in alterations that make the meat unpleasant to eat (Jasra *et al*., 2001). Deterioration of fish during frozen storage depends on many factors including fish species, storage temperature, time, and enzymatic degradation (Ang & Hultin, 1989; Hsieh & Regenstein, 1989; Badii & Howell, 2001). Additionally, those alterations determine the quality of frozen fish, especially in terms of protein functionality (Benjakul *et al.*, 2003). Therefore, products should reach the consumers as fast as possible, as fresh-chilled products to obtain maximum quality (Einen *et al*., 2002). Freezing rate (Sikorski & Sun Pan, 1992), temperature (Love, 1962), time of frozen storage (Love & Olley, 1965), and the rate

and method of thawing (Hewitt, 1969; Love & Haq, 1970) are all factors known to affect quality (Einen *et al*., 2002). Storage time and temperature are the major factors affecting the rate of loss of quality and the shelf life of fish (Whittle, 1997).

Air-blast freezing (–30°C, 4 m/s) increased the freezing rate, resulting in superior preservation of the microstructure (Cheng *et al.*, 2014). Nowadays, the evaluation of the degree of meat spoilage is usually made either subjectively, based on sensory assessment or by microbiological analyses (Byun *et al*., 2003; Dainty, 1996; Ellis & Goodacre, 2001; Nychas *et al*., 2008; Papadopoulou *et al.*, 2013). Compared with sensory evaluation, it has been proved that the textural measurements by instrumental analysis methods are better and more precise by reasons of reducing the variations during measurements arising from human factors (Cheng *et al*., 2014). Texture and structure measurements and evaluations for fish and fish fillet are of significance in freshness quality control and assurance, and product development in the seafood industry (Cheng *et al*., 2014). Most importantly, microbiological and physiochemical parameters could provide more information and further interpretation to evaluate the texture of barramundi fillets. Although good handling and storage practices are broadly known, sometimes, due to technological or economic factors, they cannot be completely followed (Careche *et al.,* 1999). Even so, to be useful and for correct interpretation of results a thorough knowledge of handling and processing conditions prior to sampling is essential (FAO, 2014). The proposed research program aims to achieve best practice by testing on barramundi and providing an optimised process back to industry. The value of barramundi production is around $A8 million at farm gate (Seafood CRC, 2014). The proposed research is to investigate the effect of pre and post freezing treatments on the quality and shelf life of barramundi. Pre and post treatments include chilling, time and temperature abuse before freezing; different, thawing techniques, ice forms, glazing, packaging, and use of phosphates.

CHAPTER 3

GENERAL METHODOLOGY

CHAPTER 3: GENERAL METHODOLOGY

This chapter describes all common and general materials and methods used in this research. This study incorporates five different experiments. Each experiment tested different treatment either before freezing or after freezing. Each treatment had four replicates. Samples were thawed under running tap water (18-20^{o}C) just before analysis. Sub samples from the thawed fish were analysed for their quality and shelf life. Sub samples from the thawed fish were analysed for its quality and shelf life.

3.1 SAMPLE PREPARATION

Marine water cultured barramundi from Marine Farms Pty Ltd, Exmouth, Western Australia, Australia (latitude 21° 54' S; longitude 114° 10' E) were used in all the experiments in the present study. The freshly Ikijimied fish were procured from the producer prior to their domestic sales. The fish were then kept at a temperature of 0-5ºC throughout harvest and shipment. The average whole weight of barramundi used was 3.35 kg. Upon arrival, each whole barramundi was washed under running tap water (18-20°C) and filleted prior to packing. The fillets were then cut into slices of approximately 2 cm thick. Each fillet portion (approximately 200 g), was then packed into a separate sealed polythene bag.

3.2 FREEZING

Fillets were individually frozen as and when needed as per the individual experiments, in an air blast freezer at -80°C for 8 hours and were then stored in a freezer at -20°C for 20 days. Microbiological and physiochemical analyses of barramundi fillets were then carried out on the 21st day from the commencement of storage. Each experimental treatment had four replicates, with a single fillet (1.05 kg) used per replicate. Sub samples from the thawed fish were analysed for their quality and shelf life. In order to measure the microbiological and physiochemical properties, the fillets were sub-sampled in the laboratory under hygienic conditions and macerated in an acid washed glass blender. Table 3.1 shows the list of chemicals used for analysis. Quality and shelf life of barramundi were evaluated using the microbiological and physiochemical analyses described below:

3.3 MICROBIOLOGICAL ANALYSIS

Total viable count (TVC) was determined using standard plate counts according to the method described by Association of Official Analytical Chemists (1995) with minor modifications. Samples of fish slices were aseptically weighed and homogenised in a 0.1% peptone solution, with a sterile glass rod. Further serial dilutions were made and 0.1 ml of each dilution was pipetted onto the surface of the plate count agar (Oxoid, Department of Agriculture, Perth, WA), after which all colonies were counted. The number of colony forming units (c.f.u.) was counted after 48 ± 3 h incubation at 25°C.

3.4 PROXIMATE ANALYSIS

The muscle was homogenized, and the moisture content of 5 g of homogenized sample was determined by drying the sample at 105°C until a constant weight was obtained (AOAC, 1990). Ash was determined using the basic AOAC (1990) method, involving heating the samples in a furnace at 550°C for 8–12 h. Total protein nitrogen content was measured by the standard method as described in AOAC (1990) with a Kjeltec Auto 1030 analyzer (Tecator, *Höganäs*, Sweden), and the final protein content is expressed on dry matter basis. The results are presented as mean ± standard error (SE) of determinations for four replicate samples.

3.5 pH

The pH of barramundi fillets was determined using a TPS WP-80 pH meter (TPS Pty, Ltd., Springwood, Qld, Australia). Five grams of barramundi meat was ground with 45 mL of distilled water in a test tube with a glass rod and pH was then measured. The mean of four replicate samples was used as mean pH.

3.6 TOTAL VOLATILE BASE NITROGEN (TVBN)

The total volatile base nitrogen (TVBN) was determined by the macro-Kjeldahl method (Pearson, 1981). The analysis was based on titration with 0.1 M sodium hydroxide of a distillate of fish muscle triturate (10 g) in water (300 mL) and magnesium oxide (2 g). The results were expressed in mg 100/g of muscle. The analyses were run in four replicate samples.

3.7 TEXTURE

3.7.1 Sample Preparation

Figure 3.1 indicates the section of the barramundi fillets analysed for rheological parameters. The middle (belly) of each fillet was collected and cut into 3.0 × 2.0 × 1.5 cm pieces with a sharp knife. Four fillets per treatment were subjected to hardness, cohesiveness, springiness, gumminess, chewiness and stiffness testing with each sample punctured on the middle of the fillet. Four determinations of each texture variable were made on each fillet. Prior to analysis, samples were allowed to thaw to equilibrate at room temperature (18–20°C, 2 h).

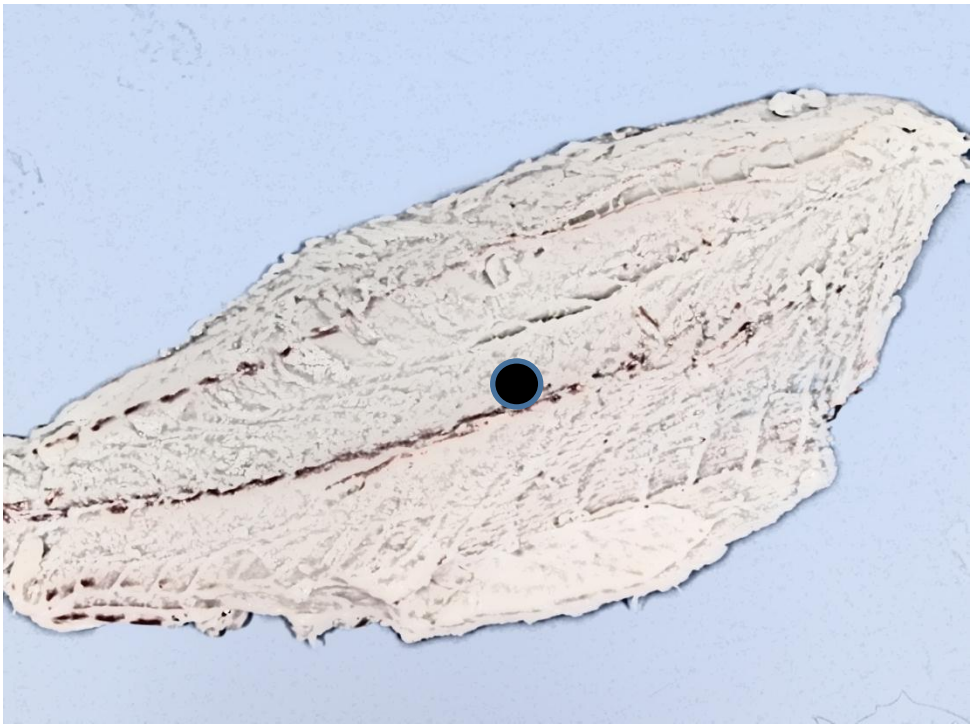

Figure 3.1: The rheological parameters of barramundi fillets were measured at the location marked on the fillet

The rheological parameters of barramundi fillets: hardness (firmness), springiness, cohesiveness, gumminess, chewiness and stiffness were measured at the location marked on the fillet.

3.7.2 Texture Profile Analysis (TPA)

Texture profile analysis was conducted using a texture analyser (TA Plus; AMETEK Lloyd Instruments Ltd., Fareham, UK). The machine interfaced to a personal computer with Nexygen™ Software (Version 4.6; AMETEK Lloyd Instruments Ltd.) following the methods explained earlier by Zaharah & Singh (2011), with a load cell of 500 N. Measurements were taken with a Magness-Taylor probe (4 mm in diameter) and the crosshead operated at a constant speed of 2 mm s^{-1} to 7.5 mm depth. A trigger force of 1 N was used to puncture the fillets for all determinations. The test conditions were two consecutive cycles of 30% compression with 5 s between cycles. Each sample was placed on top of the square-base table and the gap size between the sample and the probe was at least 2 mm. The following rheological parameters of the barramundi fillets were determined (with units in brackets): fillet hardness (firmness) (Newtons (N), springiness (cm), gumminess (kilogram force (kgf)), chewiness (kilogram force millimetre (kgf.mm)) and stiffness (kg force per millimetre (kg f mm^{-1})). No specific expressed units were used for measurements of cohesiveness.

3.8 COLOR MEASUREMENT

Colour measurements were performed on sample using a colorimeter Minolta Spectrophotometer CM-508i. The colour reading includes L* denotes lightness on a 0 to 100 scale from black to white; a* (+) red or (-) green; and b* (+) yellow or (-) blue. The instrument was calibrated using a white standard plate. The values, expressed as L* (lightness), a* (redness) and b* (yellowness) units, were obtained from 4 different areas of the upper surface for each chop, and a minimum of 3 chops per treatment block were analysed to obtain an average value.

Table 3.1: List of chemicals used

Chemical/Product Name	Manufacturer/Supplier
Ethanol	AJAX FINE CHEM
Hydrochloric acid	AJAX FINE CHEM
Magnesium Oxide	ACROS ORGANICS/THERMO FISHER
Ortho phosphoric acid	AJAX CHEMICALS
Sulphuric Acid	AJAX FINECHEM
Antifoam agent	LABCHEM/AJAX CHEMICALS PTY LIMITED

3.9 STATISTICAL ANALYSIS

All collected data were stored in Excel and Word document format. Statistical analyses were performed using Statistical Package for the Social Sciences software version 19.0 (IBM Corp., Armonk, NY). All results data were expressed as means ± SE. Analysis of variance followed by Tukey's post hoc analysis was used to determine significant differences between treatments at $\alpha < 0.05$ levels. All data were tested for homogeneity of variance by Levene's test. Regression analyses were conducted to determine correlations using Microsoft Excel.

CHAPTER 4

EFFECT OF TIME-TEMPERATURE ABUSE

CHAPTER 4: EFFECT OF TIME-TEMPERATURE ABSUE ON MICROBIOLOGICAL AND PHYSIOCHEMICAL PROPERTIES OF BARRAMUNDI (*LATES CALCARIFER*, BLOCH) FILLETS

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4.1 INTRODUCTION

Following slaughter, fish are likely to be exposed to inconsistent temperatures during transportation and subsequent storage. This temperature abuse may accelerate quality and shelf life changes in the fillets. Therefore, it is important that adequate chill/storage procedures are in place to ensure that perishable foods not only achieve their required shelf lives but are safe for consumption by the end user (Jol *et al.*, 2006). Exposure to higher temperatures and/or fluctuations of storage temperature produces cumulative adverse effects on the quality of stored foods, which is the primary cause of damage to food marketed through retail channels (Blond & Le Meste, 2004).

Temperature control of stored fish is essential, not only to maintain quality but also to minimize changes in microbiological and physiochemical properties. The optimum range for successfully handling and displaying refrigerated foods is -1 to 2°C, certainly never higher than 5°C (Almonacid-Merino & Torres, 1993). However, many of the retail display cases cycle up to 7 to 10°C (Young, 1987). Domestic refrigerator temperatures are often higher than the recommended temperature of 5°C (Notermans *et al*., 1997; Nauta *et al*., 2003). The usual method to preserve the quality of fresh fish is storage in ice fish in ice. However, during iced storage of raw fish the quality of the fish muscle will deteriorate (Hultmann & Rustad, 2007). Poor postharvest handling practices may enhance the rate of deterioration (Ashie *et al.*, 1996). Freezing and frozen storage of fish can also lead to structural and physiochemical changes that alter the properties of the fish muscle causing quality deterioration to different degrees (Burgaard & Jørgensen, 2011). In addition, the longer the storage period the softer the texture of the fish will be (Jiang *et al*., 2008). The impact of time-temperature abuse differs between species of fish. There is currently no information available on the quality and shelf life changes in barramundi (*Lates calcarifer*) fillets caused by exposure to different temperatures prior to freezing (pre-blast freezing temperatures).

The expansion of barramundi markets is presently limited because of quality loss during the freezing process (Zakhariya *et al.*, 2014). Barramundi has a reputation as a high quality commercial species, with premium eating qualities (Australian Barramundi Farmers Association, 2008). Barramundi is an important and valuable product in the Australian fish processing industry, with an estimated aquaculture farm gate value of AU$45 million per annum (Australian Barramundi Farmers Association, 2014). However, the fish may occasionally be subjected to inadequate storage conditions (temperature abuse) for a limited period during distribution from slaughter to consumer. The aim of the present experiment was to investigate the effects of different pre-blast freezing temperature periods and temperatures (time-temperature index) on the quality and shelf life of barramundi fillets.

4.2 MATERIALS AND METHODS

4.2.1 Sample Preparation

Please refer to Chapter 3 (General methodology)

4.3 EXPERIMENTAL PROCEDURE

Fillets were divided into four batches, with four replicates of each: the control (fresh) batch of barramundi fillets (BF) were analysed immediately after being received and were not subjected to freezing, the second batch underwent pre-blast freezing treatment at 5°C for 0 h, 1 h, 1 day, 2 days, 4 days, 8 days, and 16 days, the third batch underwent pre-blast freezing treatment at 0°C for the same time intervals, and the fourth batch underwent pre-blast freezing treatment at -20°C for the same time intervals before blast freezing. All barramundi fillets were then individually frozen on a polystyrene dish in an air blast freezer with 5 m/s air velocity at –80°C for 8 h at the Department of Agriculture and Environment, Curtin University, Perth, Western Australia. All the frozen fillets were subsequently stored in a freezer at –20°C at CARL for 20 days. At the end of each treatment samples were thawed under running tap water (18- 20°C). Microbiological and physiochemical analyses of barramundi fillets were then carried out. The fillets were sub sampled in the laboratory under hygienic conditions and macerated in an acid washed glass blender before being analysed for their quality and shelf life. Quality and shelf life of barramundi fillets were evaluated using the microbiological and physiochemical analyses described below:

4.4 ANALYTICAL DETERMINATIONS

Please refer Chapter 3 (General methodology)

4.4.1 Microbiological Analysis

Please refer Chapter 3 (General methodology)

4.4.2 Proximate Analysis

Please refer Chapter 3 (General methodology)

4.4.3 pH

Please refer Chapter 3 (General methodology)

4.4.4 Total Volatile Base Nitrogen (TVBN)

Please refer Chapter 3 (General methodology)

4.4.5 Texture

Please refer Chapter 3 (General methodology)

4.4.5.1 Sample Preparation

Please refer Chapter 3 (General methodology)

4.4.5.2 Texture Profile Analysis (TPA)

Please refer Chapter 3 (General methodology)

4.4.6 Color Measurement

Please refer Chapter 3 (General methodology)

4.5 STATISTICAL ANALYSIS

Please refer Chapter 3 (General methodology)

4.6 RESULTS

The proximate composition of fresh barramundi fillets was 72.38±0.93% w.b. (wet basis) moisture, 1.02±0.04 % ash and 62.54±0.47 % d.b. (dry basis) protein. Fillet moisture content increased over time and was significantly higher ($P<0.05$) after 16 days of PBF temperature period than in fresh fillets for each of the PBF temperatures (Table 4.1). The increase in mean % moisture content of fillets subjected to 16 days of PBF temperature period at 5°C, 0°C and -20°C was 5.24% w.b., 3.86% w.b. and 3.17% w.b., respectively. One day of PBF temperature period resulted in a significant ($P<0.05$) increase in fillet ash content at each tested temperature except at 5°C. Ash content then decreased significantly ($P<0.05$) by 2 days PBF temperature period at 5°C and 0°C, and by 4 days PBF temperature period at -20°C (Table 4.1).

Table 4.1: Changes in the moisture content % w.b., ash content % and protein content % d.b. of barramundi (*Lates calcarifer*) fillets before freezing (BF) and pre-blast freezing treatments (PBF) at 0°C, 5°C and, -20°C for 0h, 1h, 1 day, 2 days, 4 days, 8 days and 16 days.

Moisture content %							
	0h	1h	1 day	2 days	4days	8days	16days
5°C	$72.38\pm0.93^{a}_{A}$	$72.52\pm0.39^{a}_{A}$	$73.57\pm0.11^{a}_{A}$	$73.92\pm0.12^{a}_{A}$	$74.03\pm0.90^{a}_{A}$	$75.34\pm0.09^{ab}_{B}$	$77.62\pm0.12^{b}_{A}$
0°C	$72.38\pm0.93^{a}_{A}$	$71.78\pm0.31^{a}_{A}$	$72.53\pm0.30^{a}_{A}$	$72.27\pm0.05^{a}_{A}$	$73.79\pm0.39^{ab}_{A}$	$74.00\pm0.31^{ab}_{A}$	$76.24\pm0.03^{b}_{B}$
-20°C	$72.38\pm0.93^{ab}_{A}$	$71.79\pm0.25^{a}_{A}$	$72.45\pm0.96^{ab}_{A}$	$73.08\pm0.74^{abc}_{A}$	$74.69\pm1.09^{abc}_{A}$	$75.37\pm0.40^{bc}_{A}$	$75.55\pm0.16^{c}_{C}$
Ash content %							
	0h	1h	1 day	2 days	4days	8days	16days
5°C	$1.02\pm0.04^{abc}_{A}$	$1.18\pm0.08^{bc}_{A}$	$1.24\pm0.07^{c}_{A}$	$0.96\pm0.01^{ab}_{B}$	$0.92\pm0.01^{a}_{B}$	$0.91\pm0.02^{a}_{B}$	$0.90\pm0.00^{a}_{B}$
0°C	$1.02\pm0.04^{a}_{A}$	$1.11\pm0.05^{ab}_{A}$	$1.26\pm0.07^{b}_{A}$	$1.02\pm0.00^{a}_{B}$	$1.10\pm0.03^{ab}_{A}$	$1.00\pm0.02^{a}_{A}$	$0.97\pm0.02^{a}_{A}$
-20°C	$1.02\pm0.04^{ac}_{A}$	$1.10\pm0.04^{ac}_{A}$	$1.33\pm0.03^{b}_{A}$	$1.18\pm0.06^{bc}_{A}$	$1.09\pm0.04^{ac}_{A}$	$0.96\pm0.02^{ac}_{AB}$	$0.92\pm0.01^{a}_{AB}$
Protein content %							
	0h	1h	1 day	2 days	4days	8days	16days
5°C	$62.54\pm0.47^{a}_{A}$	$61.15\pm0.29^{a}_{A}$	$60.26\pm0.36^{ab}_{B}$	$60.63\pm0.11^{a}_{AB}$	$58.05\pm0.37^{bc}_{B}$	$57.33\pm0.22^{c}_{C}$	$55.99\pm0.16^{c}_{C}$
0°C	$62.54\pm0.47^{a}_{A}$	$62.23\pm0.63^{a}_{A}$	$62.03\pm0.50^{a}_{AB}$	$61.08\pm0.69^{ab}_{B}$	$61.28\pm0.20^{ab}_{C}$	$61.27\pm0.27^{ab}_{B}$	$59.26\pm0.36^{b}_{B}$
-20°C	$62.54\pm0.47^{ab}_{A}$	$61.47\pm0.72^{a}_{A}$	$62.53\pm0.71^{ab}_{A}$	$63.04\pm0.89^{abc}_{A}$	$64.25\pm0.53^{bc}_{A}$	$64.98\pm0.31^{bc}_{A}$	$65.08\pm0.02^{c}_{A}$

All values are the means ± SE of four replicates, n=4
Values followed by different superscript letters in the same row are significantly different at α=0.05
Values followed by different subscript capital letters in the same column, within a subset are significantly different at α=0.05

The protein content of fillets decreased as pre-blast freezing temperature period increased at 5°C and 0°C, with protein content significantly lower ($P<0.05$) after 16 days at 0°C and after 4 days and longer at 5°C compared to fresh fillets. Conversely, the protein content increased over time at -20°C, with protein content significantly higher ($P<0.05$) after 16 days treatment than in fresh fillets and after 4 days than at the other temperatures (Table 4.1). pH increased significantly ($P<0.05$) over the pre-blast freezing temperature period (0-16 days) from 6.34±0.00 to 6.78±0.00 at 5°C and to 6.68±0.00 at 0°C. However, pH increased to a much lesser degree over 16 days at -20°C from 6.34±0.00 to 6.49±0.01 (Table 4.2). Fillets that underwent PBF temperature period at 5°C for 16 days had significantly higher ($P<0.05$) pH than at 0°C and -20°C.

Table 4.2:Changes in the pH value of barramundi (*Lates calcarifer*) fillets before freezing (BF) and pre-blast freezing treatments (PBF) at 0°C, 5°C and, -20°Cfor 0h, 1h, 1 day, 2 days, 4 days, 8 days and 16 days.

Treatment	0h	1h	1day	2days	4days	8days	16days
5°C	$6.34\pm0.00^{a}{}_{A}$	$6.34\pm0.01^{a}{}_{A}$	$6.49\pm0.00^{cde}{}_{B}$	$6.51\pm0.00^{def}{}_{A}$	$6.56\pm0.01^{efg}{}_{B}$	$6.63\pm0.00^{gh}{}_{B}$	$6.78\pm0.00^{i}{}_{C}$
0°C	$6.34\pm0.00^{a}{}_{A}$	$6.39\pm0.02^{ab}{}_{A}$	$6.42\pm0.01^{abcd}{}_{A}$	$6.46\pm0.01^{bcd}{}_{A}$	$6.55\pm0.00^{efg}{}_{B}$	$6.58\pm0.00^{fg}{}_{B}$	$6.68\pm0.00^{h}{}_{B}$
-20°C	$6.34\pm0.00^{a}{}_{A}$	$6.38\pm0.02^{ab}{}_{A}$	$6.40\pm0.00^{abc}{}_{A}$	$6.44\pm0.02^{bcd}{}_{A}$	$6.41\pm0.04^{abc}{}_{A}$	$6.44\pm0.01^{bcd}{}_{A}$	$6.49\pm0.01^{cde}{}_{A}$

All values are the means ± SE of four replicates, n=4
Values followed by different superscript letters in the same row are significantly different at α=0.05
Values followed by different subscript capital letters in the same column are significantly different at α=0.05

The TVBN of barramundi fillets rose from 6.25±0.02 to 54.14±0.18 mg 100 g^{-1}, and 49.19±0.05 mg 100 g^{-1} after 16 days when subjected to PBF temperature period at 5°C and 0°C, respectively, but only to 11.63±0.23 mg 100 g^{-1} at -20°C. TVBN levels increased significantly ($P<0.05$) compared to fresh fillets when fillets were exposed to PBF temperature period for one hour and longer at all temperatures (Table 4.3).

Table 4.3: Changes in the TVBN value (mg 100 g^{-1}) of barramundi (*Lates calcarifer*) fillets before freezing (BF) and pre-blast freezing (PBF) treatments at 0°C, 5°C and, -20°C for 0h, 1h, 1 day, 2 days, 4 days, 8 days and 16 days.

Treatment	0h	1h	1day	2days	4days	8days	16days
5°C	$6.25\pm0.02^{a}_{A}$	$10.45\pm0.02^{cd}_{B}$	$12.01\pm0.05^{f}_{B}$	$17.55\pm0.11^{g}_{C}$	$29.28\pm0.22^{i}_{C}$	$41.46\pm0.22^{j}_{C}$	$54.14\pm0.18^{l}_{C}$
0°C	$6.25\pm0.02^{a}_{A}$	$9.61\pm0.23^{bc}_{A}$	$12.38\pm0.19^{f}_{B}$	$12.51\pm0.25^{f}_{B}$	$17.37\pm0.07^{g}_{B}$	$19.17\pm0.05^{h}_{B}$	$49.19\pm0.05^{k}_{B}$
-20°C	$6.25\pm0.02^{a}_{A}$	$9.44\pm0.22^{b}_{A}$	$10.14\pm0.01^{bcd}_{A}$	$10.24\pm0.06^{bcd}_{A}$	$10.56\pm0.30^{d}_{A}$	$10.77\pm0.27^{de}_{A}$	$11.63\pm0.23^{ef}_{A}$

All values are the means ± SE of four replicates, n=4
Values followed by different superscript letters in the same row are significantly different at α=0.05
Values followed by different subscript capital letters in the same column are significantly different at α=0.05

TVC on fresh fillets was 2.44±0.03 log CFU g^{-1}. 16 days PBF temperature period at 5°C, 0°C, and -20°C resulted in TVC values increasing significantly ($P<0.05$) to 8.58±0.20, 9.96±0.12 and 4.18±0.06, log CFU g^{-1} respectively. TVC increased significantly ($P<0.05$) between 0 days and 4 days, and between 4 days and 16 days PBF temperature period at 5°C and 0°C. However PBF temperature treatment at -20°C had relatively minimal impact as TVC was significantly lower ($P<0.05$) with treatment at -20°C than at 0 and 5°C for 8 days and longer (Table 4.4).

Table 4.4: Changes in the TVC (log CFU g^{-1}) of barramundi (*Lates calcarifer*) fillets before freezing (BF) and pre-blast freezing treatments (PBF) at 0°C, 5°C and, -20°C for 0h, 1h, 1 day, 2 days, 4 days, 8 days and 16 days.

Treatment	0h	1h	1day	2days	4days	8days	16days
5°C	$2.44\pm0.03^{a}_{A}$	$2.62\pm0.15^{ab}_{A}$	$3.33\pm0.16^{bc}_{A}$	$3.42\pm0.22^{cd}_{A}$	$3.76\pm0.08^{cd}_{A}$	$6.38\pm0.12^{f}_{B}$	$8.58\pm0.20^{g}_{B}$
0°C	$2.44\pm0.03^{a}_{A}$	$2.61\pm0.18^{ab}_{A}$	$3.29\pm0.14^{bc}_{A}$	$3.49\pm0.23^{cd}_{A}$	$5.11\pm0.12^{e}_{B}$	$8.17\pm0.33^{g}_{C}$	$9.96\pm0.12^{h}_{C}$
-20°C	$2.44\pm0.03^{a}_{A}$	$2.51\pm0.06^{a}_{A}$	$3.52\pm0.07^{cd}_{A}$	$3.73\pm0.09^{cd}_{A}$	$3.80\pm0.02^{cd}_{A}$	$3.86\pm0.05^{cd}_{A}$	$4.18\pm0.06^{d}_{A}$

All values are the means ± SE of four replicates, n=4
Values followed by different superscript letters in the same row are significantly different at α=0.05
Values followed by different subscript capital letters in the same column are significantly different at α=0.05

The mean L* value of the fresh fillets was 50.19±0.00, the mean a* value was -2.43±0.16 and the mean b* value was 0.28±0.00. L*, a* and b* increased significantly ($P<0.05$) when subjected to PBF temperature period at 0°C and 5°C from 0h to 16 days (Table 4.5). Fillets that underwent PBF temperature period at 5°C and 0°C for 16 days had significantly higher ($P<0.05$) L*value (lighter) than at -20°C. Fillets that underwent PBF temperature period at

5°C, had higher a* values (more greenish) and b* value (more yellowish) than fillets that underwent PBF temperature period at 0°C and -20°C after 16 days.

Table 4.5: Changes in the L*, a* and b* values of barramundi (*Lates calcarifer*) fillets before freezing (BF) and pre-blast freezing (PBF) treatments at 0°C, 5°C and, -20°C for 0h, 1h, 1 day, 2 days, 4 days, 8 days and 16 days.

L*							
	0h	1h	1day	2days	4days	8days	16days
5°C	$50.19\pm0.00^{a}_{A}$	$51.32\pm0.04^{b}_{A}$	$52.38\pm0.09^{c}_{A}$	$52.78\pm0.05^{c}_{A}$	$54.81\pm0.11^{e}_{A}$	$56.48\pm0.18^{f}_{A}$	$58.89\pm0.01^{g}_{A}$
0°C	$50.19\pm0.00^{a}_{A}$	$50.68\pm0.10^{a}_{B}$	$50.31\pm0.08^{a}_{C}$	$51.49\pm0.13^{b}_{B}$	$52.44\pm0.08^{c}_{B}$	$53.51\pm0.12^{d}_{B}$	$56.64\pm0.14^{f}_{B}$
-20°C	$50.19\pm0.00^{a}_{A}$	$50.17\pm0.03^{a}_{C}$	$51.56\pm0.11^{b}_{B}$	$51.54\pm0.19^{b}_{B}$	$52.77\pm0.08^{c}_{B}$	$53.65\pm0.17^{d}_{B}$	$54.62\pm0.07^{e}_{C}$
a*							
	0h	1h	1day	2days	4days	8days	16days
5°C	$-2.43\pm0.16^{a}_{A}$	$-2.23\pm0.02^{b}_{AB}$	$-2.47\pm0.05^{a}_{B}$	$-1.74\pm0.04^{c}_{A}$	$-1.50\pm0.03^{d}_{A}$	$-1.42\pm0.20^{d}_{A}$	$-0.54\pm0.01^{e}_{A}$
0°C	$-2.43\pm0.16^{a}_{A}$	$-2.37\pm0.06^{a}_{B}$	$-2.49\pm0.10^{a}_{B}$	$-1.24\pm0.01^{e}_{A}$	$-1.20\pm0.02^{e}_{A}$	$-1.37\pm0.16^{e}_{A}$	$-1.47\pm0.19^{e}_{B}$
-20°C	$-2.43\pm0.16^{a}_{A}$	$-2.17\pm0.02^{b}_{A}$	$-2.11\pm0.00^{b}_{A}$	$-2.34\pm0.21^{a}_{B}$	$-2.40\pm0.16^{a}_{B}$	$-2.13\pm0.01^{b}_{B}$	$-2.06\pm0.01^{c}_{C}$
b*							
	0h	1h	1day	2days	4days	8days	16days
5°C	$0.28\pm0.00^{a}_{A}$	$0.32\pm0.00^{a}_{A}$	$0.57\pm0.06^{ab}_{A}$	$1.17\pm0.02^{c}_{AB}$	$2.51\pm0.03^{d}_{A}$	$3.48\pm0.16^{ef}_{A}$	$5.52\pm0.20^{g}_{A}$
0°C	$0.28\pm0.00^{a}_{A}$	$0.32\pm0.02^{a}_{A}$	$0.52\pm0.09^{a}_{A}$	$1.00\pm0.05^{bc}_{B}$	$2.22\pm0.14^{d}_{A}$	$3.23\pm0.04^{e}_{A}$	$3.92\pm0.03^{f}_{B}$
-20°C	$0.28\pm0.00^{a}_{A}$	$0.33\pm0.00^{a}_{A}$	$0.43\pm0.08^{a}_{A}$	$1.30\pm0.11^{c}_{A}$	$2.25\pm0.10^{d}_{A}$	$2.37\pm0.07^{d}_{B}$	$3.52\pm0.13^{ef}_{B}$

All values are the means ± SE of four replicates, n=4
Values followed by different superscript letters in the same row are significantly different at α=0.05
Values followed by different subscript capital letters in the same column are significantly different at α=0.05
L- (Lightness); a*(redness); b*- (Yellowness)*

Each rheological parameter decreased significantly (P<0.05) after 16 days of PBF temperature period at 5°C, 0°C and -20°C, compared to fresh fillets. The most significant (P<0.05) decrease in rheological parameters (hardness, cohesiveness, springiness, gumminess, chewiness and stiffness) occurred between fresh fillets and fillets exposed to between 1 hour and 1 day PBF temperature period at all temperatures. With the exception of hardness, which decreased to a greater degree at 5°C and 0°C than at -20°C, each PBF temperature treatment had a similar effect on rheological parameters (Table 4.6).

Table 4.6:Changes in the hardness (N), cohesiveness, springiness (cm), gumminess (kgf), chewiness (kgf.mm), and stiffness (kgf/mm) of barramundi (*Lates calcarifer*) fillets before freezing (BF) and pre-blast freezing (PBF) treatments at 0°C, 5°C and, -20°C for 0h, 1h, 1 day, 2 days, 4 days, 8 days and 16 days.

Hardness (N)							
Treatment	0h	1h	1day	2days	4days	8days	16days
5°C	$1.98\pm0.04^{a}_{A}$	$1.76\pm0.01^{b}_{A}$	$1.68\pm0.02^{bc}_{A}$	$1.66\pm0.00^{bc}_{B}$	$1.59\pm0.02^{ce}_{B}$	$1.46\pm0.01^{d}_{C}$	$1.46\pm0.02^{de}_{B}$
0°C	$1.98\pm0.04^{a}_{A}$	$1.72\pm0.02^{b}_{A}$	$1.71\pm0.00^{b}_{A}$	$1.71\pm0.01^{b}_{B}$	$1.64\pm0.02^{b}_{B}$	$1.60\pm0.02^{bc}_{B}$	$1.48\pm0.00^{c}_{B}$
-20°C	$1.98\pm0.04^{a}_{A}$	$1.79\pm0.02^{b}_{A}$	$1.75\pm0.01^{b}_{A}$	$1.84\pm0.0^{b}_{A}$	$1.84\pm0.01^{b}_{A}$	$1.84\pm0.01^{b}_{A}$	$1.76\pm0.01^{b}_{A}$
Cohesiveness							
Treatment	0h	1h	1day	2days	4days	8days	16days
5°C	$0.08\pm0.00^{a}_{A}$	$0.07\pm0.00^{a}_{A}$	$0.06\pm0.00^{a}_{A}$	$0.01\pm0.00^{b}_{B}$	$0.00\pm0.00^{b}_{A}$	$0.00\pm0.00^{b}_{A}$	$0.00\pm0.00^{b}_{A}$
0°C	$0.08\pm0.00^{a}_{A}$	$0.07\pm0.00^{a}_{A}$	$0.05\pm0.00^{ab}_{A}$	$0.03\pm0.00^{bc}_{A}$	$0.01\pm0.00^{c}_{A}$	$0.01\pm0.00^{c}_{A}$	$0.01\pm0.00^{c}_{A}$
-20°C	$0.08\pm0.00^{a}_{A}$	$0.04\pm0.01^{b}_{A}$	$0.01\pm0.00^{bc}_{A}$	$0.00\pm0.00^{c}_{B}$	$0.00\pm0.00^{c}_{A}$	$0.00\pm0.00^{c}_{A}$	$0.00\pm0.00^{c}_{A}$
Springiness (cm)							
Treatment	0h	1h	1day	2days	4days	8days	16days
5°C	$0.08\pm0.00^{a}_{A}$	$0.03\pm0.00^{b}_{A}$	$0.01\pm0.00^{c}_{A}$	$0.01\pm0.00^{c}_{A}$	$0.01\pm0.00^{c}_{A}$	$0.01\pm0.00^{c}_{A}$	$0.01\pm0.00^{c}_{A}$
0°C	$0.08\pm0.00^{a}_{A}$	$0.04\pm0.00^{b}_{A}$	$0.01\pm0.00^{c}_{A}$	$0.01\pm0.00^{c}_{A}$	$0.01\pm0.00^{c}_{A}$	$0.01\pm0.00^{c}_{A}$	$0.01\pm0.00^{c}_{A}$
-20°C	$0.08\pm0.00^{a}_{A}$	$0.02\pm0.00^{b}_{A}$	$0.01\pm0.00^{c}_{A}$	$0.01\pm0.00^{c}_{A}$	$0.01\pm0.00^{c}_{A}$	$0.01\pm0.00^{c}_{A}$	$0.01\pm0.00^{c}_{A}$
Gumminess (kgf)							
Treatment	0h	1h	1day	2days	4days	8days	16days
5°C	$0.10\pm0.00^{a}_{A}$	$0.05\pm0.02^{ab}_{A}$	$0.00\pm0.00^{b}_{A}$	$0.00\pm0.00^{b}_{A}$	$0.00\pm0.00^{b}_{A}$	$0.00\pm0.00^{b}_{A}$	$0.00\pm0.00^{b}_{A}$
0°C	$0.10\pm0.00^{a}_{A}$	$0.07\pm0.02^{a}_{A}$	$0.00\pm0.00^{b}_{A}$	$0.00\pm0.00^{b}_{A}$	$0.00\pm0.00^{b}_{A}$	$0.00\pm0.00^{b}_{A}$	$0.00\pm0.00^{b}_{A}$
-20°C	$0.10\pm0.00^{a}_{A}$	$0.07\pm0.02^{a}_{A}$	$0.00\pm0.00^{b}_{A}$	$0.00\pm0.00^{b}_{A}$	$0.00\pm0.00^{b}_{A}$	$0.00\pm0.00^{b}_{A}$	$0.00\pm0.00^{b}_{A}$
Chewiness (kgf.mm)							
Treatment	0h	1h	1day	2days	4days	8days	16days
5°C	$0.04\pm0.00^{a}_{A}$	$0.02\pm.00^{ab}_{A}$	$0.02\pm0.00^{ab}_{A}$	$0.01\pm0.00^{ab}_{B}$	$0.01\pm0.00^{b}_{B}$	$0.01\pm0.00^{b}_{A}$	$0.01\pm0.00^{b}_{A}$
0°C	$0.04\pm0.00^{ab}_{A}$	$0.03\pm.00^{ab}_{A}$	$0.05\pm0.00^{a}_{A}$	$0.05\pm0.00^{a}_{A}$	$0.03\pm0.00^{ab}_{A}$	$0.01\pm0.00^{b}_{A}$	$0.01\pm0.00^{b}_{A}$
-20°C	$0.04\pm0.00^{a}_{A}$	$0.03\pm0.01^{ab}_{A}$	$0.02\pm0.00^{ab}_{A}$	$0.02\pm0.00^{ab}_{B}$	$0.01\pm0.00^{b}_{B}$	$0.01\pm0.00^{b}_{A}$	$0.01\pm0.00^{b}_{A}$
Stiffness (kgf/mm)							
Treatment	0h	1h	1day	2days	4days	8days	16days
5°C	$0.25\pm0.01^{a}_{A}$	$0.06\pm0.00^{b}_{B}$	$0.06\pm0.00^{b}_{B}$	$0.04\pm0.00^{bc}_{A}$	$0.01\pm0.00^{c}_{B}$	$0.02\pm0.00^{bc}_{A}$	$0.01\pm0.00^{bc}_{A}$
0°C	$0.25\pm0.01^{a}_{A}$	$0.07\pm0.00^{b}_{AB}$	$0.07\pm0.00^{bc}_{AB}$	$0.05\pm0.01^{bcd}_{A}$	$0.03\pm0.00^{cd}_{AB}$	$0.02\pm0.00^{d}_{A}$	$0.02\pm0.00^{d}_{A}$
-20°C	$0.25\pm0.01^{a}_{A}$	$0.10\pm0.00^{b}_{A}$	$0.09\pm0.00^{b}_{A}$	$0.06\pm0.01^{bc}_{A}$	$0.04\pm0.00^{c}_{A}$	$0.03\pm0.00^{c}_{A}$	$0.03\pm0.00^{c}_{A}$

All values are the means ± SE of four replicates, n=4
Values followed by different superscript letters in the same row are significantly different at α=0.05
Values followed by different subscript capital letters in the same column at every subset are significantly different at α=0.05

4.7 DISCUSSION

Temperature control is a critical parameter to retard quality deterioration of perishable foodstuffs, such as fresh fish, during storage and transport from processing to consumers (Margeirsson *et al.*, 2012). Zakhariya *et al*. (2014) demonstrated that it is important to prevent temperature variations or abuse during freezing and transport to avoid the detrimental effect of freezing and thawing so as to extend the quality and shelf-life of barramundi fillets. Temperature abuse may shorten the freshness period and storage life of fish products (Margeirsson *et al.*, 2012). Thus, fillets in the present study exposed to PBF temperature period at 5°C deteriorated more rapidly than did fillets exposed to PBF temperature period at 0°C and -20°C. This may have involved post-mortem myofibrillar degradation of the fish muscle, which is a major problem for the fisheries industry (Jasra *et al.,* 2001).

Studies on carp (*Labeo rohita*) (Gandotra *et al.*, 2012a), crab (*Scylla serrata*) (Zamir *et al.*, 1998), Arctic char (*Salvelinus alpinus*) (Bao *et al.*, 2007) and snakehead (*Puntius* spp.) (Siddique *et al.*, 2011) have shown that flesh moisture content increases with freezing time. Zamir *et al.* (1998) attributed this increase to the loss of water holding capacity of the tissue. Fish with higher flesh moisture content have a higher proportion of loosely bound water (Odoli, 2009). There was a gradual increase in moisture content in the present study. This increase in moisture content as spoilage progressed could be due to activities of proteolytic enzymes (Fazal & Ramesh*,* 2013). However, ash content only increased to one day, then decreased over time during PBF temperature period at all temperatures in the present study. Studies conducted by Okeyo *et al.* (2009) on Nile perch (*Lates niloticus*) and Emire & Gebremariam (2009) on tilapia *(Oreochromis niloticus)* reported a decrease in total ash content during its frozen storage. Drip loss during the thawing process might be the reason for the decrease in the ash and protein contents in the present study (Beklevik *et al.,* 2005).

The decrease in the crude protein content of barramundi fillets in the present study from 0 to 16 days of PBF temperature period at 0°C and 5°C can be attributed to the leaching of the soluble components, especially water-soluble protein and urea, from the fillets (Ashok Kumar *et al.,* 2000; Singh & Balange, 2005). Benjakul & Bauer (2001) reported that the proteins in fish flesh are soluble proteins, which are localised in the cell and released when the cells are damaged. This muscle drip loss can lower acceptability due to the loss of tasteful constituents, e.g. some amino acids or nucleotides (Benjakul & Bauer, 2001). Maria Macedo Viegas *et al.* (2013) stated that increase in protein content contained in drip loss in frozen cod

fillets is the result of muscle protein denaturation and disruption of membranes, cytoskeleton, and extracellular matrix leading to loss of intracellular compounds along with proteins. In contrast fillets exposed to PBF temperature period at -20°C had higher protein content after 16 days in the present study. This increase in protein content has also been observed during the frozen storage of fish cutlets, fish burgers and fish sticks (Raju *et al*., 1999; Vanitha *et al*., 2013), and fish fingers from perches (Lakshminatha *et al*., 1992) and this could be due to the release of oxidative enzymes and pro-oxidants from various ruptured cellular organelles (Xia *et al.,* 2009).

Post mortem pH of fish flesh varies from 6.0 to 7.1 (Simeonidou *et al.*, 1998; Ozogul *et al.*, 2005). This was confirmed for barramundi fillets in the present study (pH: 6.34 – 6.78). Abbas *et al*. (2009) stated that pH can act as an indicator of fish freshness as pH is low at the early stages of storage when the nutritional state is still good and then increases after storage for a certain period of time. Fillet pH increased significantly ($P<0.05$) with increasing storage time and temperature in the present study (0-16 days), indicating that alkaline compounds were accumulated through autolytic activities or microbial metabolism (Pons-Sanchez-Cascado *et al.*, 2006). The pH is an important determinant of microbial growth and seafood with a high pH has a high spoilage potential and a short shelf life (Newton & Gell, 1981).

The level of TVBN in freshly caught fish is generally between 5 and 20 mg N 100 g^{-1} muscle (Ozogul *et al.*, 2005). The TVBN value of PBF (0 h) barramundi fillets in the present study, 6.26±0.11 mg 100 g^{-1}, was within this range. A level of 30-35 mg 100 g^{-1} is considered the upper limit, above which fish products are considered unfit for human consumption (Ludorf & Meyer 1973; Oehlenschlager, 1992). This is as a result of microorganisms influencing changes in some volatile nitrogen bases, causing fillet deterioration (Odoli, 2009). In the present study, TVBN increased with time at each PBF treatment temperature, but to a much greater extent at 5°C and 0°C. TVBN increased above the safe limit for human consumption (30-35 mg 100 g^{-1}) between 4 and 8 days PBF temperature period at 5°C, between 8 and 16 days PBF temperature period at 0°C and remained below this limit for 16 days at -20°C. This confirms that temperature abuse may shorten the freshness period and storage life of barramundi fillets particularly at 0°C and above. 10^4-10^6 TVC/cm^2 or g^{-1} is considered an acceptable range of TVC in the Australian meat industry (Meat Standards Committee, 2002). Therefore, the TVC of the barramundi fillets in the present study was unacceptable after 8 days PBF temperature period at 0°C and 5°C, 6.38±0.12 and 8.17±0.33, respectively but

remained acceptable (less than 10^7 cfu g^{-1}) at -20°C, even after 16 days. The growth in microbial load, as represented by TVC, accelerated with increasing temperature in the present study, demonstrating that enzymatic and microbiological processes are greatly influenced by temperature (Huss, 1995). This demonstrates the significant effect that time-temperature abuse has on barramundi fillet deterioration.

Color changes in cod (*Gadus morhua*) include loss of surface glossiness, muscle opacity, or chalky appearance and are thought to be due to irreversible changes in the muscle proteins (Shenouda, 1980). Dias *et al.* (1994) stated that colour changes in black scabbard fish (*Aphanopus carbo*) and silver scabbard fish (*Lepidopus caudatus*) can occur during frozen storage due to lipid oxidation and pigment degradation processes (Dias *et al.* 1994). During 12 d of refrigerated storage, the yellow discoloured catfish (*Ictalurus punctatus*) fillets became darker and more yellow (Li *et al.*, 2013). Similarly, fillets were more yellowish after 16 d at 5°C than at 0 and -20°C in the present study. Fillets were also lighter and more greenish after 16 d at 5°C than at 0 and -20°C. The present study confirms that although fillet colour changes are slow at freezer temperatures, the rate of change is still temperature dependent and the colder the storage temperature, the slower the color change (Spooncer *et al.*, n.d.). Haard (1992) suggested that texture of fish flesh was influenced by many factors including postmortem pH decline, proteolysis, fat content, composition and its distribution in the fish muscle (Liu *et al.*, 2010). Hardness decreased significantly ($P<0.05$) as a result of 16 days of PBF temperature period treatment at all temperatures in the present study. Schubring (2002) stated that the increasing softness during refrigerated storage is a result of proteolysis caused by endogenous and microbial enzymes. These enzymes caused increased proteolysis and resultant lower hardness at 0 and 5°C than at 20°C in the present study. The decrease in firmness as well as in elasticity may be due partly to the muscle softening as a result of proteolytic activity. Texture softening is mainly influenced by the autolysis and denaturation of muscle protein during chilled and frozen storage (Tsuchiya *et al.*, 1992; Benjakul *et al.*, 1997). The decrease in rheological parameters in the present study demonstrates that time-temperature abuse or just freezing at -20°C results in significant changes in barramundi fillet texture over time. The decrease in fillet cohesiveness, springiness, gumminess, chewiness and stiffness values (Table 4.6) after PBF treatments at 0°C, 5°C and, -20°C for 16 days in the present study could be due to the corresponding softening of fillets.

4.8 CONCLUSIONS

Based upon microbiological analysis of barramundi fillets, the maximum PBF temperature shelf life was 8 days for fillets at 0˚C and 5˚C. In contrast, fillets subjected to PBF temperature period at -20°C have a shelf life of more than 16 days PBF temperature period. PBF temperature period at all temperatures deteriorated the L*, a*, b* values, and rheological parameters. TVC, TVBN, pH, protein, colour and rheological parameters deteriorated significantly after 16 days PBF temperature period at 0°C and 5°C. PBF treatment at -20°C from 0h to 16 days had only a minor effect on the microbiological and physiochemical properties. This observation, combined with the subsequent 20 day storage period, demonstrates that barramundi fillets stored at -20°C remain acceptable in terms of TVC and pH, TVBN, protein, and colour for at least 36 days. The largest detrimental changes to fillets in the present study occurred through PBF temperature period at 5°C, followed by 0°C. This demonstrates the inadequacy of storage at these higher temperatures for maintaining the quality and shelf life of barramundi fillets.

CHAPTER 5

EFFECTS OF TWO FORMS OF ICE

CHAPTER 5: THE EFFECTS OF TWO FORMS OF ICE ON MICROBIOLOGICAL AND PHYSIOCHEMICAL PROPERTIES OF BARRAMUNDI (*LATES CALCARIFER*, BLOCH) FILLETS

5.1 INTRODUCTION

Significant deterioration of sensory quality and loss of nutritional value occur during chilled storage of fish as a result of changes in chemical constituents that can reduce the commercial value (Whittle *et al*., 1990; Ashie *et al.*, 1996; Olafsdottir *et al.,* 1997; Losada *et al.*, 2005). Utilisation of ice just after landing and storage in a refrigerated plant (Nunes *et al.*, 1992) has a significant impact on the quality of fish and fish products. With the aim of preserving the greatest quantity of fish immediately after slaughter appropriately, several on-board handling systems, including storage in flake ice (Nunes *et al.*, 1992), refrigerated seawater (Kraus, 1992), or the addition of chemicals (Ponce de Leo´n *et al.*, 1993; Hwang & Regenstein, 1996), or the incorporation of chemical preservative agents (McEvily *et al*., 1991), have been suggested.

Recently, use of slurry ice, also known as liquid ice or flow ice has been reported to be a valuable technique for the chilling and refrigeration of fish species derived from both marine and aquaculture origin (Múgica *et al.,* 2008). Today, there are over 700 ice slurry systems installed in the fishery industry- one of the largest markets for ice slurry technology. Iceland, Japan and Norway are the top three users (Kauffeld *et al.*, 2010). Ice slurry is increasingly used for chilling, storage and transportation of fish on board fishing vessels and barges, at farms, and inside processing plants (Kauffeld *et al.*, 2010). Success has been reported for almost all major fish species such as tuna, yellowtail, salmon, cod, haddock, hake, herring, mackerel, sardine, shrimp, mussel and lobster (Wang & Goldstein, 2003; Pineiro *et al.*, 2004; Kauffeld *et al.*, 2010). Research by the Norwegian Institute of Fisheries and Aquaculture discovered that cod placed in ice slurry for three days (the maximum allowable time for such storage) became on average 4% heavier without any change in quality (Joensen *et al.,* 2001).

Two main features of slurry ice are its faster chilling rate, deriving from its higher heat-exchange capacity, and the reduced physical damage caused to seafood products by its microscopic spherical particles, as compared with flake ice (Rodriguez *et al*., 2003). The overall covering of the fish surface by the slurry ice mixture also protects the fish from the

action of oxygen (Kılınc *et al*., 2007). This method causes the fish to absorb water without reducing quality (Gregersen, 2001). Recent studies have reported significant inhibitory effects of slurry ice on microbiological and biochemical mechanisms responsible for fish spoilage, as compared to traditional flake ice. For example, inhibitory effects on quality loss mechanisms were reported for sardine, with an increased shelf life of 15 days in ice slurry as compared to 8 days in flake ice (Carmen *et al.*, 2005). This has led to increases in the shelf life of a broad variety of chilled aquatic food products such as lean fish (Losada *et al.*, 2004), medium-fat fish (Losada *et al.,* 2005), fatty fish (Price *et al.*, 1991; Losada *et al*., 2005) and crustaceans (Chinivasagam *et al.*, 1998). During processing and storage, fish quality may decline as a result of several factors (Aubourg & Medina, 1999). Even though autolytic processes onset the degradation process in fish as result of endogenous enzymatic changes, the spoilage of fish held in ice is predominantly due to bacterial growth. Bacteria are considered to play a dominant role in fish spoilage (Alfred, 1998).

Barramundi or Asian seabass is a commercially important aquaculture species throughout the Indo-Pacific region with significant industries in Thailand, Indonesia, Malaysia, Philippines, Taiwan and Australia (Glencross, 2006). In Australia, this species is cultured predominately in the tropical regions of Queensland, Northern Territory and Western Australia. The reputation and demand for barramundi have made it a major contributor to Australian aquaculture production. Barramundi was traditionally produced as a plate fish for the restaurant trade, but the majority is now being sold as fillets with a new market developing around direct sales to the major supermarkets (Department of Fisheries, 2011). The different points of transport, from cold storage to the retail outlet, and then to the consumer refrigerator, are critical points for the overall quality and safety of barramundi meat (Nychas *et al.*, 2008). Storage time and conditions have large impact on the quality of fish and fish products and the storage stability depends on the composition of the fish (Ashie *et al.*, 1996). With the aim of improving the freshness quality of fish, diverse storage methods, such as flake ice and slurry ice, are necessary to prolong the shelf life of fish products and avoid the corruption of fish (Jain *et al.*, 2007).

Fish preservation factors that have previously been studied are the conditions and length of pre-freezing storage (Bilinski *et al.*, 1981; Kolakowska, 1981), the degree of tissue disruption (Smith *et al.,* 1980; Kolakowska, 1981), freezing methods (Vyncke, 1978; Jhaveri & Constantinides, 1981), and storage temperature (Ke *et al.*, 1977). However, the effect of the

duration of pre- freezing storage using two different forms of ice (flake ice and slurry ice) on barramundi fillets is unreported. The present work was conducted to compare the shelf-life of barramundi fillets stored in two different ice forms, flake ice and slurry ice. The effect of storage duration in these ice forms on the microbiological and physiochemical quality parameters of barramundi fillets was investigated.

5.2 MATERIALS AND METHODS

5.2.1 Sample Preparation

Please refer to Chapter 4 (General methodology)

5.2.2 Flake Ice and Slurry Ice

Flake ice was prepared with an ice maker FM-150KE device (Hoshizaki, Japan). The average temperature of the barramundi fillets stored in flake ice was in the range of 0°C/+1°C. The temperature of the barramundi fillets stored in slurry ice (40% ice and 60% water, prepared from filtered seawater (salinity: 3.3%)) were in the range of −1.0 /-1.5°C. When necessary, the flake ice and the slurry ice mixture were renewed and both batches if fillets were kept in two different thermocol boxes.

5.3 EXPERIMENTAL PROCEDURE

The control (fresh) fillets (0h = before freezing [BF]) were analysed immediately after being received and were not subjected to freezing. The filleted barramundi meat was divided into two batches. In batch one- fillets were kept in flaked ice for 0h, 1h, 1 day, 2 days, 4 days, 8 days and 16 days before freezing in a blast freezer whereas in batch two- fillets were kept in slurry ice for the same time intervals before freezing.

After chilled-storage treatments, fillets were individually frozen in an air blast freezer at -80°C for 8 hours and were then stored in a freezer at -20°C for 20 days. Microbiological and physiochemical analyses of barramundi fillets were then carried out on the 21st day from the commencement of frozen storage. Each experimental treatment had four replicates, with a single fillet (1.05 kg) used per replicate. Samples were thawed under running tap water (18-20^{o}C) just before analysis. The experimental design is shown in Figure 5.1.

Sub samples from the thawed fish were analysed for their quality and shelf life. In order to measure the microbiological and physiochemical properties, the fillets were sub-sampled in the laboratory under hygienic conditions and macerated in an acid washed glass blender. Quality and shelf life of barramundi were evaluated using the microbiological and physiochemical analyses described below:

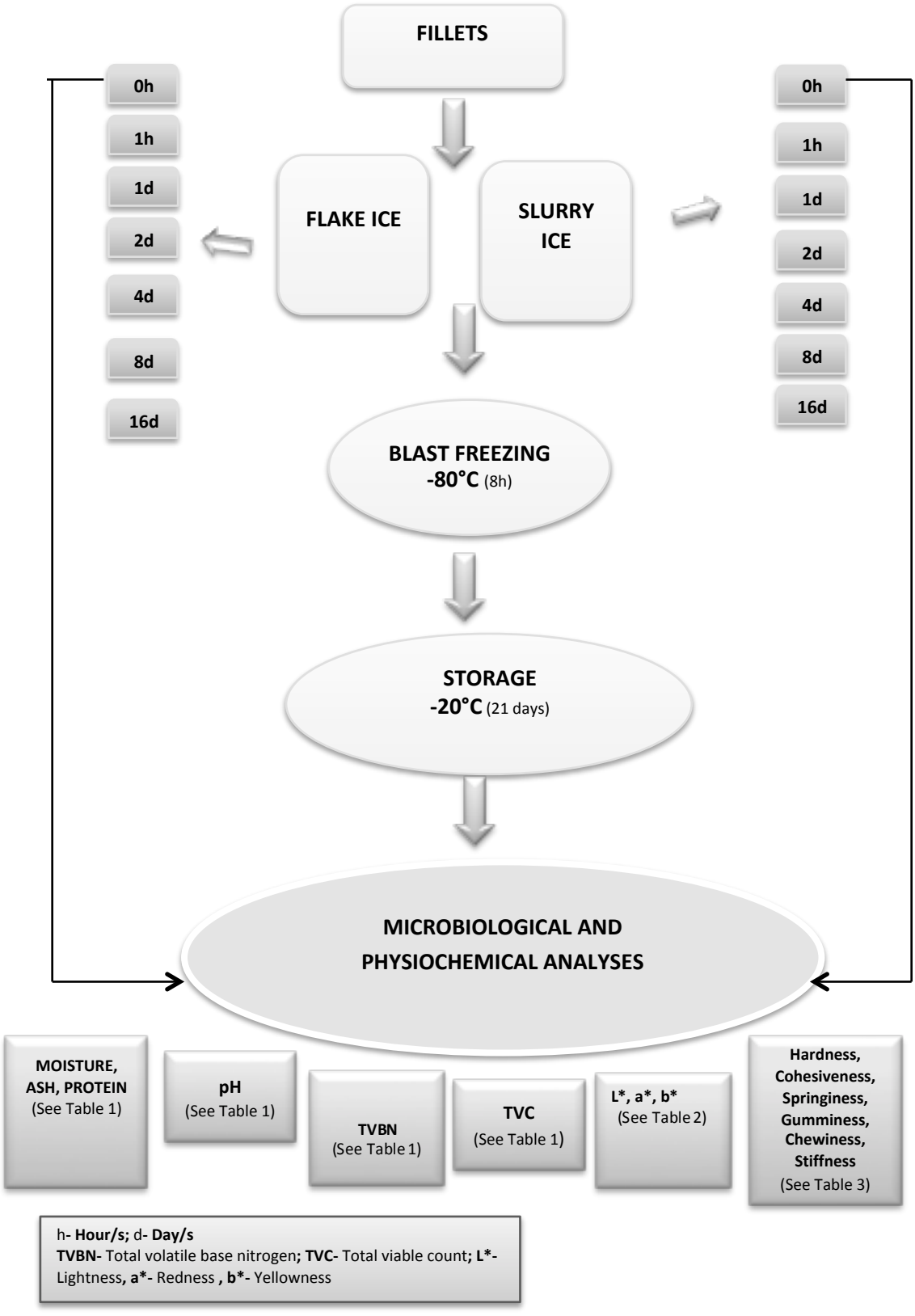

Figure 5.1: A flow diagram showing the experimental design

5.4 ANALYTICAL DETERMINATIONS

Please refer Chapter 3 (General methodology)

5.4.1 Microbiological Analysis

Please refer Chapter 3 (General methodology)

5.4.2 Proximate Analysis

Please refer Chapter 3 (General methodology)

5.4.3 pH

Please refer Chapter 3 (General methodology)

5.4.4 Total Volatile Base Nitrogen (TVBN)

Please refer Chapter 3 (General methodology)

5.4.5 Texture

Please refer Chapter 3 (General methodology)

5.4.5.1 Sample Preparation

Please refer Chapter 3 (General methodology)

5.4.5.2 Texture Profile Analysis (TPA)

Please refer Chapter 3 (General methodology)

5.4.6 Color Measurement

Please refer Chapter 3 (General methodology)

5.5 STATISTICAL ANALYSIS

Please refer Chapter 3 (General methodology)

5.6 RESULTS

The initial (pre-freezing) mean moisture, ash and protein content of barramundi fillets were 71.48±0.25% wet basis (w.b.) (Table 5.1), 1.07±0.02 % (Table 5.1) and 62.71±0.18 % dry basis (d.b.) (Table 5.1), respectively. The moisture content of fillets subjected to pre-freezing treatments in SI and FI increased to 78.01±0.08% and 77.27±0.16% (w.b.), respectively with 16 days pre-freezing chilled storage. There was significant ($P<0.05$), difference between treatments in moisture content and ash content, similarly increased significantly ($P < 0.05$), as storage time progressed in both FI and SI groups compared to BF. Moisture content and pH increased significantly with chilling time; ash and protein content decreased significantly with chilling time; Moisture content was significantly higher in SI than FI for 2-days and longer chilling; protein content was significantly higher and pH significantly lower in SI than FI after 16 days chilling; there was no significant difference in ash content between SI and FI.

TVBN increased significantly ($P<0.05$) with increasing chilling time, from 6.13±0.04 mg 100 g^{-1} (BF) to 45.69±0.69 mg 100 g^{-1} in FI and 21.57±0.62 mg 100 g^{-1} in SI after 16-days (Table 5.1). TVBN was significantly higher ($P<0.05$) in fillets chilled in FI than in SI for 1 hour and longer. There was gradual increase in pH value according to storage time. Maximum pH value was observed in fillets exposed to pre-freezing treatment in FI (16d) which was 6.63±0.01 and 6.55±0.01 pH value was noted in fillets subjected to SI and indicated a significant difference ($P < 0.05$) compared to BF. TVC increased significantly ($P<0.05$) with increasing chilling time, from 1.14±0.14 log CFU g^{-1} (BF) to 7.07±0.03 log CFU g^{-1} in FI and 4.18±0.06 log CFU g^{-1} in SI after 16-days (Table 5.1). TVC was significantly higher ($P<0.05$) in fillets chilled in FI than in SI for 1 day and longer.

Table 5.1: Changes in the moisture content % w.b., ash content %, protein content %, pH, TVBN (mg 100 g^{-1}), and TVC (log CFU g^{-1}) of barramundi (*Lates calcarifer*) fillets before freezing (0h=BF) and pre-blast freezing treatments exposed to flake ice (FI) and slurry ice (SI) for 0h, 1h, 1 day, days, 4 days, 8 days, 16 days.

Moisture							
	0h	1h	1 day	2days	4days	8days	16days
FI	$71.48\pm0.25^{a}{}_{A}$	$72.27\pm0.09^{b}{}_{A}$	$73.20\pm0.06^{de}{}_{A}$	$73.59\pm0.28^{e}{}_{A}$	$74.61\pm0.35^{f}{}_{A}$	$75.36\pm0.08^{g}{}_{A}$	$77.27\pm0.16^{h}{}_{A}$
SI	$71.48\pm0.25^{a}{}_{A}$	$72.64\pm0.13^{ab}{}_{A}$	$74.35\pm0.20^{bc}{}_{A}$	$75.17\pm0.37^{cd}{}_{B}$	$76.53\pm0.10^{de}{}_{B}$	$77.84\pm0.34^{e}{}_{B}$	$78.01\pm0.08^{fg}{}_{B}$
Ash							
	0h	1h	1 day	2days	4days	8days	16days
FI	$1.07\pm0.02^{a}{}_{A}$	$1.01\pm0.45^{a}{}_{A}$	$0.96\pm0.00^{ab}{}_{A}$	$0.96\pm0.14^{ab}{}_{A}$	$0.96\pm0.58^{ab}{}_{A}$	$0.95\pm0.34^{cd}{}_{A}$	$0.92\pm0.01^{c}{}_{A}$
SI	$1.07\pm0.02^{c}{}_{A}$	$1.03\pm0.01^{bcd}{}_{A}$	$1.06\pm0.02^{c}{}_{A}$	$0.98\pm0.27^{abc}{}_{A}$	$0.96\pm0.03^{abc}{}_{A}$	$0.96\pm0.11^{ab}{}_{A}$	$0.95\pm0.01^{a}{}_{A}$
Protein							
	0h	1h	1 day	2days	4days	8days	16days
FI	$62.71\pm0.18^{e}{}_{A}$	$61.26\pm0.40^{bcde}{}_{A}$	$61.07\pm0.36^{bcd}{}_{A}$	$61.18\pm0.12^{bcd}{}_{A}$	$60.55\pm0.59^{bc}{}_{A}$	$60.63\pm0.11^{bc}{}_{A}$	$58.74\pm0.28^{a}{}_{A}$
SI	$62.71\pm0.18^{e}{}_{A}$	$62.34\pm0.11^{de}{}_{A}$	$61.44\pm0.17^{bcde}{}_{A}$	$61.63\pm0.21^{cde}{}_{A}$	$60.60\pm0.46^{bc}{}_{A}$	$60.39\pm0.20^{bc}{}_{A}$	$60.09\pm0.03^{ab}{}_{B}$
pH							
	0h	1h	1 day	2days	4days	8days	16days
FI	$6.26\pm0.00^{a}{}_{A}$	$6.38\pm0.02^{c}{}_{A}$	$6.42\pm0.00^{cd}{}_{A}$	$6.45\pm0.01^{de}{}_{A}$	$6.53\pm0.00^{f}{}_{A}$	$6.55\pm0.01^{f}{}_{A}$	$6.63\pm0.01^{g}{}_{A}$
SI	$6.26\pm0.00^{a}{}_{A}$	$6.31\pm0.00^{ab}{}_{B}$	$6.36\pm0.00^{bc}{}_{B}$	$6.36\pm0.01^{bc}{}_{B}$	$6.46\pm0.01^{de}{}_{B}$	$6.50\pm0.01^{ef}{}_{B}$	$6.55\pm0.01^{f}{}_{B}$
TVBN							
	0h	1h	1 day	2days	4days	8days	16days
FI	$6.13\pm0.04^{a}{}_{A}$	$9.66\pm0.19^{cd}{}_{A}$	$11.63\pm0.98^{def}{}_{A}$	$12.71\pm0.16^{ef}{}_{A}$	$16.62\pm0.61^{g}{}_{A}$	$18.91\pm0.35^{h}{}_{A}$	$45.69\pm0.69^{j}{}_{A}$
SI	$6.13\pm0.04^{a}{}_{A}$	$7.47\pm0.12^{ab}{}_{B}$	$8.86\pm0.10^{bc}{}_{B}$	$9.38\pm0.19^{bc}{}_{B}$	$10.76\pm0.15^{cde}{}_{B}$	$13.17\pm0.05^{f}{}_{B}$	$21.57\pm0.62^{i}{}_{B}$
TVC							
	0h	1h	1 day	2days	4days	8days	16days
FI	$1.44\pm0.14^{a}{}_{A}$	$2.48\pm0.19^{b}{}_{A}$	$3.29\pm0.17^{cd}{}_{A}$	$3.76\pm0.06^{de}{}_{A}$	$3.94\pm0.02^{de}{}_{A}$	$5.38\pm0.12^{f}{}_{A}$	$7.07\pm0.03^{g}{}_{A}$
SI	$1.44\pm0.14^{a}{}_{A}$	$2.41\pm0.21^{b}{}_{B}$	$2.54\pm0.13^{b}{}_{B}$	$2.61\pm0.18^{bc}{}_{B}$	$3.33\pm0.15^{d}{}_{B}$	$3.86\pm0.05^{de}{}_{B}$	$4.18\pm0.06^{de}{}_{B}$

All values are the means ± SE of four replicates, n=4
Values followed by different superscript letters in the same row in each subset are significantly different at α=0.05
Values followed by different subscript capital letters in the same column in each subset are significantly different at α=0.05

L* values increased as chilling time increased, from 50.19±0.00 (BF) to 56.37±0.18 in FI and 54.62±0.07 in SI after 16 days (Table 5.2). a* values increased as chilling time increased, from -2.35±0.17 (BF) to -1.13±0.02 in FI and -1.35±0.12 in SI after 16 days (Table 2). b* values increased as chilling time increased, from 0.35±0.07 (BF) to 3.23±0.06 in FI and 2.11±0.04 in SI after 16 days (Table 5.2). Therefore fillets chilled in FI became significantly ($P<0.05$) lighter, redder and more yellow than those chilled in SI as chilling time increased.

Table 5.2: Changes in the L*, a* and b* values of barramundi (*Lates calcarifer*) fillets before freezing (BF) and pre-blast freezing treatments exposed to flake ice and slurry ice for 0h, 1h, 1 day, 2 days, 4 days, 8 days and 16 days.

L*							
	0h	1h	1 day	2days	4days	8days	16days
FI	$50.19\pm0.00^{a}{}_{A}$	$50.59\pm0.26^{ab}{}_{A}$	$50.97\pm0.40^{ab}{}_{A}$	$51.54\pm0.19^{bc}{}_{A}$	$52.77\pm0.08^{de}{}_{A}$	$53.89\pm0.35^{f}{}_{A}$	$56.37\pm0.18^{g}{}_{B}$
SI	$50.19\pm0.00^{a}{}_{A}$	$50.32\pm0.13^{a}{}_{A}$	$50.57\pm0.23^{ab}{}_{A}$	$51.07\pm0.03^{ab}{}_{A}$	$52.26\pm0.14^{cd}{}_{A}$	$53.65\pm0.17^{ef}{}_{A}$	$54.62\pm0.07^{f}{}_{A}$
a*							
	0h	1h	1 day	2days	4days	8days	16days
FI	$-2.35\pm0.17^{ab}{}_{A}$	$-2.42\pm0.09^{a}{}_{A}$	$-2.07\pm0.06^{f}{}_{B}$	$-1.84\pm0.03^{bcd}{}_{B}$	$-1.53\pm0.14^{de}{}_{B}$	$-1.34\pm0.04^{de}{}_{B}$	$-1.13\pm0.02^{e}{}_{B}$
SI	$-2.35\pm0.17^{ab}{}_{A}$	$-2.43\pm0.16^{a}{}_{A}$	$-2.35\pm0.04^{ab}{}_{A}$	$-2.22\pm0.08^{abc}{}_{A}$	$-2.03\pm0.02^{f}{}_{A}$	$-1.77\pm0.05^{cd}{}_{A}$	$-1.35\pm0.12^{de}{}_{A}$
b*							
	0h	1h	1 day	2days	4days	8days	16days
FI	$0.35\pm0.07^{a}{}_{A}$	$0.54\pm0.18^{ab}{}_{B}$	$0.62\pm0.42^{ab}{}_{B}$	$1.51\pm0.24^{cd}{}_{B}$	$2.48\pm0.17^{ef}{}_{B}$	$3.13\pm0.03^{f}{}_{B}$	$3.23\pm0.06^{f}{}_{B}$
SI	$0.35\pm0.07^{a}{}_{A}$	$0.42\pm0.09^{a}{}_{A}$	$0.47\pm0.13^{ab}{}_{A}$	$1.09\pm0.03^{abc}{}_{A}$	$1.30\pm0.11^{bcd}{}_{A}$	$1.52\pm0.15^{cd}{}_{A}$	$2.11\pm0.04^{de}{}_{A}$

All values are the means ± SE of four replicates, n=4
Values followed by different superscript letters in the same row in each subset are significantly different at α=0.05
Values followed by different subscript capital letters in the same column in each subset are significantly different at α=0.05

All textural parameters decreased significantly ($P<0.05$) in both pre-freezing treatments during chilled storage compared to BF. Fillet hardness was significantly lower in FI than in SI for 1 day and longer chilling (Table 5.3). The textural parameters, cohesiveness, springiness, gumminess, chewiness stiffness, decreased as storage time progressed when barramundi fillets were exposed to both flake ice and slurry ice.

Table 5.3: Changes in the hardness (N), cohesiveness, springiness (cm), gumminess (kgf), chewiness (kgf.mm), and stiffness (kgf/mm) of barramundi (*Lates calcarifer*) fillets before freezing (BF) and pre-blast freezing treatments exposed to flake ice and slurry ice for 0h, 1h, 1 day, 2 days, 4 days, 8 days and 16 days.

Hardness (N)							
	0h	1h	1 day	2days	4days	8days	16days
FI	$2.08\pm0.04^{a}_{A}$	$1.72\pm0.02^{b}_{A}$	$1.71\pm0.00^{b}_{A}$	$1.68\pm0.01^{b}_{A}$	$1.66\pm0.02^{b}_{A}$	$1.58\pm0.02^{bc}_{A}$	$1.35\pm0.00^{c}_{A}$
SI	$2.08\pm0.04^{a}_{A}$	$1.81\pm0.02^{b}_{A}$	$1.80\pm0.01^{b}_{B}$	$1.77\pm0.00^{b}_{B}$	$1.77\pm0.01^{b}_{B}$	$1.70\pm0.01^{b}_{B}$	$1.69\pm0.01^{b}_{B}$
Cohesiveness							
	0h	1h	1 day	2days	4days	8days	16days
FI	$0.08\pm0.00^{a}_{A}$	$0.04\pm0.01^{b}_{A}$	$0.01\pm0.00^{bc}_{A}$	$0.00\pm0.00^{c}_{A}$	$0.01\pm0.00^{c}_{A}$	$0.01\pm0.00^{c}_{A}$	$0.01\pm0.00^{c}_{A}$
SI	$0.08\pm0.00^{a}_{A}$	$0.04\pm0.01^{b}_{A}$	$0.05\pm0.00^{ab}_{B}$	$0.04\pm0.00^{bc}_{B}$	$0.00\pm0.00^{c}_{A}$	$0.00\pm0.00^{c}_{A}$	$0.00\pm0.00^{c}_{A}$
Springiness (cm)							
	0h	1h	1 day	2days	4days	8days	16days
FI	$0.07\pm0.00^{a}_{A}$	$0.04\pm0.00^{b}_{A}$	$0.01\pm0.00^{c}_{A}$	$0.01\pm0.00^{c}_{A}$	$0.01\pm0.00^{c}_{A}$	$0.01\pm0.00^{c}_{A}$	$0.01\pm0.00^{c}_{A}$
SI	$0.07\pm0.00^{a}_{A}$	$0.02\pm0.00^{b}_{A}$	$0.03\pm0.00^{c}_{A}$	$0.02\pm0.00^{c}_{A}$	$0.01\pm0.00^{c}_{A}$	$0.01\pm0.00^{c}_{A}$	$0.01\pm0.00^{c}_{A}$
Gumminess (kgf)							
	0h	1h	1 day	2days	4days	8days	16days
FI	$0.11\pm0.00^{a}_{A}$	$0.05\pm0.02^{a}_{A}$	$0.00\pm0.00^{b}_{A}$	$0.00\pm0.00^{b}_{A}$	$0.00\pm0.00^{b}_{A}$	$0.00\pm0.00^{b}_{A}$	$0.00\pm0.00^{b}_{A}$
SI	$0.11\pm0.00^{a}_{A}$	$0.07\pm0.02^{a}_{B}$	$0.04\pm0.00^{b}_{A}$	$0.01\pm0.00^{b}_{A}$	$0.01\pm0.00^{b}_{A}$	$0.01\pm0.00^{b}_{A}$	$0.01\pm0.00^{b}_{A}$
Chewiness (kgf.mm)							
	0h	1h	1 day	2days	4days	8days	16days
FI	$0.06\pm0.00^{ab}_{A}$	$0.04\pm.00^{ab}_{A}$	$0.03\pm0.00^{a}_{A}$	$0.03\pm0.00^{a}_{A}$	$0.03\pm0.00^{ab}_{A}$	$0.01\pm0.00^{b}_{A}$	$0.01\pm0.00^{b}_{A}$
SI	$0.06\pm0.00^{a}_{A}$	$0.04\pm0.01^{ab}_{A}$	$0.04\pm0.00^{ab}_{A}$	$0.05\pm0.00^{ab}_{A}$	$0.04\pm0.00^{b}_{A}$	$0.01\pm0.00^{b}_{A}$	$0.01\pm0.00^{b}_{A}$
Stiffness (kgf/mm)							
	0h	1h	1 day	2days	4days	8days	16days
FI	$0.28\pm0.01^{a}_{A}$	$0.05\pm0.00^{b}_{A}$	$0.07\pm0.00^{bc}_{A}$	$0.05\pm0.01^{bcd}_{A}$	$0.04\pm0.00^{cd}_{A}$	$0.02\pm0.00^{d}_{A}$	$0.02\pm0.00^{d}_{A}$
SI	$0.28\pm0.01^{a}_{A}$	$0.10\pm0.00^{b}_{B}$	$0.09\pm0.00^{b}_{B}$	$0.08\pm0.01^{bc}_{B}$	$0.07\pm0.00^{c}_{B}$	$0.04\pm0.00^{c}_{A}$	$0.03\pm0.00^{c}_{A}$

All values are the means ± SE of four replicates, n=4
Values followed by different superscript letters in the same row in each subset are significantly different at α=0.05
Values followed by different subscript capital letters in the same column in each subset are significantly different at α=0.05

5.7 DISCUSSION

The moisture content of the fillets chilled in SI for 16 days increased up to 78.01±0.08% due to absorption of water from the melting ice (Nunes *et al.*, 1992) and this increase in moisture was than those chilled in FI (77.27±0.16). There was a decrease in protein content and an increase in TVBN content in the fillets of both groups FI and SI. These changes coincided with an increase in the bacterial counts which could be due to the proteolytic breakdown of the protein molecules releasing volatile nitrogenous compounds determined as TVBN (Okeyo *et al.*, 2009).

Fillets chilled in FI for 16 days lost significantly more protein than those chilled in SI. The endogenous proteolytic enzymes in fillets will generally be more active under temperature abuse conditions, or under inadequate chilling conditions such as in flake ice, than at temperatures experienced during iced storage (Cepeda *et al.*, 1990; Ashie *et al.*, 1996). During the storage period, the proteolytic activity increased significantly in the abused fillets on Atlantic cod (*Gadus morhua*) (Hultmann & Rustad, 2007). Thus, as observed in the present study, in comparison with turbot stored in flake ice, the storage of turbot specimens in slurry ice implies a better maintenance of protein extractability and stability even during advanced periods of storage, this coinciding with a better maintenance of texture as determined by sensory analysis (Kılınc *et al.*, 2007). Moreover, a better stabilisation of the protein fraction above 94 kDa was achieved in turbot muscle subjected to storage in slurry ice as compared with flake ice (Losada *et al.,* 2005).

The pH of live fish muscle is close to the value 7.0. Nevertheless post-mortem pH can vary from 6.0 to 7.1, depending on season and species (Simeonidou *et al.*, 1998). Increases in pH indicate the accumulation of alkaline compounds, such as ammonia compounds and TMA, mainly derived from microbial action (Hebard *et al.*, 1982; Kılınc *et al.*, 2007). The initial low pH is due to the initial production of lactic acid, while the increase in the pH at the end of the period of frozen storage is due to the production of basic components caused by the enzymatic degradation of the fish muscle content (Simeonidou *et al.*, 1997). In one study, the pH of European hake stored in slurry ice and flake ice for 15 days increased from 6.67 to 6.8 and 7.4, respectively (Rodriguez *et al.*, 2004). Similar small increases in pH have also been reported for turbot (Pineiro *et al.*, 2003; Rodriguez *et al.*, 2003) and horse mackerel (Losada *et al.*, 2003). The TVBN and pH of fillets increased significantly as chilled storage time increased in the present trial.

The pH of barramundi fillets stored for 16 days in flake ice (6.63±0.01) was considerably higher than in slurry ice (6.55±0.01) in the present study. Similarly, Rodrıguez *et al.* (2006) reported the lowest pH values for turbot muscle stored in slurry ice were significantly below those of turbot muscle stored in flake ice, indicating superior control of both endogenous and microbial alkalinising mechanisms in turbot muscle as a consequence of storage in slurry ice, as compared to storage in flake ice. Previous reports have also described steady increases in the pH value for other fish species stored in flake ice (Nunes *et al.*, 1992; Ruı´z-Capillas & Moral, 2001).

TVBN levels were low (6.13±0.04 mg 100 g^{-1}) at the beginning of the period of frozen storage since all fish were fresh. TVBN is well documented as an index of the quality of fresh fish because its increase is related to bacterial spoilage. TVBN indicates the production of ammonia, mono-, di- and trimethylamine nitrogen, which are found in the common pattern of spoilage. Therefore the TVBN of fillets in the present trial increased significantly as chilled storage time increased in both SI and FI due to bacterial spoilage. This has also been reported in other studies on Nile perch (Gram *et al.*, 1987; Karungi *et al.*, 2004). The values for this index increased slowly until day 10 of storage when a sharp and significant increase was observed. The formation of TVBN is generally associated with the activity of microorganisms and tends to be high at high microbial population (Benjakul *et al.*, 2003). The changes in TVBN content may also be related to changes in pH (Hultmann & Rustad 2004). The initial lower levels of TVBN observed could be due to lower levels of endogenous ammonia due to reduced microbial activity during the first 10 days of storage of the fish in ice (Pacheco-Aguilar *et al.,* 2000). The increase in the TVBN could also be due to bacterial and enzymatic activity, especially the psychrophylic bacteria (Huss, 1995). The concentration of TVBN in fresh fillets is typically between 5 and 20 mg N 100 g^{-1}, whereas levels of 30–35 N 100 g^{-1} flesh are generally regarded as the limit of acceptability for ice-stored cold water fish (Huss, 1988; Connell, 1995). After 16 days of pre-freezing storage in FI, TVBN values (45.69±0.69 mg 100 g^{-1}) exceeded the legal limits (35 mg 100 g^{-1}) set for TVBN for consumption (Directive 95/149/EEC) while fillets exposed to pre-freezing storage in slurry ice were below this limit (21.57±0.62 mg 100 g^{-1}).

The prevailing of a particular microbial association, of meat depends on factors that persist during processing, transportation and storage in the market (Nychas *et al.*, 2008). Biochemical changes by endogenous enzymes cause loss of fish freshness and normally precede and are independent of bacterial deterioration (Ocaño-Higuera *et al.*, 2011), Normally, the deterioration of fish follows four stages: rigor mortis, dissolution of rigor, autolysis (loss of freshness) and bacterial spoilage (Ocaño-Higuera *et al.*, 2011). These stages occur fast or slow depending on the species, physiological condition of fish, microbial contamination and temperature. TVCs increased throughout the storage period in the present trial. Fish decomposition is a progressive proteolysis of the muscle tissue brought about primarily by the action of microorganisms and, to a lesser extent, by autolytic enzymes. Fillet TVC increased from 1.44±0.14 log cfu g^{-1} before chilling to 4.18±0.06 log cfu g^{-1} and 7.07±0.03 log cfu g^{-1}after 16 days chilling in SI and FI, respectively. Therefore, the TVC of

fillets chilled in FI exceeded 7 log cfu g^{-1} after 16 days which is considered the maximum level for acceptability of freshwater and marine fish (ICMSF, 1986). This increase correlated with the rise in pH and TVBN. The high post mortem pH and the chilled storage temperatures in the present trial were factors that could have contributed favourably to the sharp increase in the TVC (Martinsdóttir *et al.*, 2001). The increase in TVCs would also have led to an increase in the breakdown of nitrogenous components resulting in increased TVBN.

Haard (1992) reported that the initial colour of fishing products changed during storage in ice, affecting the quality. The coloration of flesh in darker fishes such as salmon is due to the predominance of carotenoid pigments, notably astaxanthin and canthaxanthin (Skrede & Storebakken, 1986). These pigments are degraded during frozen storage causing a decrease in a∗ values (Sheehan *et al.*, 1998). The L* and b* values of both SI and FI treated fillets increased significantly as storage time progressed in the present study. Several authors have attributed colour loss in fish muscle during storage to the oxidation of proteins with haemo groups, such as haemoglobin and myoglobin (Chaijan *et al.*, 2005; Richards *et al.*, 2002). Haemoglobin is a possible catalyst of lipid oxidation, due to its auto-oxidative capacity (Richards *et al.,* 2002).

Textural parameters are also frequently employed to examine and evaluate fish quality along the fish value chain, which mainly displays an assessment of the influence of handling and processing methods on the shelf life of fish products and partiality and satisfactoriness of consumers. It was noted that, the loss of texture during storage of fishery products has been revealed by Sato *et al.* (1991). The most common texture defects are muscle softening and gaping formation caused by pre-and post-mortem treatment (Cheng *et al.*, 2014). The existing problems are mostly associated with the changes of chemical compositions and the degradation of muscle proteins (Aussanasuwannakul *et al.*, 2010). Since the autolytic processes softens the muscle structure during rigor mortis (Montero & Borderias, 1990; Ando *et al.*, 1991; Ando *et al*., 1995), it is likely that the connective tissues of the myocommata of post-rigor compared to pre-rigor muscle is more susceptible to gaping (Einen *et al*., 2002). Sigurgisladottir *et al*. (2001) found that fillets from frozen salmon were softer than those of fresh salmon, and that freezing/thawing caused fibre shrinkage. Moreover, Ayala *et al.* (2010) measured the textural parameters of the dorsal muscle of the left location of sea bream dorsal muscle fillet by compression using a texturometer and the results indicated that, except springiness, all parameters changed with post-mortem storage, and most of them decreased

significantly within 5 d of storage. In particular, the values of hardness, gumminess, and chewiness all decreased sharply by about one-half, in comparison with the values observed at pre-rigor. In addition, this study found a similar softening due to freezing in pre-rigor fillets. In agreement with these results, in the present study hardness, cohesiveness, springiness, gumminess, chewiness and stiffness decreased significantly in all groups as storage time progressed, with the largest fall observed in fillets that underwent 16 d pre-freezing treatments in both SI and FI groups and indicating highest decrease in hardness in FI group fillets. The decrease in textual parameters may be due to greater activation of the enzymatic systems of the fillets (Simeonidou *et al.*, 1997). During ice storage the fish muscle softens or tenderises, due to a weakening of the Z-discs of the myofibrils, a degradation of connective tissue, or a weakening of myosin–actin junctions (Kato *et al.*, 1974; Wang *et al.*, 1998; Hultmann & Rustad, 2002). The texture of fish muscle is influenced by several factors such as rate of pH decline and extent of proteolysis causing breakdown of myofibrils (Hultmann & Rustad, 2002). The decrease in textural parameters could be due to the slow freezing of fish flesh during a long storage period. When some of the water freezes out, the concentration of solutes in unfrozen solutions increases (Duun & Rustad, 2008). This may lead to increased enzymatic activity, denaturation of the muscle proteins and structural damage of membranes, which can result in increased drip loss, loss of water holding capacity and textural changes (Foegeding *et al.*, 1996).

5.8 CONCLUSIONS

Fillets that underwent pre-freezing treatment in slurry ice had superior microbiological and physiochemical quality and thus extended shelf life compared to those subjected to pre-freezing treatment in flake ice. Low TVC values, a stable pH and low TVBN values were observed in fillets exposed to chilled storage in SI compared to FI. In addition to this, TVBN, pH, TVC, L* and b* increased to a greater extent from BF to 16 days in fillets stored in FI than in SI. On the basis of the results obtained, pre-freezing treatment in slurry ice maintained better quality in comparison to the fillets that underwent pre-freezing treatment in flake ice for up to 16 days. Thus, chilling barramundi fillets in slurry ice is recommended since this treatment can maintain higher quality fish products and would guarantee a longer shelf (at least for 16 days) compared to using flake ice.

CHAPTER 6

EFFECT OF RE-FREEZING CYCLES

CHAPTER 6: EFFECTS OF REFREEZING ON MICROBIOLOGICAL AND PHYSIOCHEMICAL PROPERTIES OF BARRAMUNDI (*LATES CALCARIFER*, BLOCH) FILLETS

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6.1 INTRODUCTION

The purpose of freezing seafood is to extend its shelf life by limiting microbial and enzymatic activities that cause deterioration (Makarios-Laham & Lee, 1993). Although freezing is an effective and important method of preserving seafood (Benjakul & Bauer, 2001), some deterioration in frozen seafood quality occurs during frozen storage (Boonsumrej *et al*., 2007) that can affect the structural and chemical properties of meats (Lawrie, 1979), which, in turn, may influence the quality of the final products (Verma *et al*., 1985). Temperature fluctuation or abuse generally occurs during transportation, storage or consumption, and repeated freeze thaw is also especially common at retail outlets, in homes and at restaurants (Srinivasan *et al*., 1997). The abuse directly contributes to biochemical and physicochemical changes in the muscle system (Xia *et al*., 2009) and then spoilage of fish caused by microbes (Sriket *et al*., 2007). One of the most important quality characteristics of fresh fish is muscle texture, and excessively soft fillets may cause problems for the industry (Hallett & Bremner, 1988; Haard, 1992; Andersen *et al*., 1997; Sigholt *et al*., 1997; Hultmann & Rustad, 2007).

Despite microbial spoilage being effectively terminated, quality deterioration, especially in texture, flavor and color, still takes place during freezing and frozen storage because of the osmotic removal of water, myosin denaturation, mechanical damage, as well as cross-linking and aggregation of myofibrillar (Shenouda, 1980; Benjakul *et al*., 2003). Likewise, meat and fish may undergo quality losses such as protein denaturation, color deterioration, weight decrement, oxidation of lipids and extensive textural changes because of freezing and thawing processes (Foegeding *et al*., 1996). Texture changes are the result of denaturation of muscle proteins, particularly those in the myofibrillar fraction (Haard, 1992).

Although many papers have been published on the effects of freezing thawing on the chemical and physical quality of fish muscle (Sigurgisladottir *et al*., 2000; Rouillé *et al*., 2002; Schubring *et al*., 2003; Kilinc & Cakli, 2004; Turhan *et al*., 2006; Boonsumrej *et al*., 2007), little has been reported about the effects of repeated freeze–thaw cycles on the

biological and physiochemical properties of barramundi. Consumers consider Australian barramundi to be a premium, great tasting, appealing, authentic and classy fish (Lawley, 2010). Barramundi is a commercially important aquaculture species throughout the Indo-Pacific region with significant industries in Thailand, Indonesia, Malaysia, Philippines, Taiwan and Australia (Glencross, 2006). The Australian Barramundi Farmers Association estimates that the Australian farmed barramundi industry produces around 5,000 tons of product and it has an estimated value of production at around $45 million at farm gate (Australian Barramundi Farmers Association, 2008). In Western Australia, one of the key edible aquaculture species produced in 2008–2009 included barramundi (453 tons, valued at $4.8 million) (ABARE-BRS, 2010). The purpose of this study was to investigate the effects of refreezing on the microbiological and physicochemical properties of barramundi fillets.

6.2 MATERIALS AND METHODS

6.2.1 Sample Preparation

Please refer to Chapter 3 (General methodology)

6.3 EXPERIMENTAL PROCEDURE

Three freeze-thawing cycles were tested in the present experiment. The control (fresh) fillets (cycle 0 = before freezing [BF]) were analyzed immediately after being received and were not subjected to freezing. Each experimental treatment had four replicates and was tested after 20 days of frozen storage. Samples at the end of each cycle were thawed under running tap water (18–20C). Subsamples from the thawed fish were analyzed for their quality and shelf life. The fillets were subsampled in the laboratory under hygienic conditions and macerated in an acid-washed glass blender. The average weight of each fillet was 1.05 kg.

All barramundi fillets were individually frozen on a polystyrene dish in an air blast freezer with 5 m/s air velocity at −80°C for 8 h at the Department of Agriculture and Environment, Curtin University, Perth, WA, Australia. All the frozen fillets were then stored in a freezer at −20°C at Curtin Aquatic Research Laboratory for 20 days. Analyses of barramundi fillets were then carried out on the 21st day from the commencement of storage.

Fillets were divided into three batches. In batch 1 (cycle 1), the fillets (12 fillets) were immediately shifted to an air blast freezer and then stored at −20°C in a freezer for 20 days.

Microbiological and physicochemical analyses were carried out after 20 days of frozen storage after thawing the sample. Batch 2 (cycle 2) underwent two cycles of freezing, storage for 20 days and then thawing before microbiological and physiochemical analysis was performed. Batch 3 (cycle 3), underwent three cycles of freezing, 20-day storage and thawing before analysis. Quality and shelf life of barramundi were evaluated using the microbiological and physiochemical analyses described below.

6.4 ANALYTICAL DETERMINATIONS

Please refer Chapter 3 (General methodology)

6.4.1 Microbiological Analysis

Please refer Chapter 3 (General methodology)

6.4.2 Proximate Analysis

Please refer Chapter 3 (General methodology)

6.4.3 pH

Please refer Chapter 3 (General methodology)

6.4.4 Total Volatile Base Nitrogen (TVBN)

Please refer Chapter 3 (General methodology)

6.4.5 Texture

Please refer Chapter 3 (General methodology)

6.4.5.1 Sample preparation

Please refer Chapter 3 (General methodology)

6.4.5.2 Texture profile analysis (TPA)

Please refer Chapter 3 (General methodology)

6.4.6 Color Measurement

Please refer Chapter 3 (General methodology)

6.5 STATISTICAL ANALYSIS

Please refer Chapter 3 (General methodology)

6.6 RESULTS

The proximate composition of fresh barramundi fillets were 72.10 ± 0.84% wet basis (w.b.) moisture, 1.07 ± 0.02% ash and 67.87 ± 0.38% dry basis (d.b.) protein. The moisture content was significantly higher ($P < 0.05$) after two cycles than after one and three. The moisture contents of barramundi fillets over three freeze–thaw cycles are shown in Table 6.1. The moisture content of fillets decreased from 72.10 ± 0.84% w.b. in the fresh samples to 70.25 ± 0.55% w.b. for the fillets subjected to three freeze–thaw cycles. Fillet ash content (Table 6.1) decreased significantly ($P < 0.05$) between BF and cycle 1 and did not change significantly ($P > 0.05$) thereafter. The fillet protein content decreased significantly ($P < 0.05$) to 54.32 ± 0.58 d.b. after three freeze–thaw cycles (Table 6.1).

Table 6.1: Changes in the moisture, ash, protein, pH, TVBN and TVC of barramundi (*Lates calcarifer*) fillets before freezing (BF), after one freeze-thaw cycle, two freeze-thaw cycles and three freeze-thaw cycles

	Moisture % w.b.	Ash %	Protein % d.b.	pH	TVB-N mg 100 g^{-1}	TVC Log CFU g^{-1}
BF	72.10±0.84^{ab}	1.07±0.02^{a}	67.87±0.38^{a}	6.26±0.00^{a}	5.22±0.06^{a}	1.45±0.17^{a}
Cycle 1	70.69±0.32^{a}	0.97±0.01^{b}	63.96±0.46^{b}	6.33±0.00^{b}	6.56±0.08^{b}	1.77±0.13^{a}
Cycle 2	74.34±0.66^{b}	0.90±0.02^{b}	55.04±0.87^{c}	6.45±0.00^{c}	7.07±0.06^{c}	3.07±0.10^{b}
Cycle 3	70.25±0.55^{a}	0.93±0.01^{b}	54.32±0.58^{c}	6.80±0.00^{d}	11.4±0.10^{d}	3.52±0.11^{b}

All values are the means ± SE of four replicates, n=4
Values followed by different superscript letters in the same column are significantly different at α=0.05
TVBN = Total Volatile Base Nitrogen, TVC = Total Viable Counts

Fresh barramundi fillets had a pH value of 6.26 ± 0.00 (Table 6.1). Fillet pH increased significantly ($P < 0.05$) after each freeze–thaw cycle from 6.26 ± 0.00 in fresh fillets to 6.80 ± 0.00 after three freeze–thaw cycles. The TVBN value of freeze-thawed barramundi

fillets increased significantly ($P < 0.05$) when compared with the fresh control and after each subsequent freeze–thaw cycle (Table 6.1). The TVBN value of fresh fillets was 5.22 ± 0.06 mg 100 g^{-1}. TVBN values of frozen-thawed fillets were between 6.56 ± 0.08 mg 100 g^{-1} (one cycle) and 11.40 ± 0.10 mg 100 g^{-1} (three cycles).

The changes in TVC on barramundi fillets, as influenced by freeze–thaw cycles, are presented in Table 6.1 TVC on fresh fillets was 1.45 ± 0.17 log CFU g^{-1} and had increased significantly ($P < 0.05$) to 3.52 ± 0.11 log CFU g^{-1} after three cycles. TVC was significantly higher ($P < 0.05$) after two and three cycles than on fresh fillets and after one cycle. TVC was significantly higher after two and three cycles than on fresh fillets and after one cycle. Regression analysis demonstrated a strong positive correlation between TVC and TVBN ($r^2 = 0.969$).

The color results for the barramundi fillets are shown in Table 6.2. The mean L* value of the fresh fillets was 54.89 ± 0.34, the mean a* value was -2.42 ± 0.12 and the mean b* value was 0.18 ± 0.04. L* increased significantly ($P < 0.05$) with increasing freeze–thaw cycles up to three cycles. a* and b* increased significantly ($P < 0.05$) to cycle 2 and did not change significantly ($P > 0.05$) thereafter. After three freeze–thaw cycles, L*, a* and b* values were 61.86 ± 1.09, -0.43 ± 0.08, and 1.18 ± 0.01, respectively.

Table 6.2: Changes in the color of barramundi (*Lates calcarifer*) fillets before freezing (BF), after one freeze-thaw cycle, two freeze-thaw cycles and three freeze-thaw cycles

	Color		
	L*	***a****	***b****
BF	54.89±0.34[a]	-2.42±0.12[a]	0.18±0.04[a]
Cycle 1	57.4±0.43[b]	-1.60±0.15[b]	0.51±0.18[a]
Cycle 2	59.04±0.47[b]	-0.41±0.07[c]	1.29±0.06[b]
Cycle 3	61.86±1.09[c]	-0.43±0.08[c]	1.18±0.01[b]

All values are the means ± SE of four replicates, n=4
Values followed by different superscript letters in the same column are significantly different at α=0.05
L = Lightness, a* = Redness, b* = Yellowness*

The rheological parameter values of the fresh barramundi fillets and fillets exposed to one, two and three cycles of freezing and thawing are presented in Table 6.3. The lowest values

for each rheological parameter were observed after three freeze–thaw cycles. The most significant ($P < 0.05$) (largest) decline in hardness, springiness and stiffness occurred between fresh and cycle 1 samples, with minimal change thereafter. The most significant ($P < 0.05$) decline in cohesiveness and gumminess occurred between cycle 1 and cycle 2 samples, with no observed change thereafter. Only a single freeze–thaw cycle had a significant impact on rheological parameters.

Table 6.3: Changes in the texture of barramundi (*Lates calcarifer*) fillets before freezing (BF), after one freeze-thaw cycle, two freeze-thaw cycles and three freeze-thaw cycles

	Hardness (N)	Cohesiveness	Springiness (cm)	Gumminess (kgf)	Chewiness (kgf.mm)	Stiffness (kgf/mm)
BF	3.14 ± 0.03^{a}	0.08 ± 0.01^{a}	0.09 ± 0.00^{a}	0.01 ± 0.00^{a}	0.03 ± 0.00^{a}	0.26 ± 0.08^{a}
Cycle 1	1.67 ± 0.09^{b}	0.05 ± 0.00^{b}	0.01 ± 0.00^{b}	0.01 ± 0.00^{a}	0.06 ± 0.00^{b}	0.06 ± 0.00^{b}
Cycle 2	2.12 ± 0.29^{b}	0.01 ± 0.00^{c}	0.01 ± 0.00^{b}	0.00 ± 0.00^{b}	0.04 ± 0.00^{a}	0.08 ± 0.01^{ab}
Cycle 3	1.58 ± 0.00^{b}	0.01 ± 0.00^{c}	0.01 ± 0.00^{b}	0.00 ± 0.00^{b}	0.01 ± 0.00^{c}	0.03 ± 0.00^{b}

All values are the means ± SE of four replicates, n=4
Values followed by different superscript letters in the same column are significantly different at α=0.05

6.7 DISCUSSION

The chemical composition of fish flesh varies both between species and between individuals, depending on sex, age, environment and season (Synnes *et al*., 2007). Gandotra *et al*. (2012a) reported that the moisture and ash content of Indian major carp (*Labeo rohita*) muscle decreased significantly during frozen storage. Alasalvar *et al*. (2002) and Orban *et al*. (2002) reported a decrease in total moisture content in sea bass (*Dicentrarchus labrax*) fillets during frozen storage. Sathivel (2005) reported a 4.1% moisture loss in pink salmon (*Oncorhynchus gorbuscha*) fillets after 3 months of frozen storage. James *et al.* (2002) stated that structural damage in meat occurred because of ice crystal formation during frozen storage (Lee *et al*., 2008). Farouk & Swan, (1998) reported that the moisture had been lost as thaw drip when frozen muscles were thawed. The decrease in moisture content is due to myofibrillar shrinkage caused by the formation of ice crystals, which damages muscle cells and cause protein denaturation (Lee *et al*., 2008). It is difficult to explain the significant increase in fillet moisture content after two freeze–thaw cycles in the present study. Zamir *et al*. (1998) reported a similar increasing trend in the moisture content of crab meat during refrigeration and attributed this to the loss of water-holding capacity of the tissue.

Ash content in BF was significantly higher than all cycles but there was no significant difference among cycles. Beklevik *et al*. (2005) and Okeyo *et al*. (2009) reported a decrease in total ash content during the frozen storage of sea bass (*Dicentrarchus labrax*) fillets and nile perch (*Lates Niloticus*), respectively. The proximate composition of fish is affected by a diversity of factors such as size, sexual maturation, temperature, salinity, exercise, ration, time and frequency of feeding, starvation, type and amount of dietary ingredients (Shearer, 1994). The decline in protein content after frozen storage observed in the present study was also reported by Beklevik *et al*. (2005) in sea bass (*Dicentrarchus labrax*); Siddique *et al*. (2011) in jatpunti (*Puntius sophore*), sarpunti (*P. sarana*) and thaisarpunti (*P. gonionotus*); and Gandotra *et al*. (2012a) in *Labeo rohita* (Ham-Buch). These researchers attributed this protein loss to amino acids and water soluble protein leaching out with melting ice. Although microbial spoilage can be terminated effectively by frozen storage, a number of changes in proteins shorten the shelf life of frozen fish (Benjakul *et al*., 2003).

The pH value of fresh fish is often between 6.0 and 6.5 (Ludorf & Meyer, 1973; Ersoy *et al*., 2008), which is similar to the present study. This increase in pH values with each cycle may be associated with the production of basic components induced by the growth of bacteria (Simeonidou *et al*., 1998), which has been attributed to the suppression of acidic groups through molecular changes in the proteins (Wierbicki *et al*., 1957). The pH is an important determinant of microbial growth and seafood with a high pH has a high spoilage potential and a short shelf life (Newton & Gell, 1981).

The quality of the frozen samples was also determined from the TVBN values. The TVBN value is affected by species, catching season and region, age and sex of fish (Ersoy *et al*., 2008). A level of 35 mg 100 g^{-1} has been considered the upper limit, above which fishery products are considered unfit for human consumption (Ludorf & Meyer, 1973). The TVBN value in the present study after three cycles was 11.4 mg 100 g^{-1}, which is acceptable for human consumption. The TVC value increased significantly with each additional freeze–thaw cycle and the TVBN value increased in a similar fashion. Therefore, there was a strong correlation between TVC and TVBN ($r^2 = 0.969$) when subjected to repeated freeze–thaw cycles. Sini *et al*. (2008) reported that the slight increase in TVBN values in samples of rohu (*Labeo rohita*) stored at ambient and refrigerated temperatures were due to enzymatic and microbial activities. Pastoriza & Sampedro (1994) stated that it would be reasonable to assume, therefore, that the TVC should give an indication of postmortem storage time, and

hence could be related to spoilage. This demonstrates that changes in TVBN and TVC are positively related.

The acceptability limits for TVC are between 10^4 and 5×10^6 cfu/g in several countries, according to varying types of fish products (Kose *et al*., 2001). According to Meat Standards Committee (2002), 10^4–10^6 TVC/cm^2 or per gram is considered an acceptable range in Australia, and TVC was within this acceptable range after freezing in the present study. In the present study, TVC increased with each freeze–thaw cycle. This was due to repeated melting and reformation of ice crystals damaging the cell membranes and organelles (Benjakul & Bauer, 2001). In addition, Benjakul & Bauer (2001) reported that the loss of native conformation of muscle proteins, due to the freeze–thaw process, could make them more susceptible to enzymic hydrolysis.

Generally, color changes can occur during frozen storage because of lipid oxidation and pigment degradation processes (Dias *et al*., 1994). In pork, a decrease in *a** value (red color) and an increase in *L** value (lightness) and *b** value (yellow color) of all samples were observed with the concomitant increase in thiobarbituric acid-reactive substances formation (Xia *et al*., 2009). Further, the decrease in redness and increase in yellowness induced by the freeze–thaw cycles in pork were possibly caused by the formation of metmyoglobin (Xia *et al*., 2009). Van Laack (1994) reported that the dark tissue of chicken skin was possibly brighter because of freeze burns (Lee *et al*., 2008). The color of the frozen fillets of broiler breast tended to be darker, redder and less yellow than the control, with increased storage duration (Lee *et al*., 2008). Frozen storages resulted in color changes that are due to changes in light absorption and light scattering caused by freeze denaturation (Francis, 1975; Lopkulkiaert *et al*., 2009). Frozen herring fillets became discolored because the lipid oxidation induced the formation of yellow fluorescent pigments in the fillet (Hamre *et al*., 2003; Thanonkaew *et al*., 2006). In this present study, fillets became lighter than the fresh fillets after three freeze–thaw cycles. The *a** (redder) and *b** (yellowish) values increased up to two cycles and did not change significantly afterward. The higher *L** and *b** values may be attributed to freezer burn (Lee *et al*., 2008).

Casas *et al*. (2006) reported that (1) if a standard sample is used to measure texture, it should always be taken from the same location in the fillet and (2) back or belly regions should be used instead of the tail location as the latter shows a wider range of textures. Likewise, in the present study the rheological parameters of barramundi fillets were taken from the belly

regions. Szczesniak (1963) reported that hardness, springiness and cohesiveness are the primary mechanical variables for characterizing the texture properties of food. When the texture of raw fish is measured, hardness and springiness are often the major variables (Botta, 1991). Protein denaturation, water loss and toughening of fish flesh are associated with frozen storage (Gill *et al*., 1979; Howgate, 1979; Kreuger & Fennema, 1989; Mackie, 1993; Nilson, 1994). This has mainly been demonstrated for fish species such as cod, haddock, hake, Alaska pollock and tilapia (Gill *et al*., 1979; Kreuger & Fennema, 1989; Hurling & McArthur, 1996). Likewise, in the present study, decrease in fillet hardness, cohesiveness, springiness, gumminess, chewiness and stiffness values occurred after freeze thawing (Table 6.3).

Hultmann & Rustad (2002) and Gallart-Jornet *et al*., (2007) reported that larger fresh salmon (*Salmo salar*) flesh hardness reduced significantly during iced storage for 11 days while cohesiveness increased. Likewise, Schubring (2002) revealed that the hardness of gutted cod (*Gadus morhua*) decreased significantly ($P < 0.05$) during storage in ice, indicating that fish becomes softer as result of proteolysis caused by endogenous and microbial enzymes. However, Hultmann *et al*. (2004) reported no significant linear dependence between textural properties and storage time during iced storage of salmon (*Salmo salar*). The increase in toughness of thawed meat has been attributed to myosin denaturation as well as to aggregation of myofibrillar proteins (Sikorski *et al*., 1976; Ramirez *et al*., 2000). In the present study, freeze thawing resulted in changes in barramundi fillet texture, as manifested by the lower rheological parameters. In general, published research on changes in rheological parameters because of the freeze thawing of fish fillets is limited and requires further investigation.

6.8 CONCLUSIONS

In the present study, fillet quality decreased with increasing number of freeze–thawing cycles, with the maximum biochemical losses being observed in the fillets subjected to three freeze–thaw cycles. The repeated freezing and thawing had a significant effect on the physical, chemical and microbiological quality of barramundi fillets. Freeze thawing of barramundi fillets caused protein denaturation and fillet discoloration. There was strong positive correlation between TVC and TVBN with each of these two variables increasing when subjected to repeated freeze–thaw cycles. The present study demonstrates that it is important to prevent temperature variations or abuse in barramundi fillets as it could

deteriorate the fillet quality. The rehydration and dehydration of the fillets would be expected to cause damage during freezing and transport with consequent loss in the quality and shelf life of barramundi fillets.

CHAPTER 7

EFFECTS OF SODIUM TRIPOLYPHOSPHATE AND VARIOUS FREEZE-THAW CYCLES

CHAPTER 7: EFFECTS OF SODIUM TRIPOLYPHOSPHATE AND VARIOUS FREEZE-THAW CYCLES ON MICROBIOLOGICAL AND PHYSIOCHEMICAL PROPERTIES OF BARRAMUNDI *(LATES CALCARIFER*, BLOCH*)* FILLETS

7.1 INTRODUCTION

Fish quality and shelf life are very important due to increasing consumer demand for fish products (Etemadian *et al.*, 2012). Population, fish species, spawning period, season, nutrition, post-harvest handling, and storage are some of the key factors that will impact the quality of a fish product (Kinsella, 1988; Nielsen *et al.*, 2002). Frozen storage is important for extending the shelf life of seafood (Lopkulkiaert *et al.*, 2009). Zakhariya *et al*. (2014) demonstrated that temperature variations or abuse during the storage of barramundi fillets could deteriorate fillet quality, which decreased with increasing number of freeze–thawing cycles.

Polyphosphates, which are polymers of phosphoric acid, are widely used as food additives and can act as buffers, emulsifiers, dispersants, antioxidants, and sequestrates (Etemadian *et al.*, 2013). Of the numerous types of phosphates used as additives in meat, poultry and seafood products, sodium tripolyphosphate (STPP) has the most satisfactory characteristics (Brotsky & Everson, 1973). Etemadian *et al.* (2013) indicated that polyphosphate solutions were the most effective method to improve the fillet quality of kutum (*Rutilus frisii kutum*). Additionally, Tanikawa *et al.* (1963) obtained a reduction in drip formation among cod fillets by soaking the fillets for 30 min in 0.5% of sodium pyrophosphate and sodium tripolyphosphate prior to freezing. However, the beneficial effects of exposing cod fillets to polyphosphate solutions prior to freezing appeared to be minimal in comparison to exposing fillets to water (Cormier & Leger, 1987).

Love & Abel (1966) reported that STPP interacts with proteins to produce a surface film on treated fillets and they theorized that such a film would seal in fluids and thus not only reduce thaw drip but also minimize moisture loss during frozen storage. The change in the surface protein layers that takes place when fish flesh is treated with STPP may not only improve the water-holding capacity but may also contribute to the overall antioxidant properties of STPP (Tims & Watts, 1958; Scheurer, 1968). The surface film could act as a barrier to oxygen resulting in a reduction in oxidation during prolonged frozen storage (Tenhet *et al.*, 1981).

Oxidation is a significant cause of quality deterioration of frozen seafood (Khayat & Schwall, 1983). Phosphate treatment of fish fillets results in increased pH and superior moisture binding (Hamm, 1960; Miller *et al*, 1968; Honikel *et al*., 1981; Young *et al*., 1999). STPP treatment can improve the shelf life of fish and fish products and can improve the quality of frozen-thawed fish (Kilinc *et al*., 2009a; Kilinc *et al*., 2009b).

However, in contrast, not all researchers are convinced of the benefits of polyphosphates (Cormier & Leger, 1987). Furthermore, there have been no published studies on the effects of STPP on microbiological and physiochemical properties of barramundi fillets subjected to various freeze-thaw cycles. Barramundi is a commercially important aquaculture species throughout the Indo-Pacific region with significant production in Thailand, Indonesia, Malaysia, Philippines, Taiwan and Australia (Glencross, 2006). The aim of the present study was to examine the effect of 0.05% STPP solution (the legally permitted concentration in Australia) (FSANZ, 2013), on the quality of barramundi fillets during three freeze-thaw cycles.

7.2 MATERIALS AND METHODS

7.2.1 Sample Preparation

Please see refer chapter 3 (General Methodology)

7.3 EXPERIMENTAL PROCEDURE

Three freeze-thawing cycles were tested in the present experiment. The control (fresh) fillets (cycle 0 = BF) were analysed immediately after being received and were not subjected to freezing. Each experimental treatment had four replicates, with a single fillet (1.05 kg) used per replicate. Samples were thawed under running tap water (18-20^{o}C) at the end of each cycle. The experimental design is shown in Figure 7.1.

7.3.1 STPP Treatment of Frozen-Thawed Barramundi Fillets

The sodium tripolyphosphate (STPP) (Vesco Foods Pty Ltd, Perth, Western Australia) used in the present study was dissolved in tap water to obtain a final concentration of 0.05 g/100 mL. STPP was then sprayed on both sides of each fillet using a spray bottle. The average weight of each fillet was 1.05 kg.

Each treatment was divided into two batches: in the first batch, samples were sprayed with 0.05% STPP; in the second batch, samples were left untreated (i.e. *Treated in STPP; Untreated*). All samples were divided into three groups. Group 1 (C1) underwent one cycle of freezing and thawing before quality and shelf life analysis. Group 2 (C2) and group 3 (C3) underwent two and three cycles of freezing and thawing respectively before analysis. Fillets in C1 were divided into two batches (*Treated in STPP [T1]; Untreated[T2]*) in Cycle 1. Fillets in C2 were divided into four batches from T1 (*Treated in STPP [T3]; Untreated [T4]*) and T2 (*Treated in STPP[T5]; Untreated[T6]*). Fillets in C3 were divided into eight batches from T3 (*Treated in STPP[T7]; Untreated[T8]*), T4 (*Treated in STPP [T9]; Untreated [T10]*), T5 (*Treated in STPP[T11]; Untreated[T12]*), and T6 (*Treated in STPP[T13]; Untreated[T14]*). Designated fillets were re-sprayed with STPP after each freeze-thaw cycle.

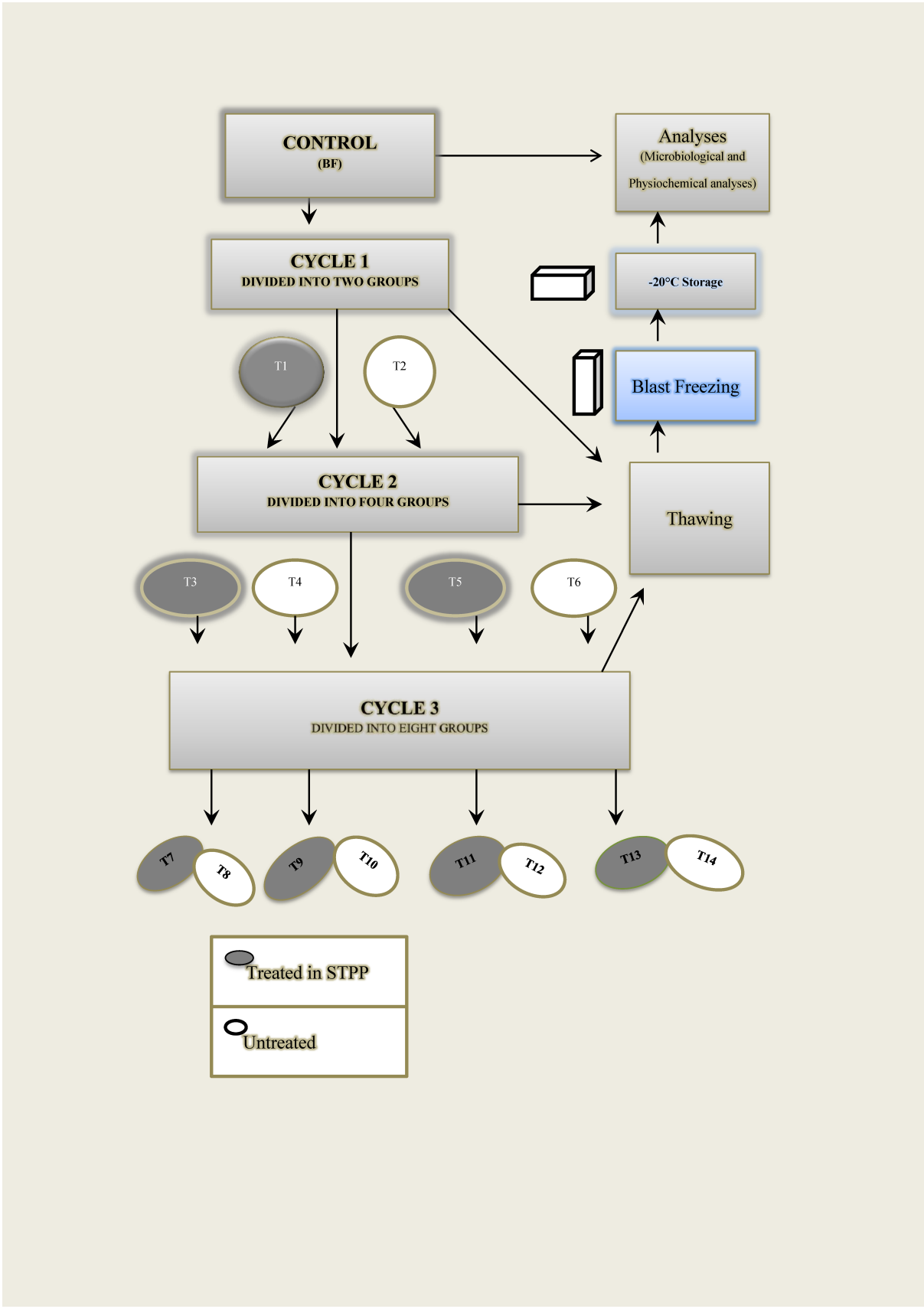


Figure 7.1: A flow diagram showing the experimental design

7.4 ANALYTICAL DETERMINATIONS

Please refer Chapter 3 (General methodology)

7.4.1 Microbiological Analysis

Please refer Chapter 3 (General methodology)

7.4.2 Proximate Analysis

Please refer Chapter 3 (General methodology)

7.4.3 pH

Please refer Chapter 3 (General methodology)

7.4.4 Total Volatile Base Nitrogen (TVBN)

Please refer Chapter 3 (General methodology)

7.4.5 Texture

Please refer Chapter 3 (General methodology)

7.4.5.1 Sample preparation

Please refer Chapter 3 (General methodology)

7.4.5.2 Texture profile analysis (TPA)

Please refer Chapter 3 (General methodology)

7.4.6 Color Measurement

Please refer Chapter 3 (General methodology)

7.5 STATISTICAL ANALYSIS

Statistical analyses were performed using SPSS version 19.0 (IBM Corp., Armonk, NY). All results data were expressed as means ± S.E. (Standard Error), of four replicated samples. Analysis of variance (ANOVA) followed by Tukey post hoc analysis was used to determine

significant differences between treatments at $\alpha < 0.05$ levels. All data were tested for homogeneity of variance by Levene's test.

7.6 RESULTS

The mean moisture content of fresh barramundi fillets was 71.63±0.08%. Fillets treated with STPP before each cycle (3-times) had the highest mean moisture content (74.32±0.21). This was significantly higher ($P<0.05$) than that of most fillets treated with STPP on less than two occasions (Table 7.1). STPP treatment of fillets (T1, T3, and T7) had no significant impact on ash contents whereas the protein contents of STPP treated fillets (T1, T3, and T7) were significantly higher ($P<0.05$) than other treatments within each cycle (Table 7.1). Fillet ash content decreased from 1.04±0.02 in the fresh samples (BF) to as low as 0.81±0.02 in T5 and 0.83±0.02 in T10. The protein content of fillets decreased from 69.45±0.18% in the fresh samples to 59.28±0.43 for STPP treated fillets (T7) and 54.04±0.01 for untreated fillets (T14) subjected to three freeze-thaw cycles. The fillet protein content of treatments T8 to T14 was significantly lower ($P<0.05$) than all other treatments (T1-7). All treatments (T1-T14) had significantly lower protein than in fresh fillets (Table 7.1).

Table 7.1: Changes in the moisture, ash, crude protein, pH, TVBN and TVC of barramundi (*Lates calcarifer*) fillets before freezing (BF), after one freeze-thaw cycle, two freeze-thaw cycles and three freeze-thaw cycles

	Moisture % w.b.	Ash %	Protein % d.b	pH	TVBN mg 100 g-1	TVC Log CFU g^{-1}
BF	71.63±0.08^{abcd}	1.04±0.02^{c}	69.45±0.18^{f}	6.25±0.00^{a}	5.45±0.09^{a}	1.37±0.09^{a}
T1*	72.91±0.51^{cde}	0.97±0.04^{abc}	63.98±0.39^{de}	6.33±0.01^{b}	7.36±0.04^{b}	1.55±0.05^{ab}
T2	70.99±0.06^{abc}	0.96±0.01^{abc}	62.11±0.35^{cd}	6.37±0.02^{ab}	7.48±0.02^{b}	2.22±0.12^{cd}
T3**	72.92±0.98^{cde}	0.96±0.01^{abc}	65.02±0.00^{e}	6.54±0.00^{de}	9.34±0.05^{c}	2.10±0.09^{bc}
T4*	70.05±0.18^{ab}	0.89±0.00^{abc}	61.55±0.50^{c}	6.38±0.00^{bc}	9.69±0.07^{c}	2.87±0.02^{defg}
T5*	69.22±0.76^{a}	0.81±0.02^{a}	61.67±0.14^{c}	6.40±0.00^{c}	9.42±0.02^{c}	3.55±0.16^{ghi}
T6	71.06±0.04^{abc}	0.90±0.03^{abc}	62.05±0.01^{cd}	6.49±0.03^{d}	9.30±0.04^{c}	3.15±0.10^{fgh}
T7***	74.32±0.21^{e}	0.95±0.00^{bc}	59.28±0.43^{b}	6.57±0.00^{ef}	11.34±0.03^{d}	2.30±0.10^{cde}
T8**	69.81±0.60^{ab}	0.92±0.02^{abc}	54.86±0.80^{a}	6.69±0.00^{gh}	12.02±0.03^{e}	2.95±0.13^{efgh}
T9**	72.04±0.28^{bcde}	0.85±0.07^{ab}	55.09±0.43^{a}	6.87±0.01^{i}	11.51±0.07^{d}	5.62±0.17^{k}
T10*	70.12±0.12^{ab}	0.83±0.02^{ab}	54.32±0.22^{a}	6.75±0.00^{h}	11.40±0.14^{d}	2.52±0.08^{cdef}
T11**	73.55±0.70^{de}	0.93±0.02^{abc}	54.47±0.62^{a}	6.55±0.00^{de}	11.55±0.09^{d}	4.32±0.20^{j}
T12*	71.41±0.58^{abcd}	0.99±0.01^{bc}	55.12±0.73^{a}	6.63±0.00^{fg}	12.33±0.18^{ef}	2.92±0.13^{efgh}
T13*	69.92±0.43^{ab}	0.85±0.06^{ab}	55.79±0.07^{a}	6.71±0.00^{h}	12.42±0.10^{ef}	4.02±0.26^{ij}
T14	70.50±0.22^{abc}	0.92±0.01^{abc}	54.04±0.01^{a}	6.90±0.01^{i}	12.65±0.13^{f}	3.60±0.04^{hi}

Values are mean ± SE, n=4
Values followed by different superscript letters in the same column are significantly different at α=0.05
TVBN = Total Volatile Base Nitrogen, TVC = Total Viable Counts
** = Treated in STPP and 'denotes' number of STPP treatments*
Dotted line indicates separate freeze-thaw cycles

STPP treated (T1, T3, and T7) fillet pH increased significantly ($P<0.05$) after each freeze-thaw cycle from 6.25±0.00 in fresh fillets to 6.57±0.00 after three freeze-thaw cycles (T7). However, the pH of treatments T8 to T14 increased to a greater extent, to between 6.69±0.00 (T8) and 6.90±0.01 (T14) when exposed to three freeze-thaw cycles (Table 7.1). T1-T14 all had significantly higher ($P<0.05$) TVBN than in fresh fillets (5.45±0.09 mg $100g^{-1}$), with TVBN increasing significantly ($P<0.05$) with each cycle. STPP had no significant impact ($P>0.05$) on TVBN over two cycles but did after three cycles ($P<0.05$), where TVBN in T7 was significantly higher ($P<0.05$) than in T8, T12, T13 and T14 (Table 7.1). Fillet TVC increased from 1.37±0.09 log $CFUg^{-1}$ on fresh fillets to between 1.55±0.05 log $CFUg^{-1}$ (T1)

after one cycle and 2.30±0.10 log $CFUg^{-1}$ (T7) after three cycles for STPP treated fillets. All the other treatments exhibited significantly higher ($P<0.05$) TVC values when compared to STPP treated (T1, T3, and T7) fillets within each cycle (Table 7.1). There was a strong positive correlation between TVBN and TVC ($r^2 = 0.899$) over the three freeze-thaw cycles. The L* values of fillets increased significantly ($P<0.05$) (ie. fillets became lighter) when subjected to each freeze-thaw cycle. The a* and b* values of fillets increased significantly ($P<0.05$) from BF to T3 (two freeze-thaw cycles) and did not change significantly ($P>0.05$) thereafter. STPP had no significant impact ($P>0.05$) on fillet color (Table 7.2). There was strong positive correlation between pH and color values (L*, a* and b*) ($r^2 = 0.981$) over repeated freeze-thaw cycles.

Table 7.2: Changes in the color of barramundi (*Lates calcarifer*) fillets before freezing (BF), after one freeze-thaw cycle, two freeze-thaw cycles and three freeze-thaw cycles

	Color		
	L*	a*	b*
BF	54.92±0.34^{a}	-2.50±0.13^{a}	0.29±0.10^{a}
T1*	57.60±0.56^{ab}	-1.18±0.06^{bc}	0.71±0.11^{a}
T2	57.35±0.14^{ab}	-1.47±0.05^{b}	0.43±0.13^{a}
T3**	59.89±0.30^{bc}	-0.64±0.18^{cd}	1.36±0.16^{b}
T4*	59.01±0.75^{b}	-0.53±0.08^{d}	1.20±0.02^{b}
T5*	58.84±0.67^{b}	-0.34±0.17^{d}	1.32±0.00^{b}
T6	58.49±0.91^{b}	-0.49±0.01^{d}	1.50±0.08^{b}
T7***	62.52±0.89^{cd}	-0.35±0.14^{d}	1.43±0.03^{b}
T8**	61.99±0.48^{cd}	-0.47±0.09^{d}	1.35±0.05^{b}
T9**	62.76±0.56^{cd}	-0.30±0.03^{d}	1.39±0.00^{b}
T10*	62.78±0.25^{d}	-0.43±0.12^{d}	1.31±0.01^{b}
T11**	63.36±0.56^{d}	-0.36±0.08^{d}	1.52±0.00^{b}
T12*	62.48±0.51^{cd}	-0.25±0.06^{d}	1.30±0.00^{b}
T13*	62.06±0.65^{cd}	-0.17±0.02^{d}	1.49±0.11^{b}
T14	62.65±0.03^{cd}	─0.43±0.16^{d}	1.43±0.20^{b}

Values are mean ± SE, n=4
Values followed by different superscript letters in the same column are significantly different at α=0.05
L = Lightness, a* = Redness, b* = Yellowness*
** = Treated in STPP and 'denotes' number of STPP treatments*
Dotted line indicates separate freeze-thaw cycles

All rheological parameters except gumminess decreased significantly ($P<0.05$) after one freeze-thaw cycle. Cohesiveness and gumminess decreased significantly ($P<0.05$) between one and two cycles. There was minimal change in parameters thereafter. STPP addition had no significant impact on rheological parameters ($P>0.05$) (Table 7.3).

Table 7.3: Changes in the texture of barramundi (*Lates calcarifer*) fillets before freezing (BF), after one freeze-thaw cycle, two freeze-thaw cycles and three freeze-thaw cycles.

	Hardness (N)	Cohesiveness	Springiness (cm)	Gumminess (kgf)	Chewiness (kgf.mm)	Stiffness (kgf/mm)
BF	3.10±0.02^{b}	0.08±0.00^{c}	0.06±0.00^{b}	0.01±0.00^{b}	0.08±0.00^{f}	0.24±0.01^{c}
T1*	1.58±0.08^{a}	0.04±0.00^{b}	0.01±0.00^{a}	0.01±0.00^{b}	0.04±0.00^{de}	0.07±0.00^{b}
T2	1.49±0.20^{a}	0.05±0.00^{b}	0.01±0.00^{a}	0.01±0.00^{b}	0.04±0.00^{cde}	0.04±0.00^{ab}
T3**	1.87±0.11^{a}	0.00±0.00^{a}	0.01±0.00^{a}	0.00±0.00^{b}	0.00±0.01^{e}	0.06±0.01^{ab}
T4*	1.85±0.24^{a}	0.01±0.00^{a}	0.01±0.00^{a}	0.00±0.00^{a}	0.03±0.00^{bcde}	0.04±0.01^{ab}
T5*	1.97±0.09^{a}	0.01±0.00^{a}	0.01±0.00^{a}	0.00±0.00^{a}	0.01±0.00^{ab}	0.02±0.00^{a}
T6	1.76±0.21^{a}	0.01±0.00^{a}	0.01±0.00^{a}	0.00±0.00^{a}	0.01±0.00^{ab}	0.03±0.00^{ab}
T7***	1.78±0.03^{a}	0.01±0.00^{a}	0.01±0.00^{a}	0.00±0.00^{a}	0.04±0.00^{cde}	0.02±0.00^{a}
T8**	1.27±0.15^{a}	0.00±0.00^{a}	0.01±0.00^{a}	0.00±0.00^{a}	0.02±0.00^{abc}	0.03±0.00^{ab}
T9**	1.41±0.10^{a}	0.01±0.00^{a}	0.01±0.00^{a}	0.00±0.00^{a}	0.02±0.00^{abcd}	0.02±0.00^{a}
T10*	1.31±0.10^{a}	0.00±0.00^{a}	0.01±0.00^{a}	0.00±0.00^{a}	0.01±0.00^{ab}	0.03±0.00^{ab}
T11**	1.47±0.22^{a}	0.00±0.00^{a}	0.01±0.00^{a}	0.00±0.00^{a}	0.01±0.00^{a}	0.02±0.00^{a}
T12*	1.36±0.13^{a}	0.00±0.00^{a}	0.01±0.00^{a}	0.00±0.00^{a}	0.01±0.00^{a}	0.03±0.00^{ab}
T13*	1.61±0.21^{a}	0.01±0.00^{a}	0.01±0.00^{a}	0.00±0.00^{a}	0.01±0.00^{a}	0.02±0.00^{a}
T14	1.63±0.10^{a}	0.00±0.00^{a}	0.01±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.03±0.00^{ab}

Values are mean ± SE, n=4
Values followed by different superscript letters in the same column are significantly different at α=0.05
** = Treated in STPP and 'denotes' number of STPP treatments*
Dotted line indicates separate freeze-thaw cycles

7.7 DISCUSSION

While much research has been conducted on the effects of STPP treatment on food products such as meats and seafood, the actual mechanism of action of STPP on proteinaceous foods is not well understood (Tenhet *et al.*, 1981). Given the various approaches of treating and evaluating the effects of sodium polyphosphates on fish fillets, it is difficult to compare the results of different researchers (Cormier & Leger, 1987). The effects of phosphates also depend on the application methodology and its concentration (Erdogdu *et al.*, 2007). Polyphosphate treatment of frozen cod (*Gadus morhus*) fillets significantly increased

expressible moisture yield after 31 weeks of storage, suggesting that polyphosphates may prevent further degradation of the protein structure during extended frozen storage on frozen cod fillets (Cormier & Leger, 1987). Woyewoda & Bligh (1986) reported rapid increases in expressible fluid among untreated cod fillets stored at -12°C; however, the apparent effectiveness of the polyphosphate treatment on other fillets was diminished after 10 weeks at this temperature and the authors found that the cryoprotectant properties were more evident at -30°C during extended storage. In the present study, treating fillets with STPP three times resulted in higher moisture content and such an increasing trend in fillet moisture content could be due to the increase in the water-holding capacity of the myofibrillar protein. The decrease in moisture content of the untreated fillets after three freeze-thaw cycles may be due to myofibrillar shrinkage caused by the formation of ice crystals, which damages muscle cells and cause protein denaturation (Lee *et al*., 2008).

Protein and ash contents do not vary as often as lipid, since they are not impacted by diet, but are mainly determined by the species, genetic characteristics and size (Haard, 1992; Shearer, 1994). In the present study, there was significant decrease in ash and protein content from BF to all other treatments (Table 7.1). Drip loss during the thawing process might be the reason for the decrease in the whole moisture, ash and protein contents (Beklevik *et al.*, 2005).

Progressive denaturation of the proteins also occurs during frozen storage (Cormier & Leger, 1987). As the water phase of the protein matrix freezes, the formation and accretion of ice crystals causes cell disruption, concentration of salts, and dehydration of protein molecules (Shenouda, 1980). In addition, Benjakul & Bauer (2001) reported that the loss of native conformation of muscle proteins, due to the freeze-thaw process, could make them more susceptible to enzymic hydrolysis. Etemadian *et al.* (2012) stated that water holding capacity of kutum fillets decreased as storage time increased due to the greater denaturation of proteins with increasing storage time. STTP significantly reduced protein denaturation of fillets in the present study. Polyphosphates may interact with the positive charges of the protein molecule to increase the net negative charge, resulting in increased water-holding capacity (Trout & Schmidt, 1984; Knipe, 2004). As a result, the repulsive forces between protein molecules may increase, leading to increased water retention (Etemadian *et al.*, 2012). STPPs exhibit a more pronounced polyionic character enabling them to attach to positive sites on protein molecules, leading to improved protein solubility and enhanced water binding (Ünal *et al.*, 2006).

The pH of all fillets in the present trial increased post-freezing (treated and untreated). This increase in pH was presumably due to production of basic amines (Reddy *et al.*, 1995; Debevere & Bosku, 1996; Manju *et al.*, 2007). Kilinc *et al.* (2009a) and Etemadian *et al.* (2011) also reported an increase in pH for samples pre-treated with phosphates. However, phosphates have been found to exhibit buffering activity in the muscle in low-sodium meat products, which could maintain the pH of muscle (Sofos, 1986). This was confirmed in the present study, as shown by T7 having a lower pH than most other cycle three treatments.

TVBN levels are useful in determining whether fish are fit for human consumption (Oelenschläger, 1992). TVBN in fish is mainly composed of ammonia and primary, secondary and tertiary amines (Manju *et al.*, 2007). The TVBN values of treated and untreated fillets increased with each freeze-thaw cycle in the current study and after three cycles were between 11.34 (T7) and 12.65 mg $100g^{-1}$ (T14), which is below the acceptable level for human consumption (30 mg 100 g^{-1}) (Castro *et al.*, 2006; Zakhariya *et al.*, 2014). TVBN increase with each cycle may be related to spoilage by bacteria and the activity of endogenous enzymes (Vareltzis *et al.*, 1997). STPP treatment limited increase in TVBN compared with other treatments after three freeze-thaw cycles (T7 / T8, T12, T13, T14) in the present study. Similarly, Kilinc *et al.* (2009b) reported that the TVBN of saithe (*Pollachius virens*) was controlled with STPP treatment. Etemadian *et al.* (2012) reported that phosphate pre-treatment showed a synergistic effect with vacuum packaging of kutum fillets on reduction of total volatile base.

STPP treated fillets exhibited lower TVC values than non-treated fillets at each cycle in the present study. The TVC of sea bass (*Dicentrarchus labrax*) and saithe (*Pollachius virens*) fillets also decreased after being placed into phosphate treatments (Kilinc *et al.* 2009b). Polyphosphates may suppress the growth of bacteria by complexing metal ions essential for cell division (Davidson & Juneja, 1990) or by changing the cellular morphology (Zaika *et al.*, 1991). The effectiveness of phosphates as antimicrobial agents in meat products depends on the type of phosphate, the amount used, the specific food product and conditions under which they are used (Sofos, 1986). The acceptability limits for TVC are between 10^4 and $5x10^6$ cfu g^{-1} in several countries, according to varying types of fish products (Kose *et al.*, 2001). The TVC of fillets in the present study were below this concentration even after three freeze-thaw cycles with or without STPP addition. Kilinc *et al.* (2009b) reported that phosphates containing two and three phosphate units were more effective at reducing microbiological

counts than a simple phosphate. TVC increased at each freeze-thaw cycle with or without STPP treatment due to repeated melting and reformation of ice crystals damaging the cell membranes and organelles (Benjakul & Bauer, 2001). In addition, the loss of native conformation of muscle proteins, due to the freeze-thaw process, could make them more susceptible to enzymic hydrolysis and ultimately spoilage (Pastoriza & Sampedro, 1994; Benjakul & Bauer, 2001).

The detrimental effects induced by multiple freeze-thaw cycles on muscle color would affect not only the subsequent processing characteristics of muscle, but the willingness of consumers to purchase the end products (Guo *et al.*, 2013). During freeze-thaw treatment of carp (*Cyprinus carpio*), the free water in tissues increased due to the formation and reformation of ice crystals, and the reflective index of light on the thawed dorsal muscle surface increased, leading to a higher L* value. The result was associated with an increase in water loss (Guo *et al.*, 2013). This would explain the increase in L* at each cycle in the present study along with associated microbiological activities (Kilinc *et al.,* 2009a). The a* and b* values in the present study increased to the second cycle and did not increase significantly thereafter. Yellow discoloration (b*) in fish muscle is a major cause of quality deterioration (Guo *et al.*, 2013). Yellow discolored catfish (*Ictalurus punctatus*) fillets became darker and more yellow during 12 d of refrigerated storage (Li *et al.*, 2013) and the results suggest that while the yellow discoloration of fresh catfish fillets is related to carotenoid contents, the intensification of the discoloration during storage appears to be related to another chemical phenomenon (Li *et al.,* 2013). Kilinc *et al.* (2009b) reported that phosphate treatment of frozen-thawed seabass and saithe increased the L* values of the samples a little but did not affect a* and b*. Similarly, STPP had no impact on fillet color in the present study; possibly because the concentration of STPP applied (0.05%) may have been too low to induce an effect.

Previous studies on pork, beef (Mancini & Hunt, 2005), and poultry (Holownia *et al.*, 2003) identified a clear positive relationship between muscle pH and color. For fish it is generally assumed that pH is of importance, as alternating color is observed in early post-mortem stages of storage, while the pH is declining (Robb *et al.*, 2000; Stien *et al.*, 2005; Guillerm-Regost *et al.,* 2006; Erikson & Misimi, 2008; Roth *et al.*, 2009). There was a strong positive correlation between pH and colour values when fillets were subjected to repeated freeze-thaw cycles in the present study.

Just one freeze-thaw cycle significantly reduced the rheological parameters of barramundi fillets in the present study. This was also reported by Zakhariya *et al.* (2014). Texture is affected by the rate and duration of rigor mortis, pH, proteolysis and storage (Haard, 1992). Fillet hardness decreases during iced storage of *Rutilus frisii kutum* fillets (Etemadian *et al.*, 2011), *Sparus auratus* fillets (Alasalvar *et al.,* 2001), and *Argyrosomus regius* fillets (Hernández *et al.*, 2009), and during refrigerated storage of *Oncorhynchus mykiss* fillets (Kilinc *et al.*, 2009a). The results of the present study concur with those of Kilinc *et al.* (2009a, 2009b), which reported that texture qualities were not influenced by phosphate treatments. However, Kilinc *et al.* (2009b) also stated that when using higher concentrations of phosphates (5%) than in the present study (0.05%), sea bass fillets became much softer, less chewable and had higher cohesiveness. Etemadian *et al.* (2011) indicated that the springiness of fillets treated with STPP with increasing storage time was lower than that of the control and the other treatments. In contrast, Young *et al.* (1987) reported that STPP increased the cohesiveness, springiness and chewiness of chicken breast meat patties at the highest phosphate level (0.05%); but, in the presence of NaCI, the phosphate tended to increase these textural attributes, especially cohesiveness and chewiness, at lower phosphate levels. Etemadian *et al.* (2013) reported that kutum fillets dipped in 5% polyphosphate solution exhibited increased textural characteristics. Thus the concentration of STPP used in the present study (0.05%) may have been too low to improve the rheological parameters of the barramundi fillets treated.

7.8 CONCLUSIONS

The present study demonstrated that treatment with STPP at each freeze-thaw cycle can maintain the quality and shelf life of fillets exposed to three freeze-thaw cycles by limiting TVC and TVBN increase, increasing/maintaining the moisture content and limiting protein denaturation. STPP had no significant impact on any other measured parameters. Freeze-thaw cycles had a negative impact on all parameters. Repeated STPP treatment of barramundi fillets (after each freeze-thaw cycle) is recommended, particularly to maintain protein content and lower TVC. The use of STPP to treat barramundi fillets under repeated freeze-thaw cycles should be examined further, at higher concentrations under commercial conditions.

CHAPTER 8

EFFECT OF GLAZING, PACKAGING AND SODIUM TRIPOLYPHOSPHATE TREATMENTS

CHAPTER 8: EFFECT OF GLAZING, PACKAGING AND SODIUM TRIPOLYPHOSPHATE TREATMENT ON MICROBIOLOGICAL AND PHYSIOCHEMICAL PROPERTIES OF BARRAMUNDI (*LATES CALCARIFER*, BLOCH) FILLETS

8.1 INTRODUCTION

Freezing is a general preservation method used to limit biochemical changes in fish that occur during storage (Sathivel *et al.*, 2007). However, during frozen or cold storage, seafood products may develop surface drying and dehydration, which may lead to freezer burn, and may suffer from quality loss owing to oxidation or rancidity (Vanhaecke *et al.*, 2010; Hyldig *et al.*, 2012; Tolstorebrov *et al.*, 2014). Phosphate treatment are required to maintain fillet quality at a higher temperature such as -20°C. Phosphates can be added to seafood for retention of natural moisture, inhibition of flavour and lipid oxidation, aiding emulsification and removal of shellfish shells and offering cryoprotection. Use of polyphosphate dips increases the water holding capacity of flesh and reduces drip loss and quality deterioration (Schnee, 2000; Aitken, 2001; Turan *et al*., 2003; Gonçalves, 2005; Gonçalves *et al.*, 2008). STPP is one of the phosphates used in the seafood industry as a humectant, i.e., substances that maintain the moisture of the product (Gonçalves *et al.*, 2008). Polyphosphates are generally used at a concentration of 10% commercially (Graham, 1982). However, Etemadian *et al.* (2012) reported that using 2% STPP solution with vacuum packaging lowered bacterial load, TBA index, and total sulfhydryl groups oxidation, and increased water holding capacity, sensory quality and shelf life of kutum (*Rutilus frisii kutum*) fillets. Kilinc *et al.* (2009b) reported that treatment with 5% sodium tripolyphosphate solution is the most effective method to reduce the bacterial loads when compared with other phosphate groups and that phosphate treatment can improve the quality of frozen-thawed sea bass (*Dicentrarchus labrax*) and saithe (*Pollachius virens*) fillets.

Another established technology generally applied during freezing and frozen storage of seafood is glazing, which involves the application of a layer of ice to the surface of a frozen product by spraying or brushing on water or by immersing the product in a water bath (Vanhaecke *et al.*, 2010). The aim of glazing is to reduce the impact of cold storage deterioration on fillet quality (Jacobsen & Fossan, 2001). Glazing is widely used to protect

products from the effects of dehydration and oxidation during cold storage (Žoldoš *et al.*, 2011). Glazing of retail products has advantages from a producer's point of view since it is simple and relatively cheap (Jacobsen & Fossan, 2001). Applying packaging materials, which prevent oxygen penetration is also an effective method for preserving quality (Tolstorebrov *et al.,* 2014). The protective effect of correctly chosen packaging methods and materials seems to improve the shelf life especially for frozen fish and meat products (Ahvenainen & Malkki, 1985). Vacuum packaging can prevent the growth of some food-borne pathogens and spoilage bacteria commonly present on meat (Church & Parsons, 1995; Labadie, 1999; Barros-Velazquez *et al.*, 2003; Venter *et al.*, 2006) and so is widely used for packaging primal cuts for distribution to retailers (Pennacchia *et al.*, 2011).

To the best of our knowledge, there have been no published studies on the effect of pre and post freezing treatments such as STPP treatment, glazing and glazing with packaging on the quality of barramundi fillets or analysis of the biochemical and physical changes that occur during these treatments. The aim of the present study is to examine the effect of glazing solution, two different concentrations of STPP (2 and 5%) and packaging on the quality of barramundi fillets during frozen storage compared to unfrozen fillets.

8.2 MATERIALS AND METHODS

8.2.1 Sample Preparation

Please see refer chapter 3 (General Methodology)

8.3 EXPERIMENTAL PROCEDURE

Each experimental treatment had four replicates, with a single fillet (1.07 kg) used per replicate. Six different treatments were employed as shown in Figure 8.1.

Glazing was carried out by dipping the fillet into the ice water (fresh distilled water (pH value equal to 7.0) at 0°C for 4-6 sec. Glazing was repeated three times with 5-10 sec intervals, and then fillets were packaged manually with shrink film (Glad product, Gow Street, Padstow,

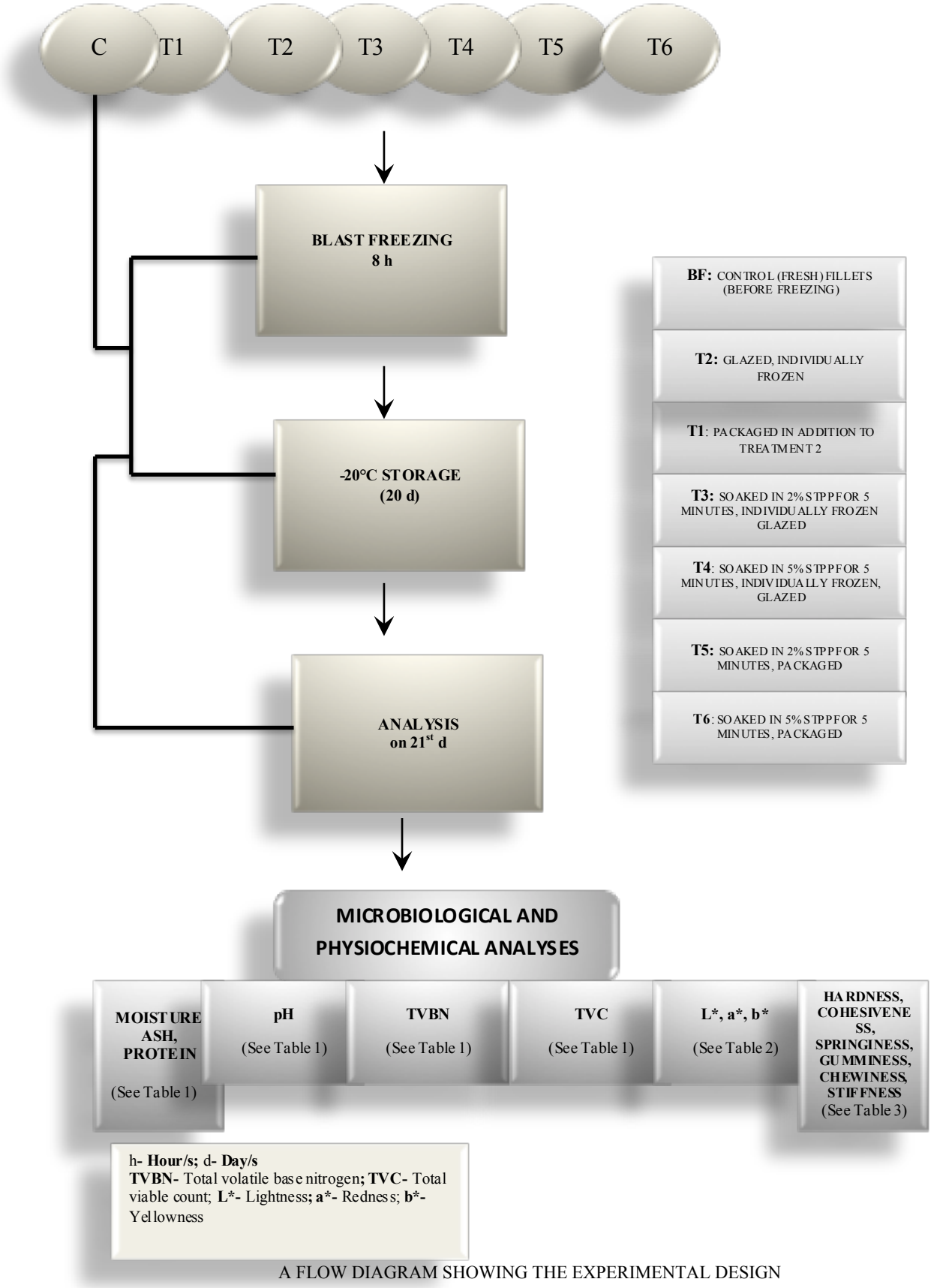


Figure 8.1: A flow diagram showing the experimental

NSW) with 3-4 layers. The sodium tripolyphosphate (STPP) (Vesco Foods Pty Ltd, Perth, Western Australia) used in the present study was dissolved in tap water to obtain final concentrations of 2 g/100 mL and 5 g/100 mL.

8.3.1 Barramundi Fillet Treatments

The experimental design is presented in Figure 1. The control (C) fillets were not subjected to or any pre-freezing treatments and were analysed immediately after freezing. The barramundi fillets were divided into six groups: all groups were washed under running tap water (18-20°C) prior to treatment; in the first group (T1), filleted samples were glazed after blast freezing; in the second group (T2), filleted samples were glazed and packaged after blast freezing; in the third group (T3), filleted samples were dipped into 2% STPP solution for 5 minutes prior to freezing and glazed after blast freezing; in the fourth group (T4), filleted samples were dipped into 5% STPP solution for 5 minutes prior to freezing and glazed after blast freezing; in the fifth group (T5) filleted samples dipped into 2% STPP solution for 5 minutes before freezing and glazed and then packaged after blast freezing; in the sixth group (T6), filleted samples were dipped into 5% STPP solution for 5 minutes before freezing and glazed and then packaged after blast freezing.

8.4 ANALYTICAL DETERMINATIONS

Please refer Chapter 3 (General methodology)

8.4.1 Microbiological Analysis

Please refer Chapter 3 (General methodology)

8.4.2 Proximate Analysis

Please refer Chapter 3 (General methodology)

8.4.3 pH

Please refer Chapter 3 (General methodology)

8.4.4 Total Volatile Base Nitrogen (TVBN)

Please refer Chapter 3 (General methodology)

8.4.5 Texture

Please refer Chapter 3 (General methodology)

8.4.5.1 Sample preparation

Please refer Chapter 3 (General methodology)

8.4.5.2 Texture profile analysis (TPA)

Please refer Chapter 3 (General methodology)

8.4.6 Color Measurement

Please refer Chapter 3 (General methodology)

8.5 STATISTICAL ANALYSIS

Please refer Chapter 3 (General methodology)

8.6 RESULTS

The moisture content of fillets in T4, T5 and T6 increased significantly ($P<0.05$) compared to C, T1 and T2 and was significantly higher ($P<0.05$) in T6 than in T3 (Table 8.1). There was no significant difference ($P>0.05$) in mean ash content between treatments (Table 8.1). Protein content was significantly lower ($P<0.05$) in all treatments than BF and significantly lower ($P<0.05$) in T4 than in T3 (Table 8.1).

The pH of fillets treated with STPP (T3, T4, T5 and T6) was significantly higher ($P<0.05$) after 20 days at -20^{o}C than those not treated with STPP (T1 and T2) (Table 8.1). Mean TVBN increased significantly ($P<0.05$) between treatments in the following order: T6 (7.52±0.02 mg 100 g^{-1}) < T2 (9.17±0.07) < T5 (10.22±0.22) < C (10.45±0.07) < T4 (11.54±0.01) < T3 (11.97±0.10) < T1 (12.35±0.14 mg 100 g^{-1}) (Table 8.2). Mean TVC was significantly higher ($P<0.05$) in all treatments than in T6 and significantly higher ($P<0.05$) in T1, T3, T4 and T5 than in T6, but did not exceed 3.93±0.12 in any treatment (Table 8.1).

Table 8.1: Changes in the proximate composition and biochemical parameters of barramundi fillets frozen (C) and when exposed to various treatments.

	Moisture % w.b.	Ash %	Protein % d.b	pH	TVBN mg 100 g^{-1}	TVC Log CFU g^{-1}
C	70.11±0.25^{a}	0.98±0.02^{a}	60.41±0.31^{c}	6.35±0.00^{a}	10.45±0.07^{c}	2.75±0.71^{b}
T1	70.82±0.16^{a}	0.99±0.01^{a}	60.15±0.11^{ab}	6.32±0.01^{a}	12.35±0.14^{e}	3.60±0.10^{c}
T2	71.20±0.43^{a}	1.46±0.28^{a}	61.30±0.10^{ab}	6.33±0.00^{a}	9.17±0.07^{b}	2.55±0.02^{b}
T3	72.21±0.39^{ab}	1.23±0.01^{a}	63.62±1.09^{b}	6.60±0.00^{b}	11.97±0.10^{d}	3.93±0.12^{c}
T4	74.02±0.53^{bc}	1.07±0.05^{a}	59.38±0.75^{a}	6.57±0.01^{b}	11.54±0.01^{d}	3.66±0.07^{c}
T5	73.52±0.75^{bc}	0.97±0.04^{a}	62.63±0.94^{ab}	6.56±0.00^{b}	10.22±0.22^{c}	3.87±0.02^{c}
T6	74.94±0.20^{c}	1.13±0.04^{a}	60.82±0.29^{ab}	6.55±0.01^{b}	7.52±0.02^{a}	1.59±0.11^{a}

All values are the means ± standard error of four replicates, n = 4.
Values followed by different superscript letters in the same column are significantly different at α = 0.05.
C, control; cfu, colony-forming unit; d.b., dry basis; TVBN, total volatile base nitrogen; TVC, total viable counts; w.b., wet basis.

Fillet lightness (L*) was significantly higher (P<0.05) in all treatments than in T1, in T2 , in T3 and in C than in T2 than in T6 (Table 8.2). Fillet redness (a*) was significantly higher (P<0.05) in all treatments than in T2 than in all other treatments and in T3 than T1. Fillet yellowness (b*) was significantly higher (P<0.05) in all treatments than in T1 and C, and in T4 and T5 than in T2 (Table 8.2).

Table 8.2: Changes in the L*, a*, and b* values of barramundi fillets on control and when exposed to various treatments.

	L*	a*	b*
BF	57.13±0.02^{bcd}	-1.44±0.07^{a}	0.56±0.41^{a}
T1	53.81±0.42^{a}	-2.11±0.02^{b}	0.39±0.03^{a}
T2	58.75±0.57^{d}	-1.14±0.05^{d}	1.01±0.02^{b}
T3	57.90±0.69^{cd}	-1.77±0.15^{c}	1.38±0.04^{bc}
T4	56.64±0.53^{bcd}	-1.80±0.04^{bc}	1.43±0.19^{c}
T5	57.25±0.06^{bcd}	-1.83±0.03^{bc}	1.44±0.03^{c}
T6	56.16±0.72^{bc}	-1.94±0.02^{bc}	1.34±0.10^{bc}

All values are the means ± standard error of four replicates, n = 4
Values followed by different superscript letters in the same column are significantly different at α = 0.05
L = Lightness, a* = Redness, b* = Yellowness*

All treatments had significantly lower (P<0.05) hardness, springiness, gumminess and stiffness than the T6 fillets. Fillets that were in control and glazed only (T1) exhibited significantly lower (P<0.05) hardness, and stiffness than most other treatments. Chewiness did not show significantly (P>0.05) changes (Table 8.3). There were no significant (P>0.05) in cohesiveness; although cohesiveness was highest in T2 and lowest in T1. Hardness was significantly higher (P<0.05) in STPP treated fillets with packaging T5 and T6) than in T1 and C.

Table 8.3: Changes in the hardness (n), cohesiveness, springiness (cm), gumminess (kgf), chewiness (kgf.mm), and stiffness (kgf/mm) of barramundi (*Lates calcarifer*) fillets on control and when exposed to various treatments.

	Hardness (N)	Cohesiveness	Springiness (cm)	Gumminess (kgf)	Chewiness (kgf.mm)	Stiffness (kgf/mm)
BF	1.50±0.32^{a}	0.01±0.00^{a}	0.01±0.00^{a}	0.01±0.00^{b}	0.05±0.00^{a}	0.04±0.01^{a}
T1	1.98±0.01^{a}	0.01±0.00^{a}	0.01±0.00^{a}	0.00±0.00^{a}	0.05±0.01^{a}	0.07±0.00^{a}
T2	2.20±0.05^{ab}	0.06±0.04^{a}	0.01±0.00^{a}	0.00±0.00^{a}	0.06±0.00^{a}	0.11±0.00^{ab}
T3	2.37±0.08^{bc}	0.03±0.00^{a}	0.01±0.00^{a}	0.00±0.00^{a}	0.04±0.00^{a}	0.12±0.02^{ab}
T4	2.46±0.04^{bc}	0.05±0.00^{a}	0.01±0.00^{a}	0.00±0.00^{a}	0.05±0.01^{a}	0.17±0.00^{b}
T5	2.66±0.13^{c}	0.05±0.00^{a}	0.01±0.00^{a}	0.00±0.00^{a}	0.04±0.00^{a}	0.15±0.00^{b}
T6	2.70±0.07^{c}	0.05±0.00^{a}	0.01±0.00^{a}	0.00±0.00^{a}	0.04±0.00^{a}	0.17±0.01^{b}

All values are the means ± standard error of four replicates, n = 4
Values followed by different superscript letters in the same column are significantly different at α = 0.05

8.7 DISCUSSION

The effectiveness of phosphates for maintaining water retention in meat products depends on the type and amount of phosphate, as well as on the type of product that was processed with their addition (Thorarinsdottir *et al.*, 2004). Numerous investigators have reported varying results on the use of polyphosphates in fish (Turan *et al.*, 2003). The main value of polyphosphates lies in increasing the water-retaining capacity of protein in fish (Turan *et al.*, 2003). Drip loss, or the release at water during thawing, implies nutrient loss. It has been reported that the usage of polyphosphate dips increases water-holding capacity of flesh and reduces drip and deterioration of the quality (Pigott & Tucker, 1990). The significantly higher (P<0.05) moisture content in T6 (5% STPP, packaged) may suggest that STPP concentration

or packaging may also increase the moisture content of fillets. As a result, the moisture % and pH value in T3 and T4- T6 was significantly higher. Fillets not treated with STPP (T1 and T2) actually maintained moisture % and pH value during frozen storage. The glaze added to all fillets helped to protect them from drying out (freezer burn) (CSIRO, 2014). Etemadian *et al.* (2012) reported that, 2% STPP pre-treatment in combination with VP (vacuum packaging) appeared to be the most effective in WHC (water holding capacity) during storage and hence increased shelf life of kutum (*Rutilus frisii kutum*) fillets in ice.

In the present study, protein content was lower in all treatments than in the control treatment; which may be due to the partial hydrolysis of protein (Zaitsev *et al.*, 1969). The decrease in the total crude protein content of fish flesh was possibly due to a decrease in salt soluble protein and water soluble protein (Chomnawang *et al.*, 2007) and this loss could also be due to autolytic deterioration associated with the actions of endogenous enzymes and bacteria (Hultman & Rustad, 2004). The functional and textural characteristics of meat depend mainly on myofibrillar proteins (Paredi & Crupkin, 2007). Myosin and actin are the major proteins which contribute to most of the functional properties of myofibrillar proteins (Mohan *et al.*, 2008).

There was an increase in pH in all STPP treatments, compared with C, T2 and T1; indicating that alkaline compounds were accumulated through autolytic activities or microbial metabolism (Pons-Sanchez-Cascado *et al.*, 2006) and this increase in pH was presumably due to the production of basic amines (Debvere & Boskou, 1996). This result is similar to that reported by Etemadian *et al.* (2011), who observed an increase in the pH of *Rutilus frisii kutum* samples pre-treated with phosphates. Etemadian *et al.* (2012) reported that regardless of phosphate pre-treatment, the pH of kutum samples stored under VP increased throughout storage in ice. Masniyom *et al.* (2005b) observed that the pH of air stored sea bass (*Lates calcalifer*) fillets increased throughout the storage time. Kilinc *et al.* (2009a) also reported that pH value in rainbow trout (*Onchorhyncus mykiss*) fillets increased according to the time of storage in sodium triphosphate.

Although the TVBN values of barramundi fillets in the present study increased significantly with storage time, up to 12.35±0.14 mg 100 g^{-1} in T1, they did not exceed the acceptable limit for human consumption (35 mg100 g^{-1}; Directive 95/149/EEC; EEC, 1995 as described in Kilinc *et al.* 2007 and Rodriguez *et al.* 2004) during the 20 day storage period. Maximum TVBN content was observed in T1 (12.35±0.14 mg 100 g^{-1}).

In many studies, TVC was close to the rejection level when bacterial counts reached 7 log cfu g^{-1} (Koutsoumanis & Nychas, 2000). The ICMSF (1986) has established a microbiological acceptability limit for human consumption of 7 log CFU g^{-1} for freshwater and marine species, which no treatments exceeded in the current study. Specifically, T6 fillets had the smallest increase in TVC over storage time compared with the other treatments. Etemadian *et al.* (2012) indicated that soaking kutum fillets in 2% STPP solution prior to VP effectively extended the shelf life of kutum fillets. Therefore, they suggested that VP and soaking fillets in phosphates, especially STPP, might prevent the growth of bacteria, leading to improved product safety. Similarly, the total viable bacteria counts on sea bass (*Dicentrarchus labrax*) fillets decreased from 3.85 to 1.98 log CFU g^{-1} after being placed in STPP solutions (Kilinc *et al.*, 2009b). However, in the present study fillets that underwent pre-freezing treatment with 2% STPP and packaging (T5) had a higher TVC value (3.87±0.02 log CFU g^{-1}) than with 5% STPP and packaging (T6) (1.59±0.11 log CFU g^{-1}).

Although the pigmentation of catfish fillets does not affect their flavor or nutritional quality, consumers may still reject the discolored product (Lovell, 1984). Kilinc *et al.* (2009a) and Molins (1991) noted that phosphates can improve fillet color. Kilinc *et al.* (2007) reported that phosphate treatment of frozen-thawed seabass and saithe did not affect a* and b*. Although the percentage of STPP used was up to 5% in the present study, STPP treatment and packaging increased lightness, redness and yellowness. The b* value in T4 and T5 was significantly higher than in T2. Lightness (L*) increased slightly, possibly due to greater water deposits on the fish surface, as the result of melting ice and liquid retention between the polyethylene film covering the fillets (Hernández *et al.* 2009) which explains the higher L* in T2. Li *et al*. (2013) suggested that other unknown chemical changes may be occurring to cause fillets to become darker and more yellow during storage. The present study also confirms that these factors require further research.

Whilst colour and texture measurements were not good indicators of deterioration, they may be used at certain times to support data obtained by physicochemical, microbiological and sensory methods (Hernández *et al.*, 2009). Hernández *et al.* (2009) observed that Lightness (L*) increased slightly as storage time progressed, possibly due to greater water deposits on the fish surface, as the result of melting ice and liquid retention between the polyethylene film covering the fillets (Hernández *et al*., 2009). Masniyom *et al.* (2005b) investigated the effect of different combinations of phosphate and modified atmosphere on refrigerated sea

bass slices, and observed that Hunter ‘a’ value of all samples gradually decreased whereas Hunter ‘L’ and ‘b’ values of seabass slices increased as storage time increased. Additionally, Etemadian *et al*. (2013) reported that Hunter L* and b* values of kutum fillets increased with increasing storage time in the fillets treated with STPP and this was probably due to the denaturation of myoglobin caused by an acidic pH (Masniyom *et al*., 2005b; Hernández *et al.*, 2009). It also may be due to drip loss emersion of respiration pigments of meat (Etemadian *et al*., 2013). The increase in b∗ value was associated with the formation of a yellowish color on the surface (Masniyom *et al.*, 2005b; Etemadian *et al*., 2013). This was presumed to be due to the oxidation of pigment under a high content of oxygen (Cort, 1974; Khayat & Schwall, 1983). Lipid oxidation was also postulated to cause the change in color (Calder, 2003; Masniyom *et al*., 2005b).

The alterations of physicochemical properties of fish can directly affect textural quality (Cheng *et al.*, 2014). Fish muscle texture depends on a number of intrinsic biological factors that are related to muscle fibre density, and involving both fat and collagen content (Hernández *et al.*, 2009). The fish death triggers autolytic and microbiological processes that make the muscle softer and less elastic (Olafsdottir *et al.*, 2004). The role of phosphate in improving the tenderness of meat is known (Mudalal *et al.*, 2014). All rheological parameters declined significantly during frozen storage in the present study. Texture changes are the result of denaturation of muscle proteins, particularly those in the myofibrillar fraction (Haard, 1992). Su´arez *et al.* (2005) studied the relation between fish firmness and the collagen content in sea bream muscle under different storage conditions. The collagen content in fish muscle decreased somewhat through the whole storage time, which was closely linked to the firmness of fish muscle. Packaging methods also affect fish product texture. Fuentes *et al.* (2012) compared the effect of air, vacuum and modified-atmosphere packaging methods on the textural properties of smoked sea bass under cold storage at 4°C for 42 d, and it was revealed that different packaging methods had a greater effect on the hardness, gumminess, and chewiness of fish samples, especially for vacuum- and modified-atmosphere packaging leading to visible decrease of textural parameters and hence it was noticed in this study all the rheological parameters decreased but not all treatments were packaged. STPP treatment at a concentration of up to 5% did not maintain the cohesiveness, springiness, gumminess chewiness or stiffness of fillets. Kilinc *et al.* (2009b) suggested that concentrations of phosphates higher than 5%, as used in the present trial, can be more effective at preserving textural parameters as dipping frozen-thawed fish species (sea bass

and saithe) in 5% phosphates improved the quality when compared with the control group. There was some improvement in T3-6 hardness and stiffness compared to all other treatments and control in the present study, with T1 (no STPP; no packaging) having significantly lower hardness, cohesiveness and stiffness than STPP treated fillets (T3, T4, T5 and T6). This confirms that STPP treatment was able to slow the deterioration of fillet rheological parameters during frozen storage.

8.8 CONCLUSIONS

STPP treatments (T3-T6) were able to slow the deterioration of fillet rheological parameters (hardness, cohesiveness and stiffness), especially as the STPP concentration increased. On the contrary glazing only (T1) was the best treatment for maintaining L*, a* and b*, and a lower TVBN and TVC but resulted in the lowest hardness, cohesiveness and stiffness. Treatments without STPP (T1 and T2) were the best for maintaining moisture content and pH (compared with BF). However, TVC and TVBN in all treatments did increase compared to BF but did not exceed the legal limit. The effect of concentrations of 5% STPP and packaging on the quality of barramundi fillets during frozen storage could prevent the increase of TVC and TVBN as compared to all other treatments and STPP treatments (T3-T6) also improved rheological parameters such as hardness, cohesiveness and stiffness.

CHAPTER 9

GENERAL DISCUSSION

CHAPTER 9: DISCUSSION

9.1 SUMMARY

The proposed research was to investigate the effects of industry-practiced pre and post freezing treatments on the quality and shelf life of barramundi fillets. These treatments include barramundi fillets exposed to various temperatures for selected time periods, use of two ice forms (flaked and slurry), freeze-thaw cycles, use of sodium tripolyphosphate (STPP), glazing and packaging. In addition, the Chapter 7 researched on effects of STPP during three freezing cycles. The quality of barramundi fillet assessed by examining microbiological and physiochemical parameters. During this research, the blast freezer was employed to freeze barramundi fillets for 8 hours, followed by the fillets getting stored in a freezer under -20°C for 21 days before they were assessed for the quality. The fillets were thawed to carry out quality assessments. If barramundi fillets were not exposed to any treatment, the fillets were considered as general controlled processed fish. The research protocol is summarised in Figure 9.1.

After slaughter, the fish are likely to be exposed to inconsistent storage conditions (temperature abuse) for a limited period during transportation and subsequent storage (Zakhariya *et al*., 2015). Total viable count, total volatile base nitrogen, pH, protein, color and rheological parameters of fillets that underwent pre-blast freezing (PBF) temperature period at 0°C and 5°C for 16 days deteriorated significantly compared with those treated at −20°C. During chilled or frozen storage, transport, and retail display, the most important factor is to maintain the temperature of the fish. Some authors have reported that during post-catch and prior to icing storage, it is common for some fish species to be temporarily exposed to higher temperatures (Albalat *et al*., 2011). This delayed icing has been shown to reduce the shelf life of fish products based on sensory, bacteriological and biochemical analysis, and the authors recommended best practice to improve fish quality onboard ship is immediate icing (Ganesan *et al*., 2005; Rezaei *et al*., 2008; Albalat *et al.,* 2011). Similar changes in TVC and TMA values have been reported in other studies on Norway lobster (*Nephrops norvegicus*), indicating that microbiological growth and consequent tissue breakdown do not become significant until at least 5–7 days if samples are kept on ice (Losada *et al.,* 2006). A slight increase in water holding capacity from 86% to 90% during storage was observed for up to

11 days of superchilled (-1°C) cod (*Gadus morhua*) fillets, suggesting that the water capacity may rise as larger proportion of loosely bound water is released from the muscles (Digre *et al.,* 2011). A loss of water holding capacity in frozen horse mackerel (*Trachurus trachurus*) muscle as storage time increased (Matos *et al.,* 2011) and a higher liquid loss in frozen rainbow trout (*Salmo gairdneri*) compared to fresh fish, associated with myosin denaturation (Digre *et al*., 2011). Unfortunately, sometimes the existing distribution channels for refrigerated foods are not always equipped for the optimum control of temperature during the distribution (Almonacid-Merino & Torres, 1993). Serious microbial stability problems exist because of the frequency of temperature abuse (Doyle, 1991). Distribution temperatures need to be lowered since they are critical in maintaining the quality and safety of the product (Young, 1987; Almonacid-Merino & Torres, 1993). Exposure of meat to increased temperature conditions undoubtedly accelerates proteases activity to breakdown muscle protein into small molecular weight peptides, and long term storage of meat is often associated with extensive softening of meat and color change independent of microorganism (Gill, 1996; Tewari *et al*., 1999).

Both enzymatic and microbiological activity are greatly influenced by temperature. Spoilage is the result of whole series of complicated deteriorative changes brought about in dead fish tissue by its own enzyme, by bacteria and by chemical action (Shewan, 1976; Ali *et al.*, 2010). Enzymes from spoilage microorganisms can metabolize the amino acids of the fish muscle producing a wide variety of volatile compounds resulting off-flavors and odors (Ali *et al.*, 2010). The combined total amount of ammonia (NH_3), dimethylamine (DMA) and trimethylamine (TMA) in fish is called the total volatile base (TVB) nitrogen content of the fish and is commonly used as an estimate of spoilage (Ali *et al.*, 2010). Total volatile base nitrogen has been extensively used as an index for freshness of fish. As the activity of spoilage bacteria increases after the death of a fish, a subsequent increase in the reduction of TMAO to TMA (Ali *et al.*, 2010). The best preservation temperature for fish can vary. Some of the important pre and post freezing treatments, which resulted in positive effects on TVBN and TVC shown in Table 9.1. These treatments indicated TVBN values reached below the legal limits (35 mg 100 g^{-1} set for TVB-N) for consumption (Directive 95/149/EEC), and reduced TVC values which remained acceptable (less than 10^7 cfu g^{-1} as described in Koutsoumanis (2001) and Olafsdottir *et al.* (1997, 2004).

Table 9.1: List of pre and post freezing treatments, which resulted in positive effects on TVBN and TVC

Treatments	Effects on TVBN and TVC
Exposure to flaked ice and/or cooling at 0° to 5°C for 8 days before freezing	+
Exposure to slurry ice or -20° C for 16 days before freezing	+
Exposure to glazing, glazing + packaging, STPP (Sodium Tripolyphosphate) treatments, STPP+ packaging	+

Slurry ice (SI), also known as fluid ice, slush ice, liquid ice or flow ice, consists of an ice–water suspension at a sub zero temperature. It may be a valuable product for preserving seafood and is of substantial interest to sea food processors compared to flake ice (FI). Results demonstrate that the post-freezing quality of fillets was better preserved following up to 16-days chilling in slurry ice than in flake ice. Therefore longer shelf life can be obtained by chilling barramundi fillets in slurry ice. Fillets exposed to pre-freezing treatments in FI demonstrated larger increase in TVBN and TVC compared to SI at the end of the frozen storage. The results also revealed that effect of concentrations of 5% STPP and packaging significantly improved the properties (reduced total viable counts and decreased total volatile base nitrogen) compared to all other treatments and STPP treatments.

Before the freezing step accomplished, adequate storage/chilling techniques of fish should be employed to reduce post-capture losses (Losada *et al.*, 2007). The differences between chilling methods are more important for product quality than perimortem stress (Digre *et al.*, 2011). Previous research has shown a strong influence of the preliminary chilling time on frozen fish quality (Aubourg *et al.*, 2002; Deng, 1978; Sankar & Viswanathan Nair, 1988; Undeland & Lingnert, 1999). The time necessary to reach subzero temperatures within 1 h at a processing plant, would be realistic when slurry is applied (Digre *et al*., 2011).

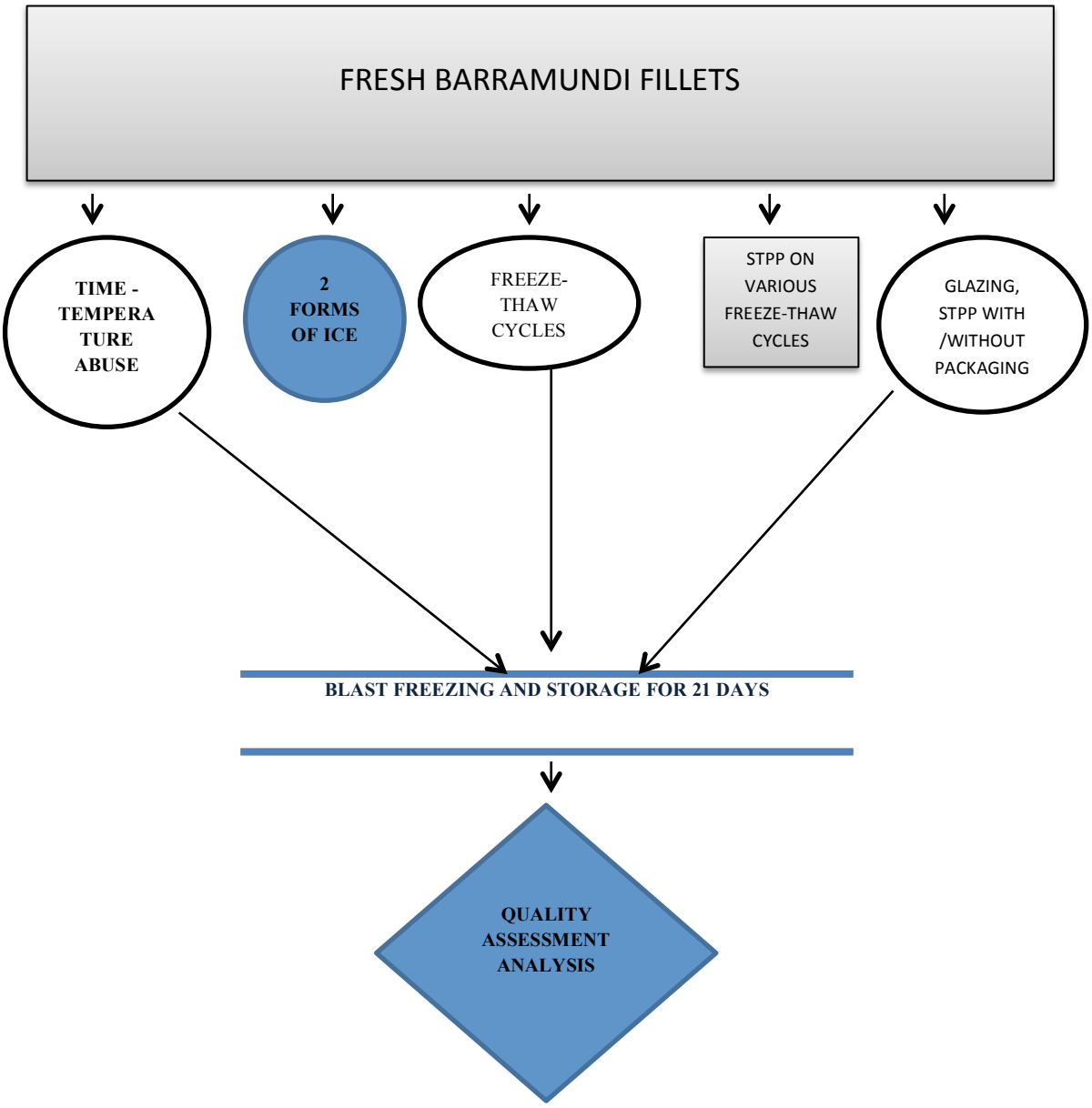


Figure 9.1: Flow diagram of experimental study

The use of slurry ice has shown profitable advantages when employed instead of traditional flake ice for the manufacture of chilled aquatic species (Losada *et al.*, 2007). The storage of horse mackerel specimens in slurry ice conditions allowed an inhibitory effect on chemical changes related to quality loss (Losada *et al.*, 2005) and thus, in their results agreed with the sensory assessment, which showed a significant increase in shelf life associated with better quality period when the slurry ice treatment was employed (Losada *et al.*, 2005). In the current research, the pre-freezing treatments of slurry ice maintained better quality than the

fillets that were exposed to flaked ice for 16 days as indicated by a longer shelf life as fillets displayed low TVC value, stable pH and decreased TVBN values. All rheological parameters fell significantly as storage time progressed in both FI and SI, with hardness falling to a greater extent in FI than SI. Fillets exposed to pre-freezing treatments in FI demonstrated smaller increase in moisture content compared to SI at the end of the frozen storage. Hultman & Rustad (2007) reported that the muscle pH was significantly higher in the abused fillets than in the control fillets throughout the storage period.

Studies have shown that repeated freeze–thawing abuse may impart changes in the texture and functional properties of the muscle proteins leading to an end in the shelf life of the frozen fish (Verma *et al.*, 1995; Benjakul *et al.*, 2005; Benjakul & Sutthipan, 2009). In the current study, the post-freezing storage time was kept constant for 20 days prior to evaluation of the fillet quality. The freezing and thawing processes can have a profound effect on muscle physicochemical characteristic (Boonsumrej *et al.*, 2007). After three freeze-thaw cycles in this study, there was significant decrease in protein content and rheological parameters. Comparably, findings in this study displayed that fillet quality lessened as the number of freeze–thaw cycles increased. Biochemical losses increased to a greater extent to the fillets noticed subjected to three freeze–thaw cycles. Repeated melting during thawing and reformation of ice crystals during freezing in multiple freeze–thaw situations was clearly detrimental to muscle tissues by causing mechanical damage to cell membranes and the loss of water holding capacity (Srinivasan *et al.*, 1997). The denaturation of muscle proteins may occur during multiple freeze-thaw cycles and a decrease in salt-soluble proteins (SSP) could be due to the denaturation of proteins caused by the interaction of free fatty acid with SSP and the consequent lower solubility of proteins (Verma & Srikar, 1994).

The proper combination of STPP and packaging materials could improve the long-term frozen storage of barramundi fillets. The use of phosphates prior to freezing fish has a significant effect on the chemical composition and physicochemical properties of the thawed product. This additive can be used in fish processing to improve yields, water holding capacity, and the sensory attributes of the final product (Nguyen *et al.*, 2012). Dehydration of the product tissue might occur leading to freezer burn, although this effect applies to dehydration during freezing rather than storage (Jacobsen & Fossan, 2001). The weight gain is not only technological benefit but also represents to the producer a gain in weight of product sold (Turan *et al.*, 2003). When used properly, phosphates in shrimp preserve the

natural moisture content of the muscle, leading to a softer, higher-quality product (Gonçalves *et al.*, 2008). Phosphate affects the muscle tissue by increasing the pH, the ionic force between proteins and water molecules, the chelation of metal ions, and the dissociation of the actomyosin complex, thus changing the dynamics of water in the tissues (Damodaran *et al.*, 2008, chap. 5; Erdogdu *et al.*, 2007; Ünal *et al.*, 2006). The content and distribution of water in fish muscle are important quality parameters because they influence color, texture and commercial value (Gonçalves & Ribeiro, 2009; Lakshmanan *et al.*, 2007; Toldrá, 2003). As polyphosphates increase the electrostatic repulsion between the peptide chains and the space between the myofibrils, more water is retained between the myofibrillar spaces, and the muscle tissue becomes more tender and springy and less cohesive (Damodaran *et al.*, 2008 chap. 5).

The softening of fish muscle that occurs post mortem leads to a reduction in fish quality (Caballero *et al.*, 2009). Flesh softening is primarily the result of protease actions on muscle proteins (Taylor *et al.*, 1995; Taylor *et al.*, 2002) and connective tissue (Ando *et al.*, 1995) that eventually cause muscle disintegration with the consequent loss of texture and freshness (Caballero *et al.*, 2009). The first and universal effect of all polyphosphate treatment is to increase the weight of the fish by retaining water (Turan *et al.*, 2003).

Weight loss by dehydration during freezing and storage is directly proportional to the exposed surface area and can be reduced by two methods: covering the surface with packaging material, and surrounding the product with a thin layer of ice (Gonçalves & Gindri Junior, 2009). As soon as seafood is removed from a freezer, they should be glazed or wrapped (unless they have been packaged before freezing) and immediately transferred to a low temperature store to rapidly refreeze and to preserve taste, smell and texture as well as to minimize thaw drip loss (Jacobsen & Fossan, 2001). In chapter 8, researched on the effects of glazing and packaging. Glazing is just a form to assure the moisture loss by sublimation during the frozen storage, which becomes an important quality and economic factor in the seafood industry (Gonçalves & Gindri Junior, 2009). Packaging and STPP treatment had a significant impact on the biological and physiochemical properties of barramundi fillets. Good packaging prevents the circulation of air over the surface of the product and protects the moisture in the surface layers of the product (Dore, 1991). STPP treatment primarily 5% STPP and packaging limited fillet quality deterioration as it reduced TVC and controlled/limited TVBN. STPP treatments had a positive impact on most of the parameters.

Therefore, STPP (2% and 5% concentration) and packaging during frozen storage can prevent fillets from quality deterioration. Similarly, the effect of STPP and various freeze-thaw cycles (3 cycles) study established that treatment with STPP at each freeze-thaw cycle could maintain the quality and shelf life of fillets when exposed to three freeze-thaw cycles and thus changes in TVC and TVBN increase, maintaining the moisture content and limiting protein denaturation were some of the positive impacts.

In this study, the TVC values of barramundi fillets after 8 d PBF treatments at 5°C and 0°C, and flake ice, the fillets were on the limit of the proposed limit for acceptance for human consumption is 5×10^6 CFU g^{-1} (ICMSF 1986). According to Gram *et al.* (1987), a large number of bacteria (10^7 to 10^8 g^{-1}) is normally found on spoiling fish, but only a part of this flora may be classified as active spoilers. In addition, a range of other conditions, such as pH, water activity, and atmosphere, may also have an impact of the growth of spoilage metabolites, such as SPB (Gram and Dalgaard 2002). Quality of the end product is related to a various technical parameters, which are measurable (Visciano *et al.* 2007); however, it is also connected to various parameters, which are linked to the consumer's expectations and preferences (Visciano *et al.* 2007). Fillets that underwent 8 d PBF treatments at 5°C and 0°C, and flake ice were higher than 30 mg 100 g^{-1} of TVBN value, the level legally permitted by some organizations for raw fish (Periago *et al.,* 2003).

Temperature prior to freezing as well as use of flaked and slurry ice has a strong relationship with values of TVBN, TVC and pH, as shown in Table 9.2. Increases in pH may indicate the accumulation of alkaline compounds such as ammonia mainly derived from microbial actions (Okeyo *et al.*, 2009). The increase may also be due to an increase in volatile bases from the decomposition of nitrogenous compounds by endogenous or microbial enzymes (Erkan & Ozden, 2007). The formation of TVBN is generally associated with the activity of microorganisms and tends to be high at high microbial population (Benjakul *et al.*, 2003). The changes in TVBN content may also be related to changes in pH (Hultmann & Rustad, 2004). The initial lower levels of TVBN observed could be due to lower levels of endogenous ammonia due to reduced microbial activity during the first 10 days of storage of the fish in ice (Pacheco-Aguilar 2000).

Table 9.2: Regression coefficient values between TVBN, TVC and pH with three pre-freezing temperatures including two forms of ice

	5°C	0°C	-20°C	Flaked ice	Slurry ice
TVBN	0.92	0.94	0.43	0.94	0.97
TVC	0.96	0.93	0.49	0.89	0.75
pH	0.84	0.86	0.71	0.72	0.79

Texture is considered to be one of the most important quality attributes in fish. During the post-mortem ageing of muscle under chilled conditions or frozen storage, degradation of muscle proteins and connective tissue contributes to the rapid softening of the fish muscle (Caballero *et al.*, 2009). Flesh texture in fish is determined by a complex set of intrinsic traits such as the muscle chemical composition (e.g. fat content and fatty acid profile, glycogen stores) and muscle cellularity (Matos *et al*., 2011). It is also strongly influenced by a variety of extrinsic factors, including pre- and post-slaughter handling procedures (Bahuaud *et al.*, 2010). Additionally, storage conditions are known to influence not only texture but also microbiological safety (Mackie, 1993). Ando *et al.* (1992) stated that the softening of the rainbow trout texture was caused mainly, by the weakening of the connective tissue in myocommata. Dunajski (1979) indicated that stress before slaughter affected several quality characteristics of fish flesh, specially fillet texture and gaping. It was evident in the current study rheological parameters decreased as storage time increased which means fillets were softer. In a study, Lee & Toledo (1984) reported that when mullet (*Mugil* spp.) stored up to 10 d on ice or at –2°C, the superchilled fish were consistently softer during the entire storage period.

9.2 CONCLUSIONS

The proposed research program aimed to achieve best treatments, which can be followed before and after the freezing of barramundi fillets. The main conclusions from this research are as follows:

1. Fillets exposed to ice slurry prior to freezing had relatively more acceptable microbiological and physiochemical quality and thus extended shelf life compared to those exposed to flake ice.
2. All rheological parameters fell as storage time progressed when barramundi fillets were exposed to both flake ice and slurry ice before freezing commenced.
3. Keeping barramundi fillets at -20°C prior to blast freezing can significantly increase the shelf life.
4. The fillets exposed to pre-blast freezing temperature of -20°C have a shelf life of more than 16 days.
5. Based on the microbiological analysis, the shelf life of barramundi fillets was 8 days when kept at 0°C and 5°C prior to blast freezing.
6. pH, TVBN, TVC, and L* (Lightness) and b* (Yellowness) values of fillets increased significantly with number of freezing cycles.
7. The number of freezing cycles reduced the certain aspects of fillet quality.
8. Ash, protein content and rheological parameters of fillets decreased significantly after three freeze–thaw cycles but it did not exceed the legal limit in terms of TVC and TVBN.
9. It is important to prevent temperature variations or abuse prior to freezing (could be during the transport) to avoid the drop in fillet quality.
10. The treatment with at each freeze-thaw cycle can maintain the quality and shelf life of fillets as sodium tripolyphosphate treated fillets were able to slow the deterioration of fillet rheological parameters, especially as the STPP concentration increased.
11. Sodium tripolyphosphate treatment limited the TVC and TVBN increases, maintained moisture content and limited the protein denaturation.
12. TVC and TVBN tests are relatively simple, fast and practical that require small samples, and have has the potential to become widely used in fish quality assessment.

9.3 RECOMMENDATIONS

In the course of this research project, there are various areas, which still need further investigations.

The areas of further research should include:

- Quantifying time temperature abuse index by exposing the fillets to wide range of temperatures and time periods. A modeller approach can be adapted for further research.
- Sodium tripolyphosphate could be experimented with as an effective cryoprotectant for frozen storage of fillets.
- Research should be conducted to quantify the relationship between the application of the sodium tripolyphosphate and moisture content in the fillets and its subsequent implications on the shelf life of the fillets.
- STPP to treat barramundi fillets under repeated freeze-thaw cycles should be examined further, under commercial conditions.
- Additional work is needed to determine the effects of temperature fluctuations during pre-freezing transport and post-freezing storage of barramundi fillets.
- Quality of the end product is related to a various technical parameters, which are measurable, however, it is also connected to various parameters, which are linked to the consumer's expectations and preferences (Visciano *et al.* 2007). Therefore, consumer preference should be evaluated.
- Organoleptic quality assessment should be incorporated into this kind of research.

REFERENCES

ABARE. (2009). Australian fisheries statistics 2008. Canberra, July.

ABARE-BRS. (2010). Australian fisheries statistics 2009. Canberra, August.

Abbas, K. A., Saleh, A. M., Mohamed, A. & Ola, L. (2009). The relationship between water activity and fish spoilage during cold storage: a review. *Journal of Food, Agriculture & Environment*, (3&4), 86-90.

Ahvenainen, R. & Malkki. Y. (1985). Influence of packaging on the shelf life of frozenfoods 2. Baltic herring fillets. *Int. J.Food Sci. Technol*., **20**, 193-201.

Aitken, A. (2001). Polyphosphates in fish processing. *Torry Advisory Note*, **31**, 1-4.

Akse, L. & Midling, K.Ø. (2001). *Slaughtering of Atlantic halibut (Hippoglossus hippo-glossus) effects on quality and storage capacity*. Farmed fish quality. Oxford, U.K.: Blackwell Science.

Alasalvar, C., Taylor, K. D. A., Oksuz, A., Garthwaite, T., Alexis, M. N. & Grigorakis, K. (2001). Freshness assessment of cultured sea bream (*Sparus aurata*) by chemical, physical and sensory methods. *Food Chem.,* **72**(1), 33-40.

Alasalvar, C., Taylor, K. D. A., Zubcov, E., Shahidi, F. & Alexis, M. (2002). Differentiation of cultured and wild sea bass (*Dicentrarchus labrax*): Total lipid content, fatty acid and trace mineral composition. *Food Chemistry,* **79**(2), 145-150.

Albalat, A., Gornik, S. G., Mullen, W., Crozier, A., Atkinson, R. J., Coombs, G. H. & Neil, D. M. (2011). Quality changes in chilled Norway lobster (*Nephrops norvegicus*) tail meat and the effects of delayed icing. *International Journal of Food Science & Technology,* **46**(7), 1413-1421.

Alfred, A. (1998). The effect of delayed icing and gutting on the quality of freshwater Arctic Charr (*Salvelinus alpinus L.*). *Fisheries Department, Ministry of Agriculture Animal Industry and Fisheries.* The United Nations University, Uganda, East Africa.

Ali, M., Sharif, M. I., Adhikari, R. K. & Faruque, O. (2010). Post mortem variation in total volatile base nitrogen and trimethylamine nitrogen between galda (*Macrobrachium rosenbergii*) and bagda (*Penaeus monodon*). *University Journal of Zoology, Rajshahi University*, **28**, 7-10.

Almonacid-Merino, S. F. & Torres, J. A. (1993). Mathematical models to evaluate temperature abuse effects during distribution of refrigerated solid foods. *Journal of Food Engineering,* **20**(3), 223-245.

Alvarez, M. D. & Canet, W. (1998). Effect of temperature fluctuations during frozen storage on the quality of potato tissue (cv. Monalisa). *Zeitschrift Fur Lebensmittel-Untersuchung Und-Forschung A-Food Research And Technology*, **206**, 52–57.

Alvarez, J. A., Pozo, R. & Pastoriza, L. (1996). Effect of a cryoprotectant agent (Sodium tripolyphosphate) on hake slices preserved in modified atmosphere packing. *Food Science Technology International*, **2**, 177–181.

Aman, M. B., Moustafa, E. K., Zoueil, M. E. & Ghaly, M. H. (1983). Effect of frozen storage on the chemical and technological characteristics of Egyptian crab meat. *European Food Research and Technology*, **177**, 97–100.

Anbalagan, M., Ganesh Prabu, P., Krishnaveni, R. E. & Manivannan, S. (2014). Effect of Low Temperature on the Bacterial Load in Chicken, Mutton and Beef Meat in Relation to Meat Spoilage. *International Journal of Research in Pure and Applied Microbiology,* **4**(1), 1-6.

Anderson, A. K. (2008). Biogenic and volatile amine-related qualities of three popular fish species sold at Kuwait fish markets. *Food Chemistry*, **107**(2), 761-767.

Andersen, U. B. & Steinsholt, K. (1992). Deep-frozen salmon: differences in quality after storage at different temperatures following different storage periods. *Norw. J. Agric. Sci*., **6**, 211 –215.

Andersen, U. B., Thomassen, M. S. & Rørå, A. M. B. (1997). Texture Properties of Farmed Rainbow Trout (*Oncorhynchus mykiss*): Effects of Diet, Muscle Fat Content and Time of Storage on Ice. *Journal of the Science of Food and Agriculture,* **74**(3), 347-353.

Ando, M., Toyohara, H., Shimizu, Y. & Sakaguchi, M. (1991). Post-mortem tenderisation of rainbow trout (*Oncorhyncus Mykiss*) muscle caused by gradual disintegration of the extracellular matrix structure. *Journal of the Science of Food and Agriculture,* **55**(4), 589-597.

Ando, M., Yoshimoto, Y., Inabu, K., Nakagawa, T. & Makinodan, Y. (1995). Postmortem change of 3-dimensional structure of collagen fibrillar network in fish muscle pericellular connective tissues corresponding to postmortem tenderization. *Fish. Sci.*, **61**(2), 327-330.

Ando, M. (1999). Correspondence of collagen to the softening of meat during refrigeration. *Journal of Food Science*, **64**, 659–662.

Ando, M., Toyohara, H. & Sakaguchi, M. (1992). Postmortem tenderization of rainbow trout muscle caused by the disintegration of collagen fibers in the pericellular connective tissue. *Nippon Suisan Gakk*., **58**, 567–570.

Ang, J. F. & Hultin, H. O. (1989). Denaturation of cod myosin during freezing after modification with formaldehyde. *Journal of Food Science*, **54,** 814–818.

Ashie, I. N. A., Smith, J. P., Simpson, B. K. & Haard, N. F. (1996). Spoilage and shelf-life extension of fresh fish and shellfish. *Critical Reviews in Food Science and Nutrition*, **36**(1-2), 87-121.

Ashok Kumar, K., Ravishankar, C. N., Badonia, R. & Solanki, K. K. (2000). Quality changes in whale shark (*Rhinodon typus* Smith) meat during storage in ice. *Fish. Technol*., **37**(1), 15–18.

Association of Official Analytical Chemists (AOAC). (1990). *Official Methods of Analysis*, Association of Official Analytical Chemists. Washington, DC.

Association Of Official Analytical Chemists (AOAC). (1995). *Official Methods of Analysis*, Association of Official Analytical Chemists. Washington, DC.

Aubourg, S. (2001). Damage detection in horse mackerel (*Trachurus trachurus*) during chilled storage. *Journal of the American Oil Chemists' Society,* **78**(8), 857-862.

Aubourg, S. P., Lehmann, I. & Gallardo, J. M. (2002). Effect of previous chilled storage on rancidity development in frozen horse mackerel (*Trachurus trachurus*). *Journal of the Science of Food and Agriculture*, **82**(15), 1764-1771.

Aubourg, S. P., Losada, V., Prado, M., Miranda, J. M. & Barros-Velázquez, J. (2007). Improvement of the commercial quality of chilled Norway lobster (*Nephrops norvegicus*) stored in slurry ice: Effects of a preliminary treatment with an antimelanosic agent on enzymatic browning. *Food chemistry*, **103**, 741-748.

Aubourg, S. & Medina, I. (1997). Quality Differences Assessment in Canned Sardine (*Sardina pilchardus*) by Fluorescence Detection. *Journal of Agricultural and Food Chemistry*, **45**(9), 3617-3621.

Aubourg, S. P., Medina, I. & Gallardo, J. M. (1998). Quality Assessment of Blue Whiting (*Micromesistius poutassou*) during Chilled Storage by Monitoring Lipid Damages. *Journal of Agricultural and Food Chemistry*, **46**(9), 3662-3666.

Aubourg, S. P. & Medina, I. (1999). Influence of storage time and temperature on lipid deterioration during cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) frozen storage. *Journal of the Science of Food and Agriculture*, **79**(13), 1943-1948.

Aussanasuwannakul, A., Kenney, P. B., Brannan, R. G., Slider, S. D., Salem, M. & Yao, J. (2010). Relating instrumental texture, determined by variable-blade and allo-kramer shear attachments, to sensory analysis of rainbow trout, *Oncorhynchus mykiss*, fillets. *Journal of Food Science*, **75**(7), S365-S374.

Australian barramundi farmers association. (2008). *Barramundi*.Available from: <http://www.abfa.org.au>. [3rd January 2008].

Australian barramundi farmers association. (2014). *Barramundi*. Available from: <http://www.abfa.org.au>. [9th July 2014].

Ayala, M. D., Abdel, I., Santaella, M., Martinez, C., Periago, M., Gil, F., Blanco, A. & Albors, O. (2010). Muscle tissue structural changes and texture development in sea bream, *Sparus aurata* L., during post-mortem storage. *LWT-Food Sci. Technol*., **43**(3), 465-475.

Azam, K., Mackie, I. & Smith, J. (1989). The effect of slaughter method on the quality of rainbow-trout (*Salmo gairdneri*) during storage on ice. *Int. J. Food Sci. Technol*., **24**(1), 69-79.

Bachhil, V. N. (1982). Influence of lower storage temperatures of keeping quality of fresh goat meat. *Indian Vet Med J*., **6**, 105–106.

Badii, F. & Howell, N. K. (2001). A comparison of biochemical changes in cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) fillets during frozen storage. *Journal of the Science of Food and Agriculture,* **82**(1), 87-97.

Badii, F. & Howell, N. K. (2002). Changes in the texture and structure of cod and haddock fillets during frozen storage. *Food Hydrocolloids*, **16**(4), 313-319.

Bahuaud, D., Mørkøre, T., Thomassen, T. K., Mørkøre, E., Østbye, M. S., Veiseth-Kent, R. & Ofstad, R. (2010). Muscle structure responses and lysosomal cathepsins B and L in farmed Atlantic salmon (*Salmo salar* L.) pre- and post-rigor fillets exposed to short and long-term crowding stress. *Food Chemistry*, **118,** 602–615.

Bao, H. N. D., Arason, S. & Þórarinsdóttir, K. A. (2007). Effects of Dry Ice and Superchilling on Quality and Shelf Life of Arctic Charr (*Salvelinus alpinus*) Fillets. *International Journal of Food Engineering*, **3**(3).

Barroso, M., Careche, M. & Borderías, A. J. (1998). Quality control of frozen fish using rheological techniques. *Trends in Food Science and Technology*, **9**(6), 223-229.

Barros-Velazquez, J., Carreira, L., Franco, C., Vazquez, B. I., Fente, C. & Cepeda, A. (2003). Microbiological and physicochemical properties of fresh retail cuts of beef packaged under an advanced vacuum skin system and stored at 4°C, *J. Food Prot*., **66** (11), 2085–2092.

Becker, B. R. & Fricke, B. A. (2005). *Design Essentials for Refrigerated Storage Facilities.*

Beklevik, G., Polat, A. & Özoğul, F. (2005). Nutritional Value of Sea Bass (*Dicentrarchus labrax*) Fillets during Frozen (-18°C) Storage. *Turkish Journal of Veterinary & Animal Sciences*, **29(**3).

Benjakul, S. & Bauer, F. (2001). Biochemical and physicochemical changes in catfish (*Silurus glanis* Linne) muscle as influenced by different freeze–thaw cycles. *Food Chemistry*, **72**(2), 207-217.

Benjakul, S., Seymour, T. A., Morrissey, M. T. & An, H. (1997). Physicochemical changes in Pacific whiting muscle proteins during iced storage. *Journal of Food Science*, **62**(4), 729-733.

Benjakul, S. & Sutthipan, N. (2009). Muscle changes in hard and soft shell crabs during frozen storage. *LWT - Food Science and Technology*, **42**(3), 723-729.

Benjakul, S., Visessanguan, W., Thongkaew, C. & Tanaka, M. (2003). Comparative study on physicochemical changes of muscle proteins from some tropical fish during frozen storage. *Food Research International*, **36**(8), 787-795.

Benjakul, S., Visessanguan, W., Thongkaew, C. & Tanaka, M. (2005) Effect of frozen storage on chemical and gel forming properties of fish commonly used for surimi production in Thailand. *Food hydrocolloids*, **19**, 197-207

Bennour M., A. El., Marrakchi, N., Bouchriti, A. Hamama, & M. El Ouadaa. (1991). Chemical and Microbiological Assessments of Mackerel (*Scomberscombrus*) Stored in Ice, *J. Food Prot.*, **54** (784), 789–792.

Beyrer, M. & Klaas, M. (2007). Influence of freezing and of frozen storage on the specific heat capacity of trout and herring fillet. *Eur. Food Res. Technol*., **224**(3), 349-353.

Bhobe, A. & Pai, J. (1986). Study of the properties of frozen shrimps. *Journal of Food Science and Technology*, **23**(3), 143-147.

Bilinski, E., Jonas, R. E. E. & Peters, M. D. (1981). Treatments affecting the degradation of lipids in frozen Pacific herring, *Clupea harengus pallasi*. *J. Inst. Can. Sci. Technol. Aliment*., **14**, 123-127.

Blond, G. & Le Meste, M. (2004). *Principles of frozen storage. Food Science And Technology.* Pp.25-54. New York: Marcel Dekker.

Bogh-Sorensen, L. (2002). Recommendations for the processing and handling of frozen foods. International Institute of Refrigeration. Paris.

Boonsumrej, S., Chaiwanichsiri, S., Tantratian, S., Suzuki, T. & Takai, R. (2007). Effects of freezing and thawing on the quality changes of tiger shrimp (*Penaeus monodon*)

frozen by air-blast and cryogenic freezing. *Journal of Food Engineering*, **80**(1), 292-299.

Boonyaratpalin, M. (1997). Nutrient requirements of marine food fish cultured in Southeast Asia. *Aquaculture*, **151**(1–4), 283-313.

Botta, J. R. (1991). Instrument for Nondestructive Texture Measurement of Raw Atlantic Cod (*Gadus Morhua*) Fillets. *Journal of Food Science*, **56**(4), 962-964.

Bracho, G. E. & Haard, N. F. (1995). Identification of two matrix metalloproteinases in the skeletal muscle of Pacific rockfish (*Sebastes* sp.). *Journal of Food Biochemistry*, **19**, 299–319.

Brandt, M. A., Skinner, E. Z. & Coleman, J. A. (1963). Texture profile method. *Journal of Food Science*, **29**, 404–409.

Bratzler, L. J. (1932). Measuring the tenderness of meat by means of a mechanical shear. Master of Science Thesis. Kansas State College (KA), USA.

Bremner, H. A. & Hallett, I. C. (1985). Muscle fiber–connective tissue junctions in the fish blue grenadier (*Macruronus novaezelandiae*). A scanning electron microscope study. *Journal of Food Science*, **50**, 975–980.

Brotsky, E. & Everson, C. (1973). Polyphosphate use in meat and other muscle foods. In: *Proceedings of the Meat Industry Conference*. (Conference Proceeding). Rahway, NJ: Merck and Company, Inc.

Brown, J. A., Watson, J., Bourhill, A. & Wall, T. (2010). Physiological welfare of commercially reared cod and effects of crowding for harvesting. *Aquaculture*, **298**, 315–324.

Browne, M. & Allen, J. (1998). Logistics of food transport. In: *Food Transportation* (edited by Heap, R., Kierstan, M. & Ford, G.), Pp. 22–50. Blackie Academic & Professional, London, U.K.

Burgaard, M. G. & Jørgensen, B. M. (2011). Effect of Frozen Storage Temperature on Quality-Related Changes in Rainbow Trout (*Oncorhynchus mykiss*). *Journal of Aquatic Food Product Technology,* **20**(1), 53-63.

Byun, J. S., Min, J. S., Kim, I. S., Kim, J. W., Chung, M. S. & Lee, M. (2003). Comparison of indicators of microbial quality of meat during aerobic cold storage. *Journal of Food Protection*, **66**, 1733–1737.

Caballero, M.J., Betancor, M., Escrig, J.C., Montero, D., Espinosa de los Monteros, A., Castro, P., Ginés, R. & Izquierdo, M.S. (2009). Post mortem changes produced in the muscle of sea bream (*Sparus aurata*) during ice storage. *Aquaculture*, **291**, 210-216.

Cai, L., Wu, X., Li, X., Zhong, K., Li, Y. & Li, J. (2014). Effects of different freezing treatments on physicochemical responses and microbial characteristics of Japanese sea bass (*Lateolabrax japonicas*) fillets during refrigerated storage. *LWT - Food Science and Technology*, **59**(1), 122-129.

Caine, W., Aalhus, J., Best, D., Dugan, M. & Jeremiah, L. (2003). Relationship of texture profile analysis and Warner-Bratzler shear force with sensory characteristics of beef rib steaks. *Meat Sci.*, **64**(4), 333-339.

Cakli, S., Kilinc, B., Cadun, A., Dincer, T. & Tolasa, S. (2007). Quality differences of whole ungutted sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*) while stored in ice. *Food Control*, **18**(5), 391-397.

Calder, B. L. (2003). The use of polyphosphates to maintain yield and quality of whole cooked, cryogenically frozen lobster (*Homarus americanus*) and the use of sorbitol and tocopherol to maintain quality of whole cooked, cryogenically frozen crab (*Cancer irroratus*). Doctoral thesis. The University of Maine.

Campañone, L., Salvadori, V. & Mascheroni, R. (2001). Weight loss during freezing and storage of unpackaged foods. *Journal of Food Engineering*, **47**(2), 69-79.

Canet, W. (1989). Quality and stability of frozen vegetables. In: *Developments in Food Preservation* (edited by Thorne, S.). Elsevier Science.

Cappeln, G., Nielsen, J. & Jessen, F. (1999). Synthesis and degradation of adenosine triphosphate in cod (*Gadus morhua*) at subzero temperatures. *Journal of the Science of Food and Agriculture*., **79**(8), 1099-1104.

Careche, M., Del Mazo, M. L., Torrejón, P. & Tejada, M. (1998). Importance of Frozen Storage Temperature in the Type of Aggregation of Myofibrillar Proteins in Cod (*Gadus morhua*) Fillets. *Journal of Agricultural and Food Chemistry*, **46**(4), 1539-1546.

Careche, M., Herrero, A. M., Rodriguez-Casado, A., Del Mazo, M. L. & Carmona, P. (1999). Structural changes of hake (*Merluccius merluccius* L.) fillets: effects of freezing and frozen storage. *J. Agric. Food Chem*, **47**(3), 952-959.

Carmen, A. C., Rodríguez, Ó., Losada, V., Aubourg, S. P. & Barros-Velázquez, J. (2005). Effects of storage in ozonised slurry ice on the sensory and microbial quality of sardine (*Sardina pilchardus*). *International Journal of Food Microbiology*, **103**(2), 121-130.

Casas, C., Martinez, O., Guillen, M. D., Pin, C. & Salmeron, J. (2006). Textural properties of raw Atlantic salmon (*Salmo salar*) at three points along the fillet, determined by different methods. *Food Control*, **17**(7), 511-515.

Castell, C. H. & Bishop, D. (1969). Effect of hematin compounds on the development of rancidity in muscle of cod, flounder, scallop, and lobster, *J. Fish Res. Board Can.*, **26**(9), 2299.

Castell C. H. & Bishop D. M. (1973). Effect of season on salt-extractable protein in muscle from trawler-caught cod and on its stability during frozen storage. *J. Fish. Res. Bd Can.*, **30**, 157-160.

Castillo-Yáñez, F. J., Pacheco-Aguilar, R., Marquez-Rios, E., Lugo-Sánchez, M. E. & Lozano-Taylor, J. (2007). Freshness loss in sierra fish (*Scomberomorus Sierra*) muscle stored in ice as affected by postcapture handling practices. *Journal of Food Biochemistry*, **31**(1), 56–67.

Castro, P., Penedo Padron, J. C., Caballero Cansino, M. J., Velazquez, E. S. & DE Larriva, R. M. (2006). Total volatile base nitrogen and its use to assess freshness in European sea bass stored in ice. *Food Control*, **17**, 245-248.

Cepeda, R., Chou, E., Bracho, G. & Haard, N. F. (1990). An immunological method for measuring collagen degradation in the muscle of fish. In: *Advances in fisheries technology and biotechnology for increased profitability: papers from the 34th Atlantic Fisheries Technological Conference and Biotechnological Workshop* (edited by M. N. Voigt & J. R. Botta). August 27–September 1, 1989.

Chaijan, M., Benjakul, S., Visessanguan, W. & Faustman, C. (2005). Changes of pigments and color in sardine (*Sardinella gibbosa*) and mackerel (*Rastrelliger kanagurta*) muscle during iced storage. *Food Chemistry*, **93**(4), 607-617.

Chambers, E. N. & Bowers, J. R. (1993). Consumer perception of sensory quality in muscle foods. *Food Technology*, **47**(11), 116–120.

Chandrasekaran, M. (1994). Methods for preprocessing and freezing of shrimps: a critical evaluation. *Journal of Food Science and Technology*, **31(**6), 441-452.

Chapman, L. (1990). Making the grade. Ice slurries get top marks for quality products. *Australian Fisheries* (July). 16–19.

Chen, J. (2009). Food oral processing-A review. *Food Hydrocolloids*, **23**(1), 1-25.

Chen, J. & Stokes, J. R. (2011). Rheology and tribology: Two distinctive regimes of food texture sensation. *Trends in Food Science and Technology*, **25**(1), 4-12.

Cheng, J. H., Sun, D. W., Han, Z. & Zeng, X. A. (2014). Texture and Structure Measurements and Analyses for Evaluation of Fish and Fillet Freshness Quality: A Review. *Comprehensive Reviews in Food Science and Food Safety*, **13**(1), 52-61.

Chenoweth, S. F., Hughes, J. M., Keenan, C. P. & Lavery, S. (1998a). *When oceans meet: a teleost shows secondary intergradation at an Indian–Pacific interface*, **265**.

Chenoweth, S. F., Jane, M. H., Clive, P. K. & Shane, L. (1998b). Concordance between dispersal and mitochondrial gene flow: isolation by distance in a tropical teleost, *Lates calcarifer* (Australian barramundi). *Heredity*, **80**(2), 187.

Chéret, R., Chapleau, N., De Lamballerie, M., Delbarre-Ladrat, C. & Verrez-Bagnis, V. (2006). Effects of high pressure on texture and microstructure of sea bass (*Dicentrarchus labrax* L.) fillets. *Journal of Food Science*, **70**(8), E477-E483.

Cheuk, W.L., Finne, G. & Nickelson, R. (1979). Stability of adenosine deaminase and adenosine monophosphate deaminase during ice storage of pink and brown shrimp from the Gulf ofMexico. *J Food Sci.*, **44**, 1625–1628.

Chinivasagam, H. N., Bremner, H. A., Wood, A. F. & Nottingham, S. M. (1998). Volatile components associated with bacterial spoilage of tropical prawns. *International Journal of Food Microbiology*, **42**(1), 45-55.

Chomnawang, C., Nantachai, K., Thawornchinsombut, S., Tungkawachara, S. & Yongsawatdigul, J. (2007). Chemical and biochemical changes of hybrid catfish fillet stored at 4°C and its gel properties. *Food Chemistry*, **103**(2), 420-427.

Chow, C. J. (1988). Effect of freezing and thawing on the discoloration of tuna meat. *Nippon Suisan Gakk*, **54**, 639-648.

Chow, C. J., Ochiai, Y. & Watabe, S. (2004). Effect of frozen temperature on autoxidation and aggregation of bluefin tuna myoglobin in solution. *J. Food Biochem*., **28,** 123-134.

Christiansen, R., Struksnæs, G., Estermann, R. & Torrissen, O. J. (1995). Assessment of flesh colour in Atlantic salmon, *Salmo salar* L. *Aquaculture Research*, **26**(5), 311-321.

Church, I. & Parsons, A. 1995. Modified atmosphere packaging technology: a review, *J. Sci. Food Agr*., **67**, 5113-5121.

CIE. (1976). Official recommendations on uniform colour space, colour difference equations and metric colour terms. Suppl. no. 2 to CIE publications no. 15. Colorimetry. Commission International de l'Eclairage, Paris.

Connell, J. J. (1975). The role of formaldehyde as a protein crosslinking agent acting during the frozen storage of cod. *Journal of the Science of Food and Agriculture*., **26**(12), 1925-1929.

Connell, J. J. (1995). *Control of fish quality,* 4th edn. Oxford.

Cormier, A. & Léger, L. W. (1987). Effect of Sodium Polyphosphates on Frozen Cod Fillets (*Gadus morhua*). *Canadian Institute of Food Science and Technology Journal*., **20**(4), 222-228.

Cort, M. (1974). Antioxidant activity of tocopherols, ascorbyl palmitate and ascorbic acid and their action. *Journal of the American Oil Chemists Society*., **51**, 321–325.

CSIRO. (2014). *Seafood: hints on buying, home freezing and preparation.* Available from: <http://www.csiro.au/Outcomes/Food-and-Agriculture/seafoods/Frozen-seafood-and-freezing-your-own.aspx >. [13th May 2013].

Culioli, J. (1995). Meat tenderness: Mechanical assessment. In: *Expression of tissue proteinases and regulation of protein degradation as related to meat quality* (edited by Ouali, A., DeMeyer, D. I. & Smulders, F. J. M.). Pp. 239–263. Utrecht (The Netherlands): ECCEAMST.

Dainty, R. H. (1996). Chemical/biochemical detection of spoilage. *International Journal of Food Microbiology*, **33**, 19–34.

Dalgaard, P. (1995). Modelling of microbial activity and prediction of shelf life for packed fresh fish. *International Journal of Food Microbiology*, **26**(3), 305-317.

Dalgaard, P., Buch, P. & Silberg, S. (2002). Seafood Spoilage Predictor—development and distribution of a product specific application software. *International Journal of Food Microbiology*, **73**(2–3), 343-349.

Dalgaard, P., Mejlholm, O., Christiansen, T. & Huss, H. H. (1997). Importance of Photobacterium phosphoreum in relation to spoilage of modified atmosphere-packed fish products. Letters in Applied Microbiology, **24**(5), 373-378.

Damodaran, S., Parkin, K. L. & Fennema, O. R. (2008). Fennema's food chemistry, 4th edn. Boca Raton: CRC Press.

Damsgård, B. (2008). Fish welfare and ethical qualities in aquaculture. In: *Improving seafood products for the consumer* (edited by Børresen, T.). Pp. 490–510. Boca Raton, FL: CRC Press.

Davidson, P. M. & Juneja, V. (1990). Antimicrobial agents. In: *Food additives* (edited by Branen, A. L., Davidson, P. M. & Salminen, S.). Pp. 83–138. Marcel Dekker.

Davies, J. R., Ledward, D. A., Bardsley, R. G. & Poulter, R. G. (1994). Species dependence of fish myosin stability to heat and frozen storage. *Int J Food Sci Technol.*, **29**, 287-301.

Davis, H. K. (1993). Modified atmosphere packing of fish. In: *Principles and applications of modified atmosphere packaging of foods* (edited by Parry, R. T.). Pp. 189-228. London: Blackie Academic and Professional.

Da-Wen Sun. (2001). *Advances in Food Refrigeration*. Leatherhead Publishing, UK.

de Huidobro, F., Miguel, E., Blazquez, B. & Onega, E. (2005). A comparison between two methods (Warner-Bratzler and texture profile analysis) for testing either raw meat or cooked meat. *Meat Sci.,* **69**(3), 527-536.

De Vido, D. M. N., Paredi, M. E. & Crupkin, M. (2001). Postmortem changes in the adductor muscle of scallop (*Chlamys tehuelchus*) in chilled and frozen storage. *Journal of Aquatic Food Product Technology*, **10**(3), 49-59.

Debevere, J. & Boskou, G. (1996.) Effect of modified atmosphere packaging on the TVB/TMA-producing microflora of cod fillets. *International Journal of Food Microbiology*, **31**(1), 221-229.

Delbarre-Ladrat, C., Chéret, R., Taylor, R. & Verrez-Bagnis, V. (2006). Trends in Postmortem Aging in Fish: Understanding of Proteolysis and Disorganization of the Myofibrillar Structure. *Critical Reviews in Food Science and Nutrition*, **46**(5), 409-421.

Del Mazo, M. L., Torrejón, P., Careche, M. & Tejada, M. (1999). Characteristics of the salt-soluble fraction of hake (*Merluccius merluccius*) fillets stored at -20 and -30 °C. *Journal of Agricultural and Food Chemistry*, **47**(4), 1372-1377.

Dempsey, P. & Bansal, P. (2012). The art of air blast freezing: Design and efficiency considerations. *Applied Thermal Engineering,* **41**, 71-83.

Deng, J. C. (1978). Effect of ice storage on free fatty acid production and lipid oxidation in mullet muscle. *J. Food Sci*., **43**, 337-340.

Department of fisheries. (2011). *Barramundi.* Available from: <http://www.fish.wa.gov.au/Documents/recreational_fishing/fact_sheets/fact_sheet_barramundi.pdf>. [13th May 2013].

Dias, J., Nunes, M. L. & Mendes, R. (1994). Effect of frozen storage on the chemical and physical properties of black and silver scabbardfish. *Journal of the Science of Food and Agriculture*, **66**(3), 327-335.

Digre, H., Erikson, U., Aursand, I. G., Gallart-Jornet, L., Misimi, E. & Rustad, T. (2011). Rested and Stressed Farmed Atlantic Cod (*Gadus morhua*) Chilled in Ice or Slurry and Effects on Quality. *Journal of Food Science*, **76**(1), S89-S100.

Digre, H., Erikson, U., Misimi, E., Lambooij, B. & van de Vis, H. (2010). Electrical stunning of farmed Atlantic cod *Gadus morhua* L.: A comparison of an industrial and experimental method. *Aquaculture Research*, **41**, 1190–1202.

Dingle, J., Keith, R. & Lall, B. (1977). Protein instability in frozen storage induced in minced muscle of flatfishes by mixture with muscle of red hake. *Canadian Institute of Food Science and Technology Journal*, **10**(3), 143-146.

Dore, I. (1991). Quality and freshness-frozen fish. In: *Fish and shellfish quality assessment*. A guide for retailers and restauranteurs. Chapter 3, In: *An Osprey Book*. Pp. 29- 42. New York: Van Nostrand Reinhold.

Doyle, M. P. (1991). Evaluating the potential risk from extended-shelf-life refrigerated foods by *Clostridium botulinum* inoculation studies. *Food Technol*., **45** (4), 154-6.

Department of Primary Industries, Queensland (DPI). (2014). *Barramundi - aquaculture prospects*. Available from: <http://www.dpi.nsw.gov.au/fisheries/aquaculture/publications/aquaculture-production-reports>. [4th August 2014].

Dubellar, C., Chow, G. & Larson, P. (2001). Relationships between inventory, sales and service in a retail chain store operation. *Int. J. Physiol. Distrib. Log. Man.*, **1**, 196–108.

Dunajski, E. (1979). Texture of fish muscle. *J. Texture Stud*., **10**, 301–317.

Dutta, C. (2009). Storage characteristics of fish balls from rohu, *Labeo rohita* at -20°C. *Indian Journal of Fisheries*, **56**(1), 39-42.

Duun, A. S. & Rustad, T. (2007). Quality changes during superchilled storage of cod (*Gadus morhua*) fillets. *Food Chemistry*, **105**(3), 1067-1075.

Duun, A. S. & Rustad, T. (2008). Quality of superchilled vacuum-packed Atlantic salmon (Salmo salar) fillets stored at -1.4 and -3.6 degrees C. *Food Chemistry*, **106**(1), 122-131.

Dziezak, J. D. (1990). Phosphates improve many foods. *Food Technology*, **44**(4), 80–91.

Ehira, S. & Uchiyama, H. (1987). Determination of fish freshness using the K value and comments on some other biochemical changes in relation to freshness. In: *Seafood quality determination* (edited by Kramer, D. E. & Liston, J.). Pp. 185–193. Elsevier Science, Amsterdam.

Ellis, D. I. & Goodacre, R. (2001). Rapid and quantitative detection of the microbial spoilage of muscle foods: Current status and future trends. *Trends in Food Science and Technology*., **12**, 414–424.

Erickson, M. (1997). *Antioxidants and their application to frozen foods*.

Einen, O., Guerin, T., Fjæra, S. O. & Skjervold, P. O. (2002). Freezing of pre-rigor fillets of Atlantic salmon. *Aquaculture*, **212**(1-4), 129-140.

Einen, O. & Thomassen, M. S. (1998). Starvation prior to slaughter in Atlantic salmon (*Salmo salar*). II. Muscle composition and evaluation of freshness, texture and colour characteristics in raw and cooked fillets. *Aquaculture*, **169**, 37–53.

Einen, O., Waagan, B. & Thomassen, M. S. (1998). Starvation prior to slaughter in Atlantic salmon (*Salmo salar*): I. Effects on weight loss, body shape, slaughter- and fillet-yield, proximate and fatty acid composition. *Aquaculture*, **166**(1-2), 85-104.

Emire, A. S. & Gebremariam, M. M. (2009). Influence of frozen period on the proximate composition and microbiological quality of Nile tilapia fish (*Oreochromis niloticus*) *Journal of Food Processing and Preservation*, **34**, 743-757.

Erdogdu, F. & Balaban, M. (2000). Thermal Processing Effects on the Textural Attributes of Previously Frozen Shrimp. *Journal of Aquatic Food Product Technology*, **9**(4), 61-84.

Erdogdu, S. B., Erdogdu, F. & Ekiz, H. I. (2007). Influence Of Sodium Tripolyphosphate (Stp) Treatment And Cooking Time On Cook Losses And Textural Properties Of Red Meats. *Journal of Food Process Engineering*, **30**(6), 685-700.

Erikson, U. & Misimi, E. (2008). Atlantic salmon skin and fillet color changes effected by perimortem handling stress, rigor mortis, and ice storage. *Journal of Food Science*, 73(2), C50-C59.

Erkan, N. (2005). Changes in quality characteristics during cold storage of shucked mussels (*Mytilus galloprovincialis*) and selected chemical decomposition indicators. *Journal of the Science of Food and Agriculture*, **85**(15), 2625-2630.

Erkan, N. & Özden, Ö. (2007). Proximate composition and mineral contents in aqua cultured sea bass (*Dicentrarchus labrax*), sea bream (*Sparus aurata*) analyzed by ICP-MS. *Food Chemistry*, **102**(3), 721-725.

Ersoy, B., Aksan, E. & Özeren, A. (2008). The effect of thawing methods on the quality of eels (*Anguilla anguilla*). *Food Chemistry*, **111**(2), 377-380.

Estrada-Flores, S. (2002). Novel cryogenic technologies for the freezing of food products. *The Official Journal of Airah*, 16–21.

Espinoza Rodezno, L. A., Sundararajan, S., Solval, K. M., Chotiko, A., Li, J., Zhang, J., Alfaro, L. & e Bankston, J. D. (2013). Cryogenic and air blast freezing techniques and their effect on the quality of catfish fillets. *LWT - Food Science and Technology*, **54**(2), 377-382.

Etemadian, Y., Shabanpour, B., Mahoonak, A. S. & Shabani, A. (2012). Combination effect of phosphate and vacuum packaging on quality parameters of *Rutilus frisii kutum* fillets in ice. *Food Research International*, **45**(1), 9-16.

Etemadian, Y., Shabanpour, B., Mahoonak, A. S. & Shabani, A. (2013). Effects of Phosphate Dip Treatment on Quality of *Rutilus frisii kutum* Fillets During Ice Storage. *J. Aquat. Food Prod. Technol*., **22**(5), 449-459.

Etemadian, Y., Shabanpour, B., Sadeghi Mahoonak, A. R., Shabani, A. & Alami, M. (2011). Cryoprotective effects of polyphosphates on *Rutilus frisii kutum* fillets during ice storage. *Food Chemistry*, **129**(4), 1544-1551.

European Commission. (2005). Commission Regulation (EC) No 2073/ 2005 of November 2005 on microbiological criteria for foodstuffs. OJ L 338, Pp. 1-22.

EC/1995/ Commission Decision 95/149/EC of 8 March 1995 fixing the total volatile basic nitrogen (TVB-N) limit values for certain categories of fishery products and specifying the analysis methods to be used. *Officinal Journal*, **LO97**, 84–87.

Food and Agriculture Organization (FAO). (1993). *Fishery Statistics Commodities*, **77**.

Food and Agriculture Organization (FAO). (2006). *Polyphosphates in fish processing*. Originated by: Torry Research Station. Available from: <http://www.fao.org/wairdocs/tan/x5909E/x5909e01.htm>. [9th February].

Food and Agriculture Organization (FAO). (2014). Cultured Aquatic Species Information Programme. *Lates calcarifer* (Block, 1790). Availble from: <http://www.fao.org/fishery/culturedspecies/Lates_calcarifer/en>. [7th July 2014].

Farouk, M. M. & Swan, J. E. (1998). Effect of rigor temperature and frozen storage on functional properties of hot-boned manufacturing beef. *Meat Science*, **49**(2), 233-247.

Fatima, R. & Qadri, R. B. (1985). Quality changes in lobster (*Panulirus polyphagus*) muscle during storage in ice. *J Agric Food Chem*., **33**,117–122.

Fazal, A. A. & Ramesh, M. (2013). Effect of storage temperatures on the quality of pink perch surimi. Innovare *Journal of Food Science*, **1**(1).

Fennema, O. R., Karel, M. & Lund, D. B. (1975). Freezing preservation. In: *Principles of food sciences part II. Physical principles of food preservation* (edited by Fennema, O. R.). Pp. 173-215. New York, NY: Marcel-Dekker.

Fisheries Research and Development Corporation (FRDC). (2012). *Barramundi Lates calcarifer.* Available from: <http://www.fish.gov.au/reports/finfish/Pages/barramundi.aspx >. [27th December 2013].

Flair flow Europe (2000). Managing the cold chain for quality and safety. Technical Manual F-FE 378A/00.

Flores, J. & Bermell, S. (1984). Propiedades funcionales de las proteínas miofibrilares: capacidad de retención de agua. *Agroquímica y Tecnología de Alimentos*, **24**(2), 151–158.

Foegeding, E.A., Lanier, T.C. & Hultin, H.O. (1996). Characteristics of edible muscle tissues. In: *Food Chemistry* (edited by Fennema, O. R.), 3rd edn. Pp. 879–942. New York, NY: Marcel Dekker, Inc.

Francis, F. J. (1975). *Food colorimetry: theory and applications* (edited by Francis, F. J. & Clydesdale, F. M.).

Food Standards Australia New Zealand (FSANZ). (2013). Australia New Zealand Food Standards Code — Standard 1.3.1: Food Additives. Miscellaneous additives permitted in accordance with GMP in processed foods specified in Schedule 1 (F2013C00138).

Fuentes, A., Fernandez-Segovia, I., Serra, J. & Barat, J. (2012). Effect of partial sodium replacement on physicochemical parameters of smoked sea bass during storage. *Food Sci. Technol. Int*., **18**(3), 207-217.

Fukuda, Y., Tarakita, Z., Kawamura, M., Kakehata, K. & Ocirc, ichi. (1981). Denaturation of Myofibrillar Protein in Chub Mackerel during Freezing and Storage. *Nippon suisan gakkaishi*, **48**(11), 1627-1632.

Fukuda, Y., Tara Kita, Z., Kawamura, M., Kakehata, K. I. & Arai, K. I. (1982). Denaturation of myofibrillar proteins in chub mackerel during freezing and storage. *Bull Jpn Soc Sci Fish*., **48**, 1627-1632 .

Gallart-Jornet, L., Rustad, T., Barat, J. M., Fito, P. & Escriche, I. (2007). Effect of superchilled storage on the freshness and salting behaviour of Atlantic salmon (*Salmo salar*) fillets. *Food Chemistry*, **103**(4), 1268-1281.

Ganesan, P., Jeyasekaran, G., Maheswari, K., Jeya Shakila, R. & Sukumar, D. (2005). Quality evaluation in chilled cuttlefish (*Sepia pharaonis*) fillets. *Journal of Aquatic Food Product Technology*, **14**, 37–49.

Gandotra, R., Koul, M., Gupta, S. & Sharma, S. (2012a). Change in proximate composition and microbial count by low temperature preservation in fish muscle of Labeo rohita (Ham-Buch). *IOSR J. Pharm. Biol. Sci. (IOSRJPBS)*, **2**, 13-17.

Gandotra, R., Sharma, S., Koul, M. & Gupta, S. (2012b). Effect of chilling and freezing on fish muscle. *IOSR Int. J. Pharm Biol. Sci.*, **2**, 5-9.

Garcıa-Arias, M., Álvarez Pontes, E., Garcıa-Linares, M., Garcıa-Fernandez, M. & Sanchez-Muniz, F. (2003a). Cooking–freezing–reheating (CFR) of sardine (*Sardina pilchardus*) fillets. Effect of different cooking and reheating procedures on the proximate and fatty acid compositions. *Food Chemistry*, **83**(3), 349-356.

García-Arias, M. T., Alvarez-Pontes, E., García-Fernández, M. C. & Sánchez-Muniz, F. J. (2003b). Freezing/defrosting/frying of sardine fillets. Influence of slow and quick

defrosting on protein quality. *Journal of the Science of Food and Agriculture*, **83**(6), 602-608.

Garthwaite, G.A. (1992). Chilling and freezing of fish. In: *Fish processing technology* (edited by Hall, G. M.). Pp. 89-112. Blackie Academic Professional, and Published in North America. New York: VCH Publishers, Inc.

Gashti, G. Z. (2002). Estimation of microbiological and chemical variation in minced fish processing of Atlantic Pollock (*Pollachiu vireos*). Iranian Fisheries Research Organization. The United Nations University, Fisheries Training Programme.

George, R. M. (1993). Freezing proceseses used in the food industry. *Trends in Food Science & Technology*, **4**(5), 134-138.

German, J. B. & Kinsella, J. E. (1985). 10 Lipid oxidation in fish tissue. Enzymatic initiation via lipoxygenase. *Journal of Agriculture Food Chemistry*, **33**, 680-683.

Gill, C. O. (1986). The control of microbial spoilage in fresh meats. In: *Advances in meat research* (edited by Pearson, A. M. & T. R. Dutson). Meat and Poultry Microbiology, **2**. Pp. 49-88. Westport, CT: AVI Publishing Co., Inc.

Gill, C. O. (1996). Extending the storage life of raw chilled meats. *Meat Science*, **43**, S99–S109.

Gill, T. A., Keith, R. A. & Lall, B. S. (1979). Textural Deterioration Of Red Hake And Haddock Muscle In Frozen Storage As Related To Chemical Parameters And Changes In The Myofibrillar Proteins. *Journal of Food Science*, **44**(3), 661-667.

Glencross, B. (2004), *The nutritional management of barramundi*, Fisheries Research Contract Report No. 8, Department of Fisheries, Western Australia, 40p.

Glencross, B. (2006). The nutritional management of barramundi, *Lates calcarifer*– a review. *Aquaculture Nutrition*, **12**, 291-309.

Gökodlu, N., Özden, Ö. & Erkan, N. (1998). Physical, chemical and sensory analyses of freshly harvested sardines (*Sardina pilchardus*) stored at 4°C. *Journal of Aquatic Food Product Technology*, **7**(2), 5-15.

Gonçalves, A. A. (2005). Estudo do processo de congelamento do camarão associado ao uso do aditivo fosfato. PhD. Thesis, Pp. 170. Universidade Federal do Rio Grande do Sul, Porto Alegre, Brasil.

Gonçalves, A. A. & Gindri Junior, C. S. G. (2009). The effect of glaze uptake on storage quality of frozen shrimp. *Journal of Food Engineering,* **90**(2), 285-290.

Gonçalves, A. A., Rech, B. T., Rodrigues, P. D. M. & Pucci, D. M. T. (2008). Quality evaluation of frozen seafood (*Genypterus brasiliensis, Prionotus punctatus, Pleoticus muelleri and perna perna*) previously treated with phosphates. *Pan-American Journal of Aquatic Sciences*, **3**(3), 248-258.

Gonçalves, A. A. & Ribeiro, J. L. D. (2009). Effects of phosphate treatment on quality of red shrimp (Pleoticus muelleri) processed with cryomechanical freezing. *LWT e Food Science and Technology*, **42**, 1435-1438.

Goswami, T. K. (2010). Role of cryogenics in food processing and preservation. *International Journal of Food Engineering*, **6**(1).

Graham, J. (1982). Freezing. In: *Fish handling & processing*. In: *Ministry of Agriculture, Fisheries & Food. Torry Research Station,* Chapter 6) (edited by Aitken, A., Mackie, I. M., Merritt, J. H. & Windsor, M. L.). Pp. 56-71. Edinburgh.

Gram, L. (1995). Bacteriological changes. Paper No. 348. In: *Quality and quality changes in fresh fish* (edited by Huss, H. H.). Pp. 51–64. Rome: FAO Fisheries Technical Papers.

Gram, L. & Dalgaard P. (2002). Fish spoilage bacteria—problems and solutions. *Current Opinion Biotech*, **13**, 262–66.

Gram, L. & Huss, H. H. (1996). Microbiological spoilage of fish and fish products. *International Journal of Food Microbiology*, **33**(1), 121-137.

Gram, L., Neergaard, C. W. & Huss, H. H. (1987). Detection of specific spoilage bacteria from fish stored at low (0°C) and high (20°C) temperatures. *Journal of Applied Bacteriology*, **80**, 65-72.

Gregersen, F. (2001). Storage in ice water increases weight. Fiskeriforskning. Info Norwegian Institute of Fisheries and Aquaculture Ltd., **1**, 1.

Guerrero, L. & Gu'ardia, M. D. (1999). La medida de las propiedades meca´ nicas en la carne y en los derivados ca´ rnicos [The assessment of the mechanical properties in meat and meat products]. *Eurocarne*, **77**, 41–49.

Guillerm-Regost, C., Haugen, T., Nortvedt, R., Carlehöug, M., Lunestad, B. T., Kiessling, A. & Rorar, A. M. B. (2006). Quality Characterization of Farmed Atlantic Halibut During Ice Storage. *Journal of Food Science*, **71**(2), S83-S90.

Guo, Y., Kong, B., Xia, X., Yu, T. & Liu, Q. (2013). Changes in physicochemical and protein structural properties of common carp (*Cyprinus carpio*) muscle subjected to different freeze–thaw cycles. *Journal of Aquatic Food Product Technology*, **23**(6), 579-590.

Haard, N. F. (1990). Biochemical reactions in fish muscle during frozen storage. In: *Seafood Science and Technology* (edited by Bligh, G.). Pp. 176-209. London, U.K.: Fishing News Books.

Haard, N. F. (1992). Control of chemical composition and food quality attributes of cultured fish. *Food Research International*, **25**(4), 289-307.

Hale, M. B. & Waters M. E. (1981). Frozen storage stability of whole and headless freshwater prawns, *Machrobranchium rosenbergii*. *Marine Fish Review*, **42**, 18–21.

Hallett, I. C. & Bremner, H. A. (1988). Fine structure of the myocommata-muscle fibre junction in hoki (*Macruronus novaezelandiae*). *Journal of the Science of Food and Agriculture*, **44**(3), 245-261.

Hamm, R. (1986). Funcional properties of the myofibrillar system and their measurements. In: *Muscle as Food: Food Science and Technology a Series of Monographs* (edited by Bechtel, P. J.). Pp. 135–199. Orlando, Florida, USA: Academic Press Inc.

Hamm R. (1960). Biochemistry of meat hydration. *Adv. Food Res*., **10**, 355–463.

Hamm, R. (1979). Delocalization of mitochondrial enzymes during freezing and thawing of skeletal muscle. In: *Proteins at low temperatures* (edited by Fennema, O. R.). Advances in chemistry series, **180**, Pp. 191. Washington, DC, American Chemical Society.

Hamre, K., Lie, Ø. & Sandnes, K. (2003). Development of lipid oxidation and flesh colour in frozen stored fillets of Norwegian spring-spawning herring (*Clupea harengus* L.). Effects of treatment with ascorbic acid. *Food Chemistry*, **82**(3), 447-453.

Hansen, E., Trinderup, R. A., Hviid, M., Darre, M. & Skibsted, L. H. (2003). Thaw drip loss and protein characterization of drip from air-frozen, cryogen-frozen, and pressure-shift-frozen pork *Longissimus dorsi* in relation to ice crystal size. *European Food Research and Technology*, **218**, 2-6.

Harada, K. (1991). How to handle albacore. *Australian Fisheries* (February), 28–30.

Hebard, C. E., Flick, G. J. & Martin, R. E. (1982). Occurrence and significance of trimethylamine oxide c and its derivatives in fish and shellfish. In: *Chemistry and biochemistry of marine food products* (edited by Martin, R. E., Flick, G. J. Hebard, C. E. & Ward, D. R.), Pp. 149–304. Westport, CO: Av.

Hernández, M., López, M., Alvarez, A., Ferrandini, E., García García, B. & Garrido, M. (2009). Sensory, physical, chemical and microbiological changes in aquacultured meagre (*Argyrosomus regius*) fillets during ice storage. *Food Chemistry*, **114**(1), 237-245.

Hernandez-Herrero, M. M., Duflos, G., Malle, P. & Bouquelet, S. (2003). Collagenase activity and protein hydrolysis as related to spoilage of iced cod (*Gadus morhua*). *Food Research International*, **36**, 141–147.

Herrero, R., de Avila, R., Herranz, R., de la Hoz, R., Ordóñez, R. & Cambero, R. (2007). Breaking strength of dry fermented sausages and their correlation with texture profile analysis (TPA) and physico-chemical characteristics. *Meat Science*, **77**(3), 331-338.

Hessami, M. A. (2004). A study of the efficiency of plate freezing vs blast freezing of boxed boneless meat in an abattoir. In: *Proceedings of the 2004 ASME Heat Transfer/Fluids Engineering Summer Conference*. (Conference proceeding). **1**. Pp. 711-715.

Hewitt, M.R. (1969). Thawing of frozen fish in water. In: *Freezing and Irradation of Fish* (edited by Kreuzer, R.). Pp. 201–205. London: Fishing New Books.

Hobbs, G. & W. Hodgkiss (1982). The bacteriology of fish handling and processing. In: *Developments in Food Microbiology* (edited by Davies, R.). Pp. 71-117. London: Applied Science Publishers.

Holownia, K., Chinnan, M. S. & Reynolds, A. E. (2003). Pink Color Defect in Poultry White Meat as Affected by Endogenous Conditions. *Journal of Food Science*, **68**(3), 742-747.

Honikel, K., Hamid, A., Fischer, C. & Hamm, R. (1981). Influence of Postmortem Changes in Bovine Muscle on the Water‐Holding Capacity of Beef. Postmortem Storage of Muscle at Various Temperatures Between 0 and 30° C. *Journal of Food Science*, **46**(1), 23-31.

Howell, N. K., Shavila, Y., Grootveld, M. & Williams, S. (1996). High resolution NMR and Magnetic Resonance Imaging of Fresh and Frozen Cod (*Gadus morhua*) and Haddock (*Melanogrammus aeglefinus*). *Journal of Science Food Agriculture*, **72**, 49-56.

Howell, N. K. (2000). Gelation properties and interactions of fish proteins. In: *Food hydrocolloids I: physical chemistry and industrial application of gels,*

polysaccharides and proteins (edited by Nishinari, K.). Pp. 399-406. Amsterdam: Elsevier.

Howgate, P. (1979). Fish. In: *Food Microscopy* (edited by Vaughan, J. G.). Pp. 343–389. London: Academic Press.

Hsieh, Y. L. & Regenstein, J. M. (1989). Texture changes of frozen stored cod and ocean perch minces. *Journal of Food Science*, **54**, 824–826.

Huang, Y. W., Kouadio, K. & Gates, K. W. (1993). Microbiological, Sensory and Chemical Assessment of Pre-Packaged Weakfish During Iced Storage. *Journal of Aquatic Food Product Technology*, **2**(1), 135-144.

Huidobro, A., Mendes, R. & Nunes, M. (2001). Slaughtering of gilthead seabream (*Sparus aurata*) in liquid ice: influence on fish quality. *Eur. Food Res. Technol*., **213**(4-5), 267-272.

Huis in't Veld, J. H. J. (1996). Microbial and biochemical spoilage of foods: an overview. *International Journal of Food Microbiology*, **33**(1), 1-18.

Hultmann, L., Phu, T. M., Tobiassen, T., Aas-Hansen, Ø. & Rustad, T. (2012). Effects of pre-slaughter stress on proteolytic enzyme activities and muscle quality of farmed Atlantic cod (*Gadus morhua*). *Food Chemistry*, **134**(3), 1399-1408.

Hultmann, L., Rørå, A. M. B., Steinsland, I., Skåra, T. & Rustad, T. (2004). Proteolytic activity and properties of proteins in smoked salmon (*Salmo salar*) - Effects of smoking temperature. *Food Chemistry*, **85**(3), 377-387.

Hultmann, L. & Rustad, T. (2002). Textural Changes During Iced Storage of Salmon (*Salmo salar*) and Cod (*Gadus morhua*). *Journal of Aquatic Food Product Technology*, **11**(3-4), 105-123.

Hultmann, L. & Rustad, T. (2004). Iced storage of Atlantic salmon (*Salmo salar*) – effects on endogenous enzymes and their impact on muscle proteins and texture. *Food Chemistry*, **87**(1), 31-41.

Hultmann, L. & Rustad, T. (2007). Effects of temperature abuse on textural properties and proteolytic activities during post mortem iced storage of farmed Atlantic cod (*Gadus morhua*). *Food Chemistry*, **104**(4), 1687-1697.

Hurling, R. & McArthur, H. (1996). Thawing, refreezing and frozen storage effects on muscle functionality and sensory attributes of frozen cod (*Gadus morhua*). *Journal of Food Science*, **61**(6), 1289-1296.

Huss, H. H. (1988). Fresh fish: Quality and quality changes. A training manual prepared for the FAO / DANDA Training program on fish technology and quality control. *FAO Fisheries series*, **29**, 27 - 59.

Huss, H. H. (1995). Quality and quality changes in fresh fish. FAO, Rome (Italy). FAO Fisheries Technical Paper, (348).

Huss , H.H., Boerresen , T., Dalgaard , P., Gram , L., Jensen , B., Joergensen , B. Nielsen , J. Baek Olsen , K. Gill , T. & Lupin, H. M. (1995). Quality and quality changes in fresh fish. FAO, Rome (Italy). Fisheries Dept., FAO Fisheries Technical Paper, (348).

Huss H., Dalgaard, P. & Gram L. (1997) Microbiology of fish and fish products. In: *Seafood from producer to consumer, intergrated approach to quality* (edited by Luten J. B., Borresen T., and Oihlenschlager J., (1997). Development in food science 38. In: *Proceedings of the International Seafood Conference on the occasion of the 25th*

anniversary of the WEFTA, held in Noordwijkerhout, The Netherlands. (Conference Proceeding). Pp. 13-16. November 1995.

Hwang, K. T. & Regenstein, J. M. (1995). Hydrolysis and Oxidation of Mackerel (*Scomber scombrus*) Mince Lipids with NaOCl and NaF Treatments. *Journal of Aquatic Food Product Technology,* **4**(4), 19-30.

Hyldig, G., Jørgensen, B. M., Nielsen, H. H., Undeland, I., Olsen, R. E. & Jónsson, Á. (2012). Sensory Properties of Frozen Herring (*Clupea harengus*) from Different Catch Seasons and Locations. *Journal of Food Science*, **77**(9), S288-S293.

International Commission on Microbiological Specifications for Foods (ICMSF). (1986). *Sampling plans for fish and shellfish. In Microorganisms in foods 2, sampling for microbiological analysis: principles and specific applications*. Toronto, Canada: University of Toronto Press.

International Commission on Microbiological Specifications for Foods (ICMSF). (1998). *Microorganisms in foods*, 6: *Microbial ecology of food commodities. The international commission on microbiological specifications for foods*. Blackie Academic and Professional, Baltimore.

IIR. (2000). *Recommendations For Chilled Storage of perishable produce*. Available from: <http://www.frisbee.szie.hu/files/FRISBEEDEL3-2-4 3ReviewChillingSuperchillingSupercooling-S1.pdf>. [1st December 2013].

Indergård, E., Tolstorebrov, I., Larsen, H. & Eikevik, T. M. (2013). The influence of long-term storage, temperature and type of packaging materials on the quality characteristics of frozen farmed Atlantic Salmon (*Salmo Salar*). *International Journal of Refrigeration*, **41**, 27-36.

Jacobsen, S. & Fossan, K. M. (2001). Temporal variations in the glaze uptake on individually quick frozen prawns as monitored by the CODEX standard and the enthalpy method. *Journal of Food Engineering*, **48**(3), 227-233.

Jain, D., Pathare, P. B. & Manikantan, M. R. (2007). Evaluation of texture parameters of Rohu fish (*Labeo rohita*) during iced storage. *Journal of Food Engineering*, **81**(2), 336-340.

James, S. J., Kerry, J. & Ledward, D. (2002). New developments in the chilling and freezing of meat. In: *Meat Processing – Improving Quality,* 297-312.

Jasra, S. K., Jasra, P. K. & Talesara, C. L. (2001). Myofibrillar protein degradation of carp (*Labeo rohita* (Hamilton)) muscle after post-mortem unfrozen and frozen storage. *Journal of the Science of Food and Agriculture*, **81**(5), 519-524.

Jay, J. M. (1986). *Modern Food Microbiology*. New Delhi: CBS Publishers and Distributors.

Jeyasekaran, G., Maheswari, K., Ganesan, P., Jeya Shakila, R. & Sukumar, D. (2005). Quality changes in ice-stored tropical wire-netting reef cod (*Epinephelus merra*). *Journal of Food Processing and Preservation*, **29**, 165-182.

Jhaveri, S. N. (1981). Constantinides, S. M. Chemical composition and shelf life study of grayfish (*Squalus acanthias*). *J. Food Sci*., **47**, 188-192.

Jiang, M., Wang, Y., van Santen, E. & Chappell, J. A. (2008). Evaluation of textural properties of channel catfish (*Ictalurus punctatus Rafinesque*) fillet with the natural contour method. *LWT - Food Science and Technology*, **41**(9), 1548-1554.

Jiang, S. T., Hwang, B. S., Moody, M. W. & Chen, H. C. (1991). Thermostability and freeze denaturation of grass prawn (*Penaeus monodon*) muscle proteins. *Journal of Agriculture and Food Chemistry*, **39**, 1998–2001.

Johnson, W.A., Nicholson, F.J., Roger, A. & Stroud, G.D. (1994). Freezing and refrigerated storage in fisheries. *FAO Fisheries Technical Paper*, **340**.

Johnston, W. A., Nicholson F. J., Roger, A. & Stroud, G. D. (1994). Freezing and refrigerated storage in fisheries. FAO, Rome. FAO Fisheries Technical Paper No. 340. Pp. 143.

Joensen, S., Sørensen, N. K. & Akse, L. (2001). Cod: Storage in Ice Water Increases Weight. In: *Fiskeriforskning Info*, **1**.

Jol, S., Kassianenko, A., Wszol, K. & Oggel, J. (2006). Issues in time and temperature abuse of refrigerated foods. *Food Safety*, **11**(6), 30-32.

Jonsson, A., Sigurgisladottir, S., Hafsteinsson, H. & Kristbergsson, K. (2001). Textural properties of raw Atlantic salmon (*Salmo salar*) fillets measured by different methods in comparison to expressible moisture. *Aquac. Nutr.,* **7**(2), 81-89.

Jørgensen, B. R., Gibson, D. M. & Huss, H. H. (1988). Microbiological quality and shelf life prediction of chilled fish. *lnt. J. Food Microbial*., **6**, 295-307.

Kaale, L. D. & Eikevik, T. M. (2013a). A study of the ice crystal sizes of red muscle of pre-rigor Atlantic salmon (*Salmo sa*lar) fillets during superchilled storage. *Journal of Food Engineering*, **119**(3), 544-551.

Kaale, L. D. & Eikevik, T. M. (2013b). A histological study of the microstructure sizes of the red and white muscles of Atlantic salmon (*Salmo Salar*) fillets during superchilling process and storage. *Journal of Food Engineering*, **114** (2), 147– 278.

Kaale, L. D., Eikevik, T. M., Kolsaker, K. & Stevik, A. M. (2013c). Modeling and simulation of food products in superchilling technology. *Journal of Aquatic Food Product Technnology*, **23**, 409–420.

Kaale, L. D., Eikevik, T. M., Bardal, T., Kjorsvik, E. & Nordtvedt, T. S. (2013d). The effect of cooling rates on the ice crystal growth in air-packed salmon fillets during superchilling and superchilled storage. *International Journal of Refrigeration*, **36** (1), 110–119.

Kaale, L. D., Eikevik, T. M., Rustad, T., Nordtvedt, T. S., Bardal, T. & Kjørsvik, E. (2013e). Ice crystal development in pre-rigor Atlantic salmon fillets during superchilling process and following storage. *Food Control*, **31** (2), 491–498.

Karungi, C., Byaruhanga, Y. B. & Muyonga, J. H. (2004). Effect of pre-icing duration on quality deterioration of iced Nile perch (*Lates niloticus*). *Food Chemistry*, **85**(1), 13-17.

Kato, N., Umemoto, S. & Uchiyama., H. (1974). Partial freezing as a means of preserving freshness of fish-II. Changes in the properties of protein during the storage of partially frozen sea bass muscle. *Bulletin of the Japanese Society of Scientific Fisheries*, **40** (12), 1263–1267.

Kauffeld, M., Wang, M. J., Goldstein, V. & Kasza, K. E. (2010). Ice slurry applications. *International Journal of Refrigeration*, **33**(8), 1491-1505.

Ke, P. J., Ackman, R. G., Linke, B. A. & Nash, D. M. (1977). Differential lipid oxidation in various parts of mackerel. *J. Food Technol*., **12**, 34-47.

Kent, M. & Stroud, G. (1999). New method for the measurement of added glaze on frozen foods. *Journal of Food Engineering*, **39**(3), 313-321.

Kemp, R. M. & Chadderton, T. (1992). Blasting ahead with cartoned beef. In: *Refrigeration and Energy Section*, MIRINZ. (Conference proceeding).

Khayat, A. & Schwall, D. (1983). Lipid oxidation in seafood. *Fd. TechnoL*, **37**(7), 130-140.

Kilinc, B. & Cakli, S. (2004). Chemical, microbiological and sensory changes in thawed frozen fillets of sardine (*Sardina pilchardus*) during marination. *Food Chemistry,* **88**(2), 275-280.

Kilinc, B., Cakli, S., Cadun, A., Dıncer, T. & Tolasa, S. (2007). Comparison of effects of slurry ice and flake ice pretreatments on the quality of aquacultured sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*) stored at 4°C. *Food Chemistry,* **104**(4), 1611-1617.

Kilinc, B., Cakli, S., Cadun, A. & Sen, B. (2009a). Effects of phosphate dip treatments on chemical, microbiological, color, textural, and sensory changes of rainbow trout (*Onchorhyncus mykiss*) fillets during refrigerated storage. *Journal of Aquatic Food Product Technology*, **18**(1-2), 108-119.

Kilinc, B., Cakli, S., Dincer, T. & Cadun, A. (2009b). Effects of phosphates treatment on the quality of frozen-thawed fish species. *Journal of Muscle Foods*, **20**(4), 377-391.

Kim, C. R., Hearnsberger, J. O., Vickery, A. P., White, C. H. & Marshall, D. L. (1995). Extending shelf life of refrigerated catfish fillets using sodium acetate and monopotassium phosphate. Journ*al of Food Protection*, **58**, 644–647.

Kinsella, J. E. (1988). Fish and seafoods: nutritional implications and quality issues. *Food Technnol*, **42**(5), 146-150.

Knipe, C. L. (2004). Use of phosphates in meat products. In: *The meat industry research conference*, Nashvill, TN. (Conference proceeding). September 30-October 2.

Kolakowska, A. (1981). The rancidity of frozen Baltic herring prepared from raw material with different initial freshness. *Refrig.Sci. Technol*., **4**, 341-348.

Kolbe, E., Craven, C., Sylvia, G. & Morrissey, M. (2004). Chilling and Freezing Guidelines to Maintain Onboard Quality and Safety of Albacore Tuna Agricultural Experiment Station. Special Report No. 1006.

Kolbe, E. & Kramer, D. (2007). Planning for seafood freezing. Fairbanks, Alaska: University of Alaska Fairbanks.

Köse, S., Karaçam, H., Kutlu, S. & Boran, M. (2001). Investigating the Shelf-Life of the Anchovy Dish Called 'Hamsikuşu' in Frozen Storage at-18±1ºC. *Turkish Journal of Veterinary and Animal Sciences*, **25**(5), 651-656.

Koutsoumanis, K. (2001). Predictive modelling of the shelf life of fish under nonisothermal conditions. *Applied and Environmental Microbiology*, **67** (4), 1821–1829

Koutsoumanis, K., Lampropoulou, K. & Nychas, G. J. E. (1999). Biogenic amines and sensory changes associated with the microbial flora of Mediterranean gilt-heat sea bream (*Sparus aurata*) stored aerobically at 0, 8, and 15C. *Journal of Food Protection*., **62**, 398–402

Koutsoumanis, K. & Nychas, G. J. E. (2000). Application of a systematic experimental procedure to develop a microbial model for rapid fish shelf life predictions. *Int. J. Food Microbiol*., **60**, 171-184.

Koutsoumanis, K. & Nychas, G. J. E. (1999). Chemical and sensory changes associated with microbial flora of Mediterranean boque *(Boops hoops)* stored aerobically at 0, 3, 7 and 10 °C. *Applied and Environment al Microbiology*, **65**, 698-706.

Koutsoumanis, K., Nychas, M. C., Giannakourou, P. S. & Taoukis, G. J. E. (2002). Application of shelf life decision system (SLDS) to marine cultured fish quality. *International Journal of Food Microbiology*, **73**(2-3), 375-382.

Koutsoumanis, K. & Taoukis, P. S. (2005). 23 - Meat safety, refrigerated storage and transport: modeling and management. In: *Improving the Safety of Fresh Meat* (edited by Sofos, J. N.). Pp. 503-561. Woodhead Publishing.

Kraus, L. (1992). Refrigerated sea water treatment of herring and mackerel for human consumption. In: *Pelagic fish. The resource and its exploitation* (edited by Burt, J. Hardy, R. & Whittle, K.), Pp. 73–81. Aberdeen, Scotland, UK: Fishing News Books.

Krueger, D. J. & Fennema, O. R. (1989). Effect of Chemical Additives on Toughening of Fillets of Frozen Alaska Pollack (*Theragra chalcogramma*). *Journal of Food Science*, **54**(5), 1101-1106.

Labadie, J. (1999). Consequences of packaging on bacterial growth. Meat is an ecological niche. *Meat Science*, **52**(3), 299-305.

Laird, W. M., Mackie, I. M. & Hattula, T. (1980). Studies of the changes in the proteins of cod-frame minces during frozen storage at -15°C. In: *Advances in Fish Science and Technology* (edited by Connell, J. J. & staff of Torry Research Station), Pp. 428-434. Jubilee Conference of the Torry Research Station, Aberdeen, U.K. Surrey, U.K.: Fishing News Books.

Lakshmanan, R., Parkinson, J. A. & Piggott, J. R. (2007). High-pressure processing and water-holding capacity of fresh and cold-smoked salmon (*Salmo salar*). *LWT e Food Science and Technology*, **40**, 544-555.

Lakshminatha, R., Setty, T. M. R. & Dora, K. C. (1992). Studies on the storage behaviour of fish fingers from croakers and perches. *Journal of Fishery Technology*, **29**, 35-39.

Lawley, D. (2010). *Repositioning Australian Farmed Barramundi: Online Consumer Survey Findings.* University of Sunshine Coast, Sunshine Coast, Queensland.

Lawrie, R. A. (1979). *Meat science,* 3rd edn. Oxford: Pergamon Press.

Lee, Y. S., Saha, A., Xiong, R., Owens, C. M. & Meullenet, J. F. (2008). Changes in Broiler Breast Fillet Tenderness, Water-Holding Capacity, and Color Attributes during Long-Term Frozen Storage. *Journal of Food Science*, **73**(4), E162-E168.

Lee, C. M. & Toledo, R. T. (1984). Comparison of shelf life and quality of mullet stored at zero and subzero temperature. *J Food Sci.*, **49**, 317–322.

Leelapongwattana, K., Benjakul, S., Visessanguan, W. & Howell, N. K. (2005). Physicochemical And Biochemical Changes In Whole Lizardfish (*Saurida Micropectoralis*) Muscles And Fillets During Frozen Storage. *Journal of Food Biochemistry*, **29**(5), 547-569.

Li, X., Li, J., Zhu, J., Wang, Y., Fu, L. & Xuan, W. (2011). Postmortem changes in yellow grouper (*Epinephelus awoara*) fillets stored under vacuum packaging at 0° C. *Food Chemistry*, **126**(3), 896-901.

Li, Y., Bell, L. N., Liu, S. & Wang, Y. (2013). Color and Carotenoid Content Changes of Yellow Discolored Channel Catfish, Ictalurus punctatus, Fillets during Refrigerated Storage. *Journal of the World Aquaculture Society*, **44**(1), 148-153.

Lim, H. & Haard, N. F. (1984). Protein insolubilization in frozen Greenland halibut. *J. Food Biochem*., **8**, 163-187.

Lindsay, R. C. (1996). Food additives. In: *Food chemistry* (edited by Fennema, O. R.). Pp. 767–823. New York: Marcel Dekker.

Liston, J. (1992). Bacterial spoilage in see foods. In: *Development in food science*, **30**. *Quality assurance in the sea fish industry.* In*: Proceedings of an international conference* (edited by Huss, H. H., Jacobsen, M. & Liston, J.). Pp. 93-105. Copenhagen, Denmark: Elsevier Science Publishing.

Liu, S., Fan, W., Zhong, S., Ma, C., Li, P., Zhou, K., Peng, Z. & Zhu, M. (2010). Quality evaluation of tray-packed tilapia fillets stored at 0°C based on sensory, microbiological, biochemical and physical attributes. *African Journal of Biotechnology*, **9**(5), 692-701.

Liu, D., Liang, L., Xia, W., Regenstein, J. M. & Zhou, P. (2013). Biochemical and physical changes of grass carp (*Ctenopharyngodon idella*) fillets stored at -3 and 0 °C. *Food Chemistry*, **140**(1-2), 105.

Londahl, G. (1997). Technological aspects of freezing and glazing shrimp. *Infofish International*, **3**, 49-56.

Lopkulkiaert, W., Prapatsornwattana, K. & Rungsardthong, V. (2009). Effects of sodium bicarbonate containing traces of citric acid in combination with sodium chloride on yield and some properties of white shrimp (*Penaeus vannamei*) frozen by shelf freezing, air-blast and cryogenic freezing. *LWT - Food Science and Technology*, **42**(3), 768-776.

Losada, V., Barros-Velázquez, J. & Aubourg, S. P. (2007). Rancidity development in frozen pelagic fish: Influence of slurry ice as preliminary chilling treatment. *LWT - Food Science and Technology*, **40**(6), 991-999.

Losada, V., Pineiro, C., Rodriguez, O., Antonia, J. M., Barros-Velazquez, J. & Auborg, S. P. (2003). Improvement of horse mackerel (*Trachurus trachurus*) quality during chilling storage by flow ice application: Assessment of chemical changes. In: *Novas perspectivas sobre conservac_ao processamento e qualidae de alimentos*. Pp. 9-333. Lisbonne, Portugal: Ipimar/SPQ.

Losada, V., Piñeiro, C., Barros-Velázquez, J. & Aubourg, S. (2004). Effect of slurry ice on chemical changes related to quality loss during European Hake (*Merluccius merluccius*) chilled storage. *European Food Research and Technology*, **219**(1), 27-31.

Losada, V., Piñeiro, C., Barros-Velázquez, J. & Aubourg, S. P. (2005). Inhibition of chemical changes related to freshness loss during storage of horse mackerel (*Trachurus trachurus*) in slurry ice. *Food Chemistry*, **93**(4), 619-625.

Losada, V., Rodríguez, Ó., Miranda, J. M., Barros‐Velázquez, J. & Aubourg, S. P. (2006). Development of different damage pathways in Norway lobster (*Nephrops norvegicus*) stored under different chilling systems. *Journal of the Science of Food and Agriculture*, **86**(10), 1552-1558.

Love, R. M. (1962). Protein denaturation in frozen fish. VI. Cold-storage studies on cod using the cell fragility method. *J. Sci. Food Agric*., **13**, 269–278.

Love, R. M. (1964). *The Technology of Fish Utilization* (edited by Kreuzer, R.). Pp. 102. London: Fishing News (Books).

Love, R. M. (1988). *The Food Fishes, Their Intrinsic Variation and Practical Implications*. Pp. 121–179. London: Farrand Press.

Love, R. M. & Abel, G. (1966). The effect of phosphate solutions on the denaturation of frozen cod muscle. *International Journal of Food Science & Technology*, **1**(4), 323-333.

Love, R. M. & Haq, M. A. (1970). The connective tissues of fish: IV. Gaping of cod muscle under various conditions of freezing, cold storage and thawing. *J. Food Technol*., **5**, 249–260.

Love, G. & Langenkamp, D. (2003). *Australian Aquaculture: Industry Profiles for Related Species*, ABARE *e*Report 03.8, Prepared for the Fisheries Resources Research Fund, Canberra, May.

Love, R. M. & Olley, J. (1965). Cold storage deterioration in several species of fish, as measured by two methods. In: *The Technology of Fish Utilization* (edited by Kreuzer, R.). Pp. 116– 118. London: Fishing New Books.

Lovell, T. (1984). The yellow fat problem in fish flesh. *Aquaculture,* **10**, 39–40.

Ludorf, M. & Meyer, W. (1973). *Fische und fischerzeugnisse*. Hamburg-Berlin: Paul Parey Verlag,

Ma, L. B. & Yamanaka, H. (1991). Studies of thaw-rigor in red-sea bream and carp muscles. *Nippon Suisan Gakkaishi*, **57**, 1365– 1368.

MacCallum, W. A, Jaffray, J. I., Churchill, D. N. & Idler, D. R. (1968). Condition of Newfoundland trap-caught cod and its influence on quality after single and double freezing. *J. Fish. Res. Board Can*., **25**, 133-75

Mackie, I. M. (1993). The effects of freezing on flesh proteins. *Food Reviews International,* **9**(4), 575-610.

Magnussen, O. M. & Johansen, S., (1995). Effect of Freezing and storage temperatures at -25 C to -60 C on fat fish quality. In: *19th International Congress of Refrigeration*. Pp.249-257.

Maguire, G. (2002). Barramundi farming status report in State of the Fisheries Report 20001/2002. Department of Fisheries (Western Australia), Perth. Pp. 182.

Majumdar, R. K., Deb, S., Dhar, B. & Priyadarshini, B. M. (2012). Chemical changes in washed mince of silver carp (*Hypophthalmichthys molitrix*) during frozen storage at −20C with or without cryoprotectants. *Journal of Food Processing and Preservation*, **37**(5), 952-961.

Makarios-Laham, I. K. & Lee, T. (1993). Protein hydrolysis and quality deterioration of refrigerated and frozen seafood due to obligately psychrophilic bacteria. *J. Food Sci*., **58**, 310–313.

Mallett, C. P. (1994). *Frozen Food Technology*. London: Blackie Academic & Professionals.

Mancini, R. A. & Hunt, M. C. (2005). Current research in meat color. *Meat Sci,* **71**(1), 100–121.

Manju, S., Jose, L., SrinivasaGopal, T., Ravishankar, C. & Lalitha, K. (2007). Effects of sodium acetate dip treatment and vacuum-packaging on chemical, microbiological, textural and sensory changes of Pearlspot (*Etroplussuratensis*) during chill storage. *Food Chemistry*, **1**(1), 27-35.

Mannapperuma, J. D., Singh, R. P., Reid, D. S. (1994). Effective surface heat transfer coefficients encountered in air blast freezing of whole chicken and chicken parts, individually and in packages. *International Journal of Refrigeration*, **17**(4), 263-272.

Mantilla, D., Kristinsson, H. G., Balaban, M. O., Otwell, W. S., Chapman, F. A. & Raghavan, S. (2008). Carbon Monoxide Treatments to Impart and Retain Muscle Color in Tilapia Fillets. *Journal of Food Science*, **73**(5), C390-C399.

Margeirsson, B., Lauzon, H. L., Pálsson, H., Popov, V., Gospavic, R., Jónsson, M. ó., Sigurgisladottir, S. & Arason, S. (2012). Temperature fluctuations and quality deterioration of chilled cod (*Gadus morhua*) fillets packaged in different boxes stored on pallets under dynamic temperature conditions. *International Journal of Refrigeration*, **35**(1), 187-201.

Maria Macedo Viegas, E., Regina Barbieri de Carvalho, M., Roberto Campagnoli de Oliveira Filho, P., Gaberz Kirschnik, P., Shindy Aiura, F. & Cristina Vargas, S. (2013). Changes During Chilled Storage of Whole Tilapia and Short-Term Frozen Storage of Tilapia Fillets. *Journal of Aquatic Food Product Technology*, **22**(2), 192-200.

Maroulis, Z. B. & Saravacos, G. D. 2003. *Food Process Design*, 0-8247-4311-3. New York, NY: Marcel Dekker, Inc.

Martin, A. M. (1994). Biotechnological applications. Canada, Department of Biochemistry Memorial University.

Martinez, O., Salmeron, J., Guillen, M. & Casas, C. (2004). Texture profile analysis of meat products treated with commercial liquid smoke flavourings. *Food Control*, **15**(6), 457–61.

Martinez, O., Salmerón, J., Guillén, M. D. & Casas, C. (2010). Effect of freezing on the physicochemical, textural and sensorial characteristics of salmon (*Salmo salar*) smoked with a liquid smoke flavouring. *LWT - Food Science and Technology*, **43**(6), 910-918.

Martinsdóttir, E., Sveinsdóttir, K., Luten, J. B., Schelvis-Smit, R. & Hyldig, G. (2001). Reference manual for the fish sector: Sensory evaluation of fish freshness. QIM Eurofish, Netherlands.

Masniyom, P., Benjakul, S. & Visessanguan, W. (2005a). Collagen changes in refrigerated sea bass muscle treated with pyrophosphate and stored in modified- atmosphere packaging. *European Food Research and Technology*, **220**(3-4), 322-325.

Masniyom, P., Soottawat, B. & Visessanguan, W. (2005b). Combination effect of phosphate and modified atmosphere on quality and shelf- life extension of refrigerated seabass slices. *J. Food Sci. Technol*., **38**, 745–756.

Mathew, S. & Shamasundar, B. (2002). Effect of ice storage on the functional properties of proteins from shark (*Scoliodon laticaudus*) meat. *Nahrung/Food*, **46,** 220-226.

Matlock, R. G., Terrel, R. N., Savell, J. W., Rhee, K. S. & Dutson, T. R. (1984). Factors affecting properties of raw-frozen pork sausage patties made with various NaCl/phosphate combination. *Journal of Food Science*, **49**, 1363–1366

Matos, E., Silva, T. S., Tiago, T., Aureliano, M., Dinis, M. T. & Dias, J. (2011).Effect of harvesting stress and storage conditions on protein degradation in fillets of farmed gilthead seabream (*Sparus aurata*): A differential scanning calorimetry study. *Food Chemistry*, **126**(1), 270-276.

Matsumoto, J. J. (1979). Denaturation of fish muscle proteins during frozen storage. In: *Proteins at Low Temperatures* (edited by Fenema, O.). Advances in Chemistry Series 180, Pp. 205-224. Washington, DC: American Chemical Society.

Matsumoto, J. J. (1980). Chemical deterioration of muscle proteins during frozen storage. In: *Chemical Deterioration of Proteins* (edited by Whitaker, J. R. & Fujimaki, M.). ACS Symposium Series 123. American Chemical Society: Washington, DC.

McEvily, A., Iyengar, R. & Otwell, S. (1991). Sulfite alternative prevents shrimp melanosis. *Food Technology*., **45***,* 82–86.

McMeekin, T. A. (1982). Microbial spoilage of meats. In: *Developments in Food Microbiology – 1* (edited by R. Davies), Pp. 1–40. London, UK: Applied Science Publishers.

Mendes, R., Quinta, R. & Nunes, M. (2001). Changes in baseline levels of nucleotides during ice storage of fish and crustaceans from the Portuguese coast. *Eur. Food Res. Technol.,* **212**(2), 141-146.

Meat Standards Committee. (2002). Microbiological testing for process monitoring in the meat industry. October 30, 2002.

Mills, A. (1975). Measuring changes that occur during frozen storage of fish: a review. *International Journal of Food Science & Technology*, **10**(5), 483-496.

Miller, W., Saffle, R. & Zirkle, S. (1968). Factors which influence water-holding capacity of various types of meat. *Food Technology*, **22**(9), 1139.

Mohan, M., Ramachandran, D., Sankar, T. V. & Anandan, R. (2008). Physicochemical characterization of muscle proteins from different regions of mackerel (*Rastrelliger kanagurta*). *Journal of Food Chemistry*, **106**, 451–457.

Molins, R. A. (1991). *Antimicrobial uses of food preservatives*. Phosphate in food. Boca Raton, FL: CRC Press, Inc.

Montero, P. & Borderias, J. (1990). Effect of rigor mortis and ageing on collagen in trout (*Salmo irideus*) muscle. *Journal of the Science of Food and Agriculture*, **52**(1), 141-146.

Mørkøre, T. & Lilleholt, R., (2007). Impact of freezing temperature on quality of farmed Atlantic Cod (*Gadus Morhua* L.). *J. Texture Stud*., **38**, 457-472.

Mørkøre, T., Vallet, J. L., Cardinal, M., Gomez-guillen, M. C., Montero, P., Torrissen, O. J., Nortvedt, R., Sigurgisladottir, S. & Thomassen, M. S. (2001). Fat content and fillet shape of Atlantic salmon: Relevance for processing yield and quality of raw and smoked products. *Journal of Food Science*, **66**(9), 1348-1354.

Mudalal, S., Petracci, M., Tappi, S., Rocculi, P. & Cavani, C. (2014). Comparison between the Quality Traits of Phosphate and Bicarbonate-Marinated Chicken Breast Fillets Cooked under Different Heat Treatments. *Food and Nutrition Sciences*, **5**(01), 35-44.

Múgica, B., Barros-Velázquez, J., Miranda, J. M. & Aubourg, S. P. (2008). Evaluation of a slurry ice system for the commercialization of ray (*Raja clavata*): Effects on spoilage mechanisms directly affecting quality loss and shelf-life. *LWT - Food Science and Technology*, **41,** 974-981.

Mukundan, M. K., Antony, P. D. & Nair, M. R. (1986). A review on autolysis in fish. *Fisheries Research*, **4**(3-4), 259-269.

Nauta, M. J., Litman, S., Barker, G. C. & Carlin, F. (2003). A retail and consumer phase model for exposure assessment of Bacillus cereus. *International Journal of Food Microbiology*, **83**(2), 205-218.

Nelson, J. S. (1994). *Fishes of the World*, 3rd edn. New York: Wiley.

Newton, K. G. & Gill, C. O. (1981). The microbiology of DFD fresh meats: A review. *Meat Science*, **5**(3), 223-232.

Nguyen, M. V., Jonsson, J. O., Thorkelsson, G., Arason, S., Gudmundsdottir, A. & Thorarinsdottir, K. A. (2012). Quantitative and qualitative changes in added phosphates in cod (*Gadus morhua*) during salting, storage and rehydration. *LWT - Food Science and Technology*, **47**(1), 126-132.

Nicholson, F. (1973). The freezing time of fish. Food and Agricultural Organization of the United Nations.

Nielsen, J., Hydlig, G., & Larsen, E. (2002). Eating quality of fish- A review. *J. Aquatic Food Prod. Technol*., **11**(3/4), 125-141.

Nielsen, J. & Jessen, F. (2007). Quality of Frozen Fish. In: *Handbook of Meat, Poultry and Seafood Quality.* Pp. 577-586. Blackwell Publishing.

Nilsson, K. & Ekstrand, B. (1993). The e€ect of storage on ice and various freezing treatments on enzyme leakage in muscle tissue of rainbow trout (*Oncorhynchus mykiss*). *Zeitschrift fuÈr Lebensmittel-Untersuchung und –Forchung*., **197**, 3-7.

Nilsson, K. & Ekstrand, B. (1995). Frozen storage and thawing methods affect biochemical and sensory attributes of rainbow trout. *Journal of Food Science*, **60**, 627-630.

Nilson, K. (1994). Quality of frozen rainbow trout. PhD Thesis, Department of Food Science, Chalmers University of Technology and SIK, Sweden.

Notermans, S., Dufrenne, J., Teunis, P., Beumer, R., te Giffel, M. & Peeters Weem, P. (1997). A risk assessment study of *Bacillus cereus* present in pasteurized milk. *Food Microbiology*, **14**(2), 143-151.

Nunes, M. L., Batista, I. & De Campos, R. M. (1992). Physical, chemical and sensory analysis of sardine (*Sardina pilchardus*) stored in ice. *Journal of the Science of Food and Agriculture*, **59**(1), 37-43.

Nychas, G-J.E., Marshall, D. & Sofos, J. (2007). Meat poultry and seafood. In: *Food Microbiology Fundamentals and Frontiers* (Chapter 6) (edited by Doyle, M. P., Beuchat , L. R. & Montville, T. J.). ASM press.

Nychas, G.-J. E., Skandamis, P. N., Tassou, C. C. & Koutsoumanis, K. P. (2008). Meat spoilage during distribution. *Meat Science*, **78**(1), 77-89.

Ocaño-Higuera, V. M., Maeda-Martínez, A. N., Marquez-Ríos, E., Canizales-Rodríguez, D. F., Castillo-Yáñez, F. J., Ruíz-Bustos, E., Graciano-Verdugo, A. Z. & Plascencia-Jatomea, M. (2011). Freshness assessment of ray fish stored in ice by biochemical, chemical and physical methods. *Food Chem*., **125**(1), 49-54.

Odoli, C. O. (2009). Optimal storage conditions for fresh farmed tilapia (*Oreochromis niloticus*) fillets. Doctoral dissertation. University of Iceland.

Oelenschläger, J. (1992). Evaluation of some well established and some underrated indices for the determination of freshness and/or spoilage of ice stored wet fish. In: *Quality Assurance in the Fish Industry* (edited by Huss, H. H.). Elsevier Science Publishers.

Oehlenschlaeger , J. (1995). Assessment of freshness and spoilage indices during iced storage of plaice (*Pleuronectes platessa*). *Inf Fischwirtschaft* (Hamburg), **42**, 94–102.

Offer, G. & Trinick, T. (1983). On the mechanism of water holding in meat: The swelling and shrinking of myofibrils. *Journal Meat Science*, 8, 245–281.

Ogata, H., Aranishi, F., Hara, K., Osatomi, K. & Ishihara, T. (1998). Proteolytic degradation of myofibrillar components by carp cathepsin L. *Journal of the Science of Food and Agriculture*, **76**(4), 499-504.

Ohmori, H., Nakamura, K., Hori, T., & Yamamura, M. (1981). A study on the thawing process of frozen fish blocks in water. *International Journal of Refrigeration*, **4**(1), 27-35.

Okeyo, G. O., Lokuruka, M. N. I. & Matofari, J. W. (2009). Nutritional composition and shelf life of the Lake Victoria Nile perch (*Lates niloticus*) stored in ice. *Afr. J. Food Agric. Nutr. Dev*., **9**, 901–919.

Olafsdottir, G., Martinsdottir, E., Oehlenschlager, J., Dalgaard, P., Jensen, B., Undeland, I., Mackie, Im., Henehan, G., Nielsen, J. & Nilsen, H. (1997). Methods to evaluate fish freshness in research and industry. *Trends Food Sci. Technol*., **8**(8), 258-265.

Olafsdottir, G., Nesvadba, P., Di Natale, C., Careche, M., Oehlenschager, J., Tryggvadottir, S. V. Schubring, R., Kroeger, M., Heia, K., Esaiassen, M., Macagnano, A. & Jorgensen, B. A. (2004). Multisensor for fish quality determination. *Trends in Food Science & Technology*, **15**(2), 86-93.

Orban, E., Sinesio, F. & Paoletti, F. (1997). The Functional Properties of the Proteins, Texture and the Sensory Characteristics of Frozen Sea Bream Fillets (Sparus aurata) from Different Farming Systems. *Lebensmittel-Wissenschaft und-Technologie*, **30**(2), 214-217.

Orban, E., Di Lena, G., Nevigato, T., Casini, I., Santaroni, G., Marzetti, A. & Caproni, R. (2002). Quality characteristics of sea bass intensively reared and from lagoon as affected by growth conditions and the aquatic environment. *J. Food Sci*., **67**, 542–546.

Owusu-Ansah, Y. J. & Hultin, H. O. (1986). Chemical and physical changes in red hake fillets during frozen storage. *J. Food Sci*., **51**, 1402-1406.

Oyelese, O. A. (2007). Drip Loss Measurements, Organoleptic Assessment And Filleting Characteristics Of The Silver Catfish *Chrysichthys nigrodigitatus* Under Low Storage Temperature Conditions. *Journal of Food Processing and Preservation*, **31**(4), 469-479.

Özogul, Y., Özyurt, G., Özogul, F., Kuley, E. & Polat, A. (2005). Freshness assessment of European eel (*Anguilla anguilla*) by sensory, chemical and microbiological methods. *Food Chemistry*, **92**(4), 745-751.

Pacheco‐aguilar, R., Lugo‐sánchez, M. E. & Robles‐burgueño, M. R. (2000). Postmortem Biochemical and Functional Characteristic of Monterey Sardine Muscle Stored at 0 °C. *Journal of Food Science*, **65**(1), 40-47.

Palmer, P. J., Burke, M. J., Palmer, C. J. & Burke, J. B. (2007). Developments in controlled green-water larval culture technologies for estuarine fishes in Queensland, Australia and elsewhere. *Aquaculture*, **272**(1–4), 1-21.

Papa, I., Alvarez, C., Verrez-Bagnis, V., Fleurence, J. & Benyamin, Y. (1996). Post mortem release of fish white muscle α-actinin as a marker of disorganisation. *Journal of the Science of Food and Agriculture*, **72**(1), 63-70.

Papa, I., Astier, R. G., Ventre, C., Lebart, F., Roustan, M. C., Benyamin, C., Papa, A., Taylor, Y., Benyamin, Y. & Ouali, Y. (1997). Dystrophin cleavage and sarcolemma detachment are early post mortem changes on sea bass (Dicentrachus labrax) white muscle. *Journal of Food Science*, **62** (5), 917–921.

Papadopoulou, O. S., Panagou, E. Z., Mohareb, F. R. & Nychas, G. J. E. (2013). Sensory and microbiological quality assessment of beef fillets using a portable electronic nose in tandem with support vector machine analysis. *Food Research International*, **50**(1), 241-249.

Paredi, M. E., & Crupkin, M. (2007). Biochemical and physicochemical properties of actomyosin from pre- and post-spawned flounder (*Paralichtys patagonicus*) stored on ice. *LWT - Food Science and Technology*, **40**(10), 1716-1722.

Parsons, C. A. (1995). Modified atmosphere packaging technology: a review*, J. Sci. Food Agr*., **67**, 5113–5121.

Pastoriza, L., & Sampedro, G. (1994). Influence of ice storage on ray (*Raja clavata*) wing muscle. *Journal of the Science of Food and Agriculture*, **64**(1), 9-18.

Pearce, K. L., Rosenvold, K., Andersen, H. J., & Hopkins, D. L. (2011). Water distribution and mobility in meat during the conversion of muscle to meat and ageing and the impacts on fresh meat quality attributes - A review. *Meat Science*, **89**(2), 111-124.

Pearson, A. B., & Lamb, R. A. (2001). Developments in refrigeration technology for spiral and blast freezers. *Proceedings of the International Institute of Refrigeration – Rapid Cooling of Food. Section*., **2**, 153–161.

Pearson, A., Love, J. & Shorland, F. (1977). Warmed-over Flavour in Meat Poultry and Fish. *Adv. Food Res.*, **23**, 2-74.

Pearson, D. (1981). *Chemical Analysis of Food.* 8th edn. Edinburgh, London, M'elborne and New York.

Penfield, M. A. & Campbell, A. M. (1990). *Evaluating food by objective methods.* In: *Experimental Food Science*, 3rd edn. Pp. 23–50, San Diego, CA: Academic Press.

Pennacchia, C., Ercolini, D. & Villani, F. (2011). Spoilage-related microbiota associated with chilled beef stored in air or vacuum pack. *Food Microbiology*, **28**(1), 84-93.

Periago, M. J., Rodrigo, J., Ros, G., Rodriguez-Jerez, J. J., & Hernandez- Herrero, M. (2003). Monitoring volatile and nonvolatile amines in dried and salted roes of tuna (*Thunnus thynnus* l.) during manufacture and storage. *Journal of Food Protection*, **66**, 335–340.

Phillips, M. (2010). The Australian Barramundi Industry Addressing Cheap Imports, A report for Nuffield Australia Farming Scholars, October 2010, Nuffield Australia Project No 1001.

Pigott, G. & Tucker, B. (1987). Science opens new horizons for marine lipids in human nutrition. *Food Reviews International*, **3**(1-2), 105-138.

Pigott, G. M. & Tucker, B. W. (1990). Seafood effects of technology on nutrition. Pp. 359. New York: Marcel Dekker, Inc.

Piñeiro, C., Barros-Velázquez, J. & Aubourg, S. P. (2004). Effects of newer slurry ice systems on the quality of aquatic food products: a comparative review versus flake-ice chilling methods. *Trends in Food Science & Technology*, **15**(12), 575-582.

Pineiro, C., Vazquez, J., Perez-Diz, A., Gallardo, J. M. & Auborg, S. P. (2003). Chemical changes related to quality loss during farmed turbot chilling by applying flow and traditional icing. In: *Proceedings of the first joint trans-Atlantic fisheries technology conference. TAFT 2003. 33rd WEFTA and 48th AFTC meetings*. (Conference proceeding). Pp. 71–72. Reykjavik,Iceland.

Platter, W., Tatum, J. D., Belk, K., Chapman, P., Scanga, J. & Smith, G. (2003). Relationships of consumer sensory ratings, marbling score, and shear force value to consumer acceptance of beef strip loin steaks. *J. Anim. Sci*., **81**(11), 2741-2750.

Ponce de Leo´n, S., Inoue, N. & Shinano, H. (1993). Effect of aceticand citric acids on the growth and activity (VB-N) of *Pseumonas* sp. and *Moraxella* sp. *Bulletin of the Faculty of Fisheries of Hokkaido University*., **44**, 80–85.

Pons-sánchez-cascado, S., Veciana-nogués, M. T., Bover-cid, S., Mariné-font, A. & Vidal-carou, M. C. (2006). Use of volatile and non-volatile amines to evaluate the freshness of anchovies stored in ice. *Journal of the Science of Food and Agriculture*, **86**(5), 699-705.

Price, R., Melvin, E. & Bell, J. (1991). Postmortem changes in chilled round, bled and dressed albacore. *Journal of Food Science*, **56**(2), 318-321.

Quaranta, H. O. & Pérez, S. S. (1983). Chemical methods for measuring changes in freeze stored fish: A review. *Food Chemistry*, **11**(2), 79-85.

Queensland government. (2014). *Barramundi aquaculture*. Available from: <https://www.business.qld.gov.au/industry/fisheries/aquaculture/aquaculture-species/barramundi-aquaculture>. [11th May 2014].

Rahman, M. S. & Al-Farsi, S. A. (2005). Instrumental texture profile analysis (TPA) of date flesh as a function of moisture content. *J. Food Eng*., **66**(4), 505-511.

Raju, C.V., Dhananjaya, S. & Reddy, G.V.S. (1999). Preparation and cold storage studies on ready-to-fry sticks from pink perch (*Nemipterus japonicus*). *Journal of Aquatic Biology*, **14**, 111-114.

Ramiarez, J. A., Martian‐polo, M. O. & Bandman, E. (2000). Fish Myosin Aggregation as Affected by Freezing and Initial Physical State. *Journal of Food Science*, **65**(4), 556-560.

Reddy, N. R., Villanueva, M. & Kautter, D. A. (1995). Shelf Life of Modified-Atmosphere-Packaged Fresh Tilapia Fillets Stored under Refrigeration and Temperature-Abuse Conditions. *Journal of Food Protection*, **58**(8), 908-914.

Refsgaard, H. H. F., Jensen, B. & Brockhoff, P. B. (1998). Sensory and Chemical Changes in Farmed Atlantic Salmon (*Salmo solar*) during Frozen Storage. *Journal of Agricultural and Food Chemistry*, **46**(9), 3473-3479.

Rehbein, H. & Karl, H. (1985). Solubilization of fish muscle proteins with buffers containing sodium dodecyl sulphate. *Z. Lebensm. Unters. Forsch*., **180**, 373-378.

Rezaei, M., Hosseini, S. F., Langrudi, H. E., Safari, R. & Hosseini, S.V. (2008). Effect of delayed icing on quality changes of iced rainbow trout (*Oncorynchus mykiss*). *Food Chemistry*, **160**, 1161–1165.

Richards, M. P., Modra, A. & Li, R. (2002). Role of deoxyhemoglobin in lipid oxidation of washed cod muscle mediated by trout, poultry and beef hemoglobins. *Meat Sci.,* **62**(2), 157-163.

Robb, D., Kestin, S. & Warriss, P. (2000). Muscle activity at slaughter: I. Changes in flesh colour and gaping in rainbow trout. *Aquaculture*, **182**(3), 261-269.

Rodríguez, Ó., Barros-Velázquez, J., Piñeiro, C., Gallardo, J. M. & Aubourg, S. P. (2006). Effects of storage in slurry ice on the microbial, chemical and sensory quality and on the shelf life of farmed turbot (*Psetta maxima*). *Food Chemistry*, **95**(2), 270-278.

Rodriguez, O., Barros-Velazquez, J., Ojea, A., Pineiro, C., Gallardo, J. M. & Aubourg, S., (2003). Effect of chilled storage in flow ice on the microbiological quality and shelf life of farmed turbot (*Psetta maxima*). Isolation and identification of major proteolytic bacteria. In: *Proceedings of the first joint trans-Atlantic fisheries technology conference. TAFT 2003, 33rd WEFTA and 48th AFTC meetings*, (Conference proceeding). Pp.73–74. Reykjavik, Iceland.

Rodríguez, Ó., Losada, V., Aubourg, S. P. & Barros-Velázquez, J. (2004). Enhanced shelf-life of chilled European hake (*Merluccius merluccius*) stored in slurry ice as determined by sensory analysis and assessment of microbiological activity. *Food Research International*, **37**(8), 749-757.

Rodrıguez, O., Losada, V., Aubourg, S. & Barros-Vela´zquez, J. (2005). Sensory, microbial and chemical effects of a slurry ice system on horse mackerel (*Trachurus trachurus*). *Journal of the Science of Food and Agriculture*, **85**, 235–242.

Rørå, A. M. B. & Einen, O. (2003). Effects of Freezing on Quality of Cold-smoked Salmon Based on the Measurements of Physiochemical Characteristics. Journal of Food Science, 68(6), 2123-2128.

Roth, B., Foss, A. & Imsland, A. K. (2009). Relationship between Muscle pH and Flesh Color of Atlantic Halibut. *Journal of Food Science*, **74**(3), S123-S125.

Rouillé, J., Lebail, A., Ramaswamy, H. S. & Leclerc, L. (2002). High pressure thawing of fish and shellfish. *Journal of Food Engineering*, **53**(1), 83-88.

Ruiz-Capillas, C. & Moral, A. (2001). Correlation between biochemical and sensory quality indices in hake stored in ice. *Food Research International*, **34**(5), 441-447.

Ruiz de Huidobro, F., Onega, E., Caneque, V., Lauzurica, S., Velasco, S., & Ptrez, C. (2001). Sensory characterization of meat texture in sucking lambs. Methodology. *Investigacion Agraria: Produccion y Sanidad Animales*, **16**(2), 245-256.

Russell, D. J. & Garrett, R. N. (1985). Early life history of Barramundi, *Lates calcarifer* (Broch), in North-eastern Queensland. *Australian Journal of Marine and Freshwater Research*, **36**(2), 191-201.

Saeed, S. & Howell, N. K. (1999). High performance liquid chromatography (HPLC) and spectroscopic studies on fish oil oxidation products extracted from frozen Atlantic mackerel. *JAOCS*, **76**, 391-397.

Saeed, S. & Howell, N. K. (2002). Effect of lipid oxidation and frozen storage on muscle proteins of Atlantic mackerel (*Scomber scombrus*). *Journal of the Science of Food Agriculture*, 82(5), 579-586.

Safari, E., Fogarty, N. M., Ferrier, G. R., Hopkins, D. L. & Gilmour, A. R. (2001). Diverse lamb genotypes. 3. Eating quality and the relationship between its objective measurement and sensory assessment. *Meat Science*, **57**(2), 153–159.

Sallam, K. I. (2007). Antimicrobial and antioxidant effects of sodium acetate, sodium lactate, and sodium citrate in refrigerated sliced salmon. *J. Food Control*., **18**, 566–575.

Sankar, T. & Viswanathan Nair, P. (1988). Effect of pre-processing iced storage on deteriorative changes in lipids of silver pomfret stored at -18oC. *Fishery Technology*, **25**, 100-104.

Sathivel, S. (2005). Chitosan and Protein Coatings Affect Yield, Moisture Loss, and Lipid Oxidation of Pink Salmon (*Oncorhynchus gorbuscha*) Fillets During Frozen Storage. *Journal of Food Science*, **70**(8), e455-e459.

Sathivel, S., Liu, Q., Huang, J. & Prinyawiwatkul, W. (2007). The influence of chitosan glazing on the quality of skinless pink salmon (*Oncorhynchus gorbuscha*) fillets during frozen storage. *Journal of Food Engineering*, **83**(3), 366-373.

Sato, K., Ohashi, C., Ohtsuki, K. & Kawabata, M. (1991). Type v collagen in trout (*Salmo gairdneri*) muscle and its solubility change during chilled storage of muscle. *Journal of Agricultural and Food Chemistry*, **39**(7), 1222-1225.

Scheurer, P. G. (1968). Penetration Gradients of Sodium Nitrite and Sodium Tripolyphosphate in Haddock Fillets. *Journal of Food Science*, **33**(5), 504-506.

Schnee, R. (2000). Budenheim Phosphates for Seafood Processing. *Chemische Fabrik Budenheim*, **11**.

Schubring, R. (1999). DSC studies on deep frozen fishery products. *Thermochimica Acta*, **337**(1-2), 89-95.

Schubring, R. (2002). Texture measurement on gutted cod during storage in ice using a hand held instrument. *Inf. Fischwirtsch. Fischereiforsch*., **49**, 25–27.

Schubring, R., Meyer, C., Schluter, O., Boguslawski, S. & Knorr, D. (2003). Impact of high pressure assisted thawing on the quality of fillets from various fish species. *Innov. Food Sci. Emerg. Technol*, **4**, 257–267.

SeaFood CRC. (2014). *Barramundi.* Available from: <http://seafoodcrctraining.com.au/for-schools/species-information/barramundi/>. [29th July 2014].

Seo, H., Endo, Y., Fujimoto, K., Moku, M. & Kawaguchi, K. (1997). Denaturation of myofibrillar protein in myoctophid fish during refrigeration and freezing storage. *Fisheries Science*, **63**, 839–840.

Shackelford, S. D., Wheeler, T. & Koohmaraie, M. (1995). Relationship Between Shear Force And Trained Sensory Panel Tenderness Ratings Of 10 Major Muscles From Bos-Indicus And Bos-Taurus Cattle. *J. Anim. Sci*., **73**(11), 3333-3340.

Shackelford, S. D., Wheeler, T. L. & Koohmaraie, M. (1999). Evaluation of slice shear force as an objective method of assessing beef longissimus tenderness. *Journal of Animal Science*, **77**(10), 2693–2699.

Shahidi, F., Chong, X. & Dunajski, E. (1994). Freshness quality of harp seal (*Phoca groenlandica*) meat. *Journal of Agriculture and Food Chemistry*, **42**(4), 868–872.

Shearer, K. D. (1994). Factors affecting the proximate composition of cultured fishes with emphasis on salmonids. *Aquaculture*, **119**(1), 63-88.

Sheehan, E., O'Connor, T. P., Sheehy, P., Buckley, D. & Fitzgerald, R. (1998). Stability of astaxanthin and canthaxanthin in raw and smoked Atlantic salmon (*Salmo salar*) during frozen storage. *Food Chem*., **63**(3), 313-317.

Shenouda, S. Y. K. (1980). Theories of protein denaturation during frozen storage of fish flesh. In: *Advance in food research* (edited by Chichester, C. O.), **26**. Pp. 275. New York: Academic Press.

Shewan, J. M. (1976). The bacteriology of fresh and spoiling fish and biochemical changes induced by bacterial action. In: *Proc. of the Conf. on the handling, processing and marketing of tropical fish. London, Tropical Products Institute*. Pp. 377-94.

Siddique, M. N., Hasan, M. J., Reza, M. Z., Islam, M. R., Boduruzaman, M., Forhadur, M. & Reza, S. (2011). Effect of freezing time on nutritional value of Jatpunti (*Puntius sophore*), Sarpunti (*P. sarana*) and Thaisarpunti (*P. gonionotus*). Bangladesh *Research Publications Journal*, **5**(4), 387-392.

Sigholt, T., Erikson, U., Rustad, T., Johansen, S., Nordtvedt, T. S. & Seland, A. (1997). Handling Stress and Storage Temperature Affect Meat Quality of Farmed-raised Atlantic Salmon (*Salmo Salar*). *Journal of Food Science*, **62**(4), 898-905.

Sigurgisladottir, S., Ingvarsdottir, H., Torrissen, O. J., Cardinal, M., & Hafsteinsson, H. (2000). Effects of freezing/thawing on the microstructure and the texture of smoked Atlantic salmon (*Salmo salar*). *Food Research International,* **33**(10), 857-865.

Sigurgisladottir, S., Hafsteinsson, H., Jonsson, A., Lie, Ø., Nortvedt, R., Thomassen, M. & Torrissen, O. (1999). Textural Properties of Raw Salmon Fillets as Related to Sampling Method. *Journal of Food Science*, **64**(1), 99-104.

Sigurgisladottir, S., Sigurdardottir, M. S., Ingvarsdottir, H., Torrissen, O., & Hafsteinsson, H. (2001). Microstructure and texture of fresh and smoked Atlantic salmon, *Salmo salar* L., fillets from fish reared and slaughtered under different conditions. *Aquac. Res.,* **32**(1), 1-10.

Sikorski, Z. E. & Kolakowska, A. (1994). Changes in proteins in frozen stored fish. In: *Seafood Proteins* (edited by Sikorski, Z. E., Sun Pan, B. & Shahidi, F.). Pp. 99-231. New York: Chapman and Hall.

Sikorski, Z. E. (1978). Protein changes in muscle foods due to freezing and frozen storage. *International Journal of Refrigeration,* **1**(3), 173-180.

Sikorski, Z. E., Olley, J. & Kostuch, S. (1976). Protein changes in frozen fish. *Crit. Rev. Food Sci. Nutr.*, **8**(1), 97-129.

Sikorski, Z. E. & Kolakowska, A. (1994). Changes in proteins in frozen stored fish. In: *Seafood proteins* (edited by Sikorski, Z. E., Sun Pan, B. & Shahidi, F.). Pp. 99-112. UK, London: Chapman & Hall.

Sikorski, Z. E. & Sun Pan, B. (1992). Preservation of seafood quality. In: *Seafoods: Chemistry, Processing Technology and Quality* (edited by Shahidi, F. & Botta, J. R.). Pp. 168– 195. London: Blackie Academic Press.

Simeonidou, S., Govaris, A. & Vareltzis, K. (1998). Quality assessment of seven Mediterranean fish species during storage on ice. *Food Research International*, **30**(7), 479-484.

Simeonidou, S., Govaris, A. & Vareltzis, K. (1997). Effect of frozen storage on the quality of whole fish and fillets of horse mackerel (*Trachurus trachurus*) and mediterranean hake (*Merluccius mediterraneus*). *Zeitschrift für Lebensmitteluntersuchung und - Forschung A*, **204**(6), 405-410.

Simpson, R., Almonacid, S., Nuñez, H., Pinto, M., Abakarov, A. & Teixeira, A. (2012). Time-Temperature Indicator To Monitor Cold Chain Distribution Of Fresh Salmon (*Salmo Salar*). *Journal of Food Process Engineering*, **35**(5), 742-750.

Singh, R. K. & Balange, A. K. (2005). Characteristics of Pink Perch (*Nemipterus Japonicus*) Surimi at Frozen Temperature. *Journal of food processing and preservation*, **29**(1), 75-83.

Sini, T. K., Santhosh, S., Joseph, A. C. & Ravisankar, C. N. (2008). Changes In The Characteristics Of Rohu Fish (*Labeo Rohita*) Sausage During Storage At Different Temperatures. *Journal of Food Processing and Preservation*, **32**(3), 429-442.

Skandamis, P. N. & Nychas, G. J. E. (2002). Preservation of fresh meat with active and modified atmosphere packaging conditions. *International Journal of Food Microbiology*, **79**, 35–43.

Skirtun, M., Sahlqvist, P. & Vieira, S. (2013). Australian fisheries statistics 2012. FRDC project 2010/208. ABARES, Canberra, November. CC BY 3.0.

Skjervold, P. O., Røra˚, A. M. B., Fjæra, S. O., Synstad, A. V., Vorre, A. & Einen, O. (2001). Pre-rigor filleting of live chilled salmon improves final product quality. *Aquaculture*, **194**, 315–326.

Skrede, G. & Storebakken, T. (1986). Characteristics of color in raw, baked and smoked wild and pen-reared Atlantic salmon. *Journal of Food Science*, **51**(3), 804-808.

Slabyj, B. M. & True, R. H. (1978). Effect of preprocess holding on the quality of canned maine sardines. *Effect of preprocess holding on the quality of canned maine sardines*., **43**(4), 1172-1176.

Smith, J. G. M., McGill, A. S., Thomson, A. B. & Hardy, R. (1980). Preliminary investigation into the chill and frozen storage characteristics of scad (*Trachurus trachurus*) and its acceptability for human consumption. In: *Advances in Fish Science and Technology* (edited by Connell, J.). Pp. 303-307. Farnham, Surrey, U.K.: Fishing News Books.

Smith, D. P. & Young, L. L. (2007). Marination Pressure and Phosphate Effects on Broiler Breast Fillet Yield, Tenderness, and Color. *Marination Pressure and Phosphate Effects on Broiler Breast Fillet Yield, Tenderness, and Color*, **86**(12), 2666-2670.

Sofos, J. N. (1986). Use of phosphates in low-sodium meat products. *Food technology (USA)*, **40**(9).

Soottawat, B. & Friedrich, B. (2001). Biochemical and physicochemical changes in catfish (*Silurus glanis* Linne) muscle as influenced by different freeze–thaw cycles. *Food Chemistry*, **72**, 207–217.

Sousa, M. B., Canet, W., Alvarez, M. D. & Tortosa, M. E. (2005). The effect of the pre-treatments and the long and short-term frozen storage on the quality of raspberry (cv. Heritage). *European Food Research and Technology*, **221**, 132–144.

Spooncer, W. F., Smith, D. R., & Powell, V.H. (n.d.) The Effect Of Chilling & Freezing On Meat Quality, CSIRO Meat Research Laboratory.

Sriket, P., Benjakul, S., Visessanguan, W. & Kijroongrojana, K. (2007). Comparative studies on the effect of the freeze-thawing process on the physicochemical properties and microstructures of black tiger shrimp (*Penaeus monodon*) and white shrimp (*Penaeus vannamei*) muscle. *Food Chemistry*, **104**(1), 113-121.

Srinivasan, S., Xiong, Y. L. & Blanchard, S. P. (1997). Effects of freezing and thawing methods and storage time on thermal properties of freshwater prawns (*Macrobrachium rosenbergii*). *Journal of the Science of Food and Agriculture*, **75**(1), 37-44.

Stien, L. H., Amundsen, A. H., Mørkøre, T., Økland, S. N. & Nortvedt, R. (2006). Instrumental colour analysis of Atlantic salmon (*Salmar salar* L.) muscle. In: *Seafood research from fish to dish—quality, safety and processing of wild and farmed fish* (edited by Luten, J. B., Jacobsen, C., Bekaert, K., Sæbø, A. & Oehlenschlager, J.). Pp. 525–39. Wageningen: Acad. Publishers.

Stien, L. H., Hirmas, E., Bjørnevik, M., Karlsen, Ø., Nortvedt, R., Rørå, A. M. B., Sunde, J. & Kiessling, A. (2005). The effects of stress and storage temperature on the colour and texture of pre-rigor filleted farmed cod (*Gadus morhua* L.). *Aquaculture Research*, **36**(12), 1197-1206.

Suárez, M. D., Abad, M. M., Ruiz-Cara, T., Estrada, J. D. & García-Gallego, M. (2005). Changes in muscle collagen content during post-mortem storage of farmed sea bream (*Sparus aurata*), influence on textural properties. *Aquacult. Int*., **13**, 315–325.

Synnes, M., Larssen, W. E. & Kjerstad, M. (2007). Chemical characterization and properties of five deep-sea fish species. *LWT – Food Sci. Technol*., **40**, 1049–1055.

Szczesniak, A. S. (1963). Classification of Textural Characteristics a. *Journal of Food Science*, **28**(4), 385-389.

Tan, F. L. & Fok, S.C. (2009). Freezing of tilapia fillets in an air blast freezer. *International Journal of Food Science & Technology*, **44(**8), 1619-1625.

Tanikawa, E., Akiba, M. & Shitamori, A. (1963). Cold storage of cod fillets treated with polyphosphates. *Food Tech*., **17**(11), 1425.

Taylor, R. G., Geesink, G. H., Thompson,V. F., Koohmaraie, M. & Goll, D. E. (1995). Is Z-disk degradation responsible for post-mortem tenderization? *J. Anim. Sci*. **73**, 1351–1367.

Taylor, R. G., Fjaera, S. O. & Skjervold, P. O. (2002). Salmon fillet texture is determined by myofiber–myofiber and myofiber–myocommata attachment. *Food Chem. Toxicol*., **67**, 2067–2071.

Tejada, M., Careche, M., Torrejo´n, P., Del Mazo, M. L., Solas, M. T., Garcı´a, M. L. & Barba, C. (1996). Protein extracts and aggregates forming in minced cod (*Gadus morhua*) during frozen storage. *Journal of Agricultural and Food Chemistry*, **44**(10), 3308-3314.

Tenhet, V., Finne, G., Ii, R. N. & Toloday, D. (1981). Penetration of sodium tripolyphosphate into fresh and prefrozen peeled and deveined shrimp. *Journal of Food Science*, **46**(2), 344-349.

Teruel, S. R. L. & Simpson, B. K. (1995). Characterization of the collagenolytic enzyme fraction from winter flounder (*Pseudopleuronectes americanus). Comparative Biochemistry and Physiology, Part B*, **112**(1)*,* 131–136.

Tewari, G., Jayas, D. S. & Holley, R. A. (1999). Centralized packaging of retail meat cuts: A review. *Journal of Food Protection*, **62**, 418–425.

Thanonkaew, A., Benjakul, S., Visessanguan, W. & Decker, E. A. (2006). The effect of metal ions on lipid oxidation, colour and physicochemical properties of cuttlefish (*Sepia pharaonis*) subjected to multiple freeze-thaw cycles. *Food Chemistry*, **95**(4), 591-599.

Thorarinsdottir, K. A., Gudmundsdottir, G., Arason, S., Thorkelsson, G. & Kristbergsson, K. (2004). Effects of Added Salt, Phosphates, and Proteins on the Chemical and Physicochemical Characteristics of Frozen Cod (*Gadus morhua*) Fillets. *Journal of Food Science*, **69**(4), E144-E152.

Thyholt, K. & Isaksson, T. (1997). Differentiation of Frozen and Unfrozen Beef Using Near-Infrared Spectroscopy. *Journal of the Science of Food and Agriculture*, **73**(4), 525-532.

Tims, M. J. & Watts, B. M. (1958). Protection of cooked meats with phosphates. *Food Technol*., **12**, 240.

Toldrá, F. (2003). Muscle foods: water, structure and functionality. *Food Science and Technology International*, **9**, 173-177.

Tolstorebrov, I., Eikevik, T. M. & Indergård, E. (2014). The influence of long-term storage, temperature and type of packaging materials on the lipid oxidation and flesh color of frozen Atlantic herring fillets (*Clupea harengus*). *International Journal of Refrigeration*, **40**, 122-130.

Trout, G. & Schmidt, G. (1984). Effect of phosphate type and concentration, salt level and method of preparation on binding in restructured beef rolls. *Journal of Food Science*, **49**, 687–694.

Tsigarida, E. & Nychas, G. J. E. (2001). Ecophysiological attributes of a *Lactobacillus* sp. and a *Pseudomonas* sp. on sterile beef fillets in relation to storage temperature and film permeability. *Journal of Applied Microbiology*, **90**, 696–705.

Tsuchiya, H., Kita, S. & Seki, N. (1992). Postmortem changes in α-actinin and connectin in carp and rainbow trout muscles. *Nippon Suisan Gakkaishi*., **58**, 793-798.

Tucker, J., Russell, D. & Rimmer, M. (2002). Barramundi culture: a success story for aquaculture in Asia and Australia. *World Aquaculture-Baton Rouge*, **33**(3), 53-59.

Turan, H., Kaya, Y. & Erkoyuncu, I. (2003). Effects of glazing, packaging and phosphate treatments on drip loss in rainbow trout (*Oncorhynchus mykiss*) during frozen storage. *Turkish Journal of Fisheries and Aquatic Sciences*., **3**, 105-109.

Turhan, S., Sule Ustun, N. & Bank, I. (2006). Effect of freeze–thaw cycles on total and heme iron contents of bonito (*Sarda sarda*) and bluefish (*Pomatomus saltator*) fillets. *Journal of Food Composition and Analysis*, **19**(4), 384-387.

Ünal, S. B., Erdoğdu, F. & Ekiz, H. İ. (2006). Effect of temperature on phosphate diffusion in meats. *Journal of food engineering*, **76**(2), 119-127.

Undeland, I., Hall, G. & Lingnert, H. (1999). Lipid oxidation in fillets of herring (*Clupea harengus*) during ice storage**.** Influence of Prefreezing Storage. *J. Agric. Food Chem*., **47**(2), 2075-2081.

Valentas, K. J., Rotstein, E. & Singh R. P. (1997). *Handbook of Food Engineering Practice*. CRC Press.

Van Laack, R. L. J. M. (1994). Spoilage and preservation of muscle foods. In: *Muscle Foods* (edited by Kinsman, D. M., Kotula, A. W. & Breidenstein, B. C.). Pp. 379–405. New York: Chapman & Hall.

Vanhaecke, L., De Brabander, H. F. & Verbeke, W. (2010). Glazing of frozen fish: Analytical and economic challenges. *Analytica Chimica Acta*, **672**(1-2), 40-44.

Vanitha, M., Dhanapal, K., Sravani, K. & Reddy, G. V. S. (2013). Quality Evaluation Of Value Added Mince Based Roducts From Catla (*Catla Catla*) During Frozen Storage. *International Journal Of Science, Environment And Technology*, **2**(3), 487.

Vareltzis, K., Koufidis, D., Gavrilidou, E. & Vasiliadou, S. (1997). Effectiveness of natural rosemary (*Rosmarinus officinalis*) extract on the stability of filleted and minced fish during frozen storage. *Z. Lebensm.-Unters.-Forsch*., **205**, 93–96.

Veland, J. & Torrissen, O. (1999). The texture of Atlantic salmon (*Salmo salar*) muscle as measured instrumentally using TPA and Warner-Brazler shear test. *J. Sci. Food Agric*., **79**(12), 1737-1746.

Venter, P., Shale, K., Lues, J. F. R. & Buys, E. M. (2006). Microbial Proliferation And Mathematical Indices Of Vacuum-Packed Bovine Meat. *Journal of Food Processing and Preservation*, **30**(4), 433-448

Verma, M. M., Alarcon Rojo, A. D., Ledward, D. A., & Lawrie, R. A. (1985). Effect of frozen storage of minced meats on the quality of sausages prepared from them. *Meat Science*, **12**(3), 125-129.

Verma, J. K., & Srikar, L. N. (1994). Protein and lipid changes in Pink Perch (*Nemipterus japonicus*) mince during frozen storage. *Journal of Food Science and Technology*, **31**(3), 238–240.

Verma, J. K., Srikar, L. N., Sudhakara, N. S. & Sarma, J. (1995). Effects of frozen storage in lipid freshness parameters and some functional properties of oil sardine (*Sardinella longiceps*) mince. *Food Research International*, **28**, 87–90 .

Visciano, P., Campana, G., Annunziata, L., Vergara, A. & Ianieri, A. (2007) Effect Of Storage Temperature On Histamine Formation In *Sardina Pilchardus* And *Engraulis Encrasicolus* After Catch**.** *Journal of Food Biochemistry*, **31**(5), 577-588.

Vogel, B. F., Ng, Y. Y., Hyldig, G., Mohr, M. & Gram, L. (2006). Potassium lactate combined with sodium diacetate can inhibit growth of *Listeria monocytogenes* in

vacuum-packed cold-smoked salmon and has no adverse sensory effects. *J. Food Protect*., **69**, 2134–2142.

Vyncke, W. (1978). Influence of chilling, freezing and thawing on fish quality-recent aspects. In: *Food Quality and Nutrition.Research Priorities for Thermal Processing* (edited by Downey, W. K.). Pp. 325-352. London, U.K.: Applied Science Publishers.

Wagner, J. R. & Anon, M. C. (1985). Effect of freezing rate on the denaturation of myofibrillar proteins. *International Journal of Food Science and Technology*, **20**, 735–744.

Wang, M. J. & Goldstein, V. (2003). Ice slurry: advanced fish chilling and preservation technology. *Am. Fis Soc. Symp*., **38**, 379-386.

Wang, D., Tang, J., Correia, L. R. & Gill, T. A. (1998). Postmortem changes of cultivated Atlantic salmon and their effects on salt uptake. *Journal of Food Science*, **63** (4), 634-637.

Warner, K. F. (1928). Progress report of the mechanical test for tenderness of meat. *Proceedings of the American Society of Animal Production*, **21**, 114.

Wells, J. H. & Singh, R. P. (1989). A Quality-Based Inventory Issue Policy For Perishable Foods. *Journal of Food Processing and Preservation*, 12(4), 271-292.

Wheeler, T. L., Shackelford, S. D., Koohmaraie, L. P., Johnson, M. F., Miller, R. K. & Miller, M. (1997). A Comparison of Warner-Bratzler Shear Force Assessment Within and among Institutions. *Journal of animal science*, 75(9), 2423-2432.

Whittle, K. J. (1997). Opportunities for improving the quality of fisheries products. In: *Seafood from Producer to Consumer, Integrated Approach to Quality* (edited by Luten, J. B., Børresen, T. & Oehlenschla¨ger, J.). In: *Proceedings of the International Seafood Conference on the occasion of the 25th anniversary of the WEFTA*, Netherlands, 13– 16 November, 1995. Pp. 549–560. Amsterdam: Elsevier.

Whittle, K., Hardy, R. & Hobbs, G. (1990). Chilled fish and fishery products, in Chilled Foods. In: *The State of the Art* (edited by Gormley, T.). Pp. 87–116. New York: Elsevier Applied Science.

Wierbicki, E., Kunkle, L. E. & Deatherage, F. E. (1957). Changes in the water holding capacity and cationic shifts during the heating and freezing and thawing of meat as revealed by a simple centrifugal method for measuring shrinkage. *Food Technol*., **11**, 69.

Woyewoda, A. D. & Bligh, E. G. (1986) . Effect of Phosphate Blends on Stability of Cod Fillets in Frozen Storage. *Journal of Food Science*, **51**(4), 932-935.

Xia, X., Kong, B., Liu, Q. & Liu, J. (2009). Physicochemical change and protein oxidation in porcine longissimus dorsi as influenced by different freeze-thaw cycles. *Meat Science*, **83**(2), 239-245.

Xiong, Y. L. (2000). Protein oxidation and implication for muscle food quality. In: *Antioxidants in muscle foods* (edited by Decker, E. A., Faustman, C. L. & Lopez-Bote, C. J.). Pp. 85–111. New York: John Wiley and Sons Inc.

Yin, X., Luo, Y., Fan, H., Wu, H. & Feng, L. (2014). Effect of previous frozen storage on quality changes of grass carp (*Ctenopharyngodon idellus*) fillets during short-term chilled storage. *International Journal of Food Science & Technology*, **49**(6), 1449-1460.

Young, L. L., Buhr, R. & Lyon, C. (1999). Effect of polyphosphate treatment and electrical stimulation on postchill changes in quality of broiler breast meat. *Poultry science*, **78**(2), 267-271.

Young, L. L. (1987). 'Next supermarket revolution' - fresh prepared food. In: *The National Provisioner.* 21 Nov. Pp. 28.

Young, L. L., Lyon, C., Searcy, G. & Wilson, R. (1987). Influence of Sodium Tripolyphosphate and Sodium Chloride on Moisture-Retention and Textural Characteristics of Chicken Breast Meat Patties. *Journal of Food Science*, **52**(3), 571-574.

Yue, G. H., Zhu, Z. Y., Lo, L. C., Wang, C. M., Lin, G., Fenf, F., Pang, H. Y., Li, J., Gong, P., Liu, H. M. Tan, J., Chou, R., Lim, H. & Orban, L. (2009). Genetic variation and population structure of Asian seabass (*Lates calcarifer*) in the Asia-Pacific region. *Aquaculture*, **293**(1–2), 22-28.

Zaharah, S. S. & Singh, Z. (2011). Post-harvest fumigation with nitric oxide at the pre-climacteric and climacteric-rise stages influences ripening and quality in mango fruit. *J. Horticult. Sci. Biotechnol*., **86**(6), 645-653.

Zaitsev, V. I., Kizevetter, L., Laqunov. T., Makarava, T., Minder, L. & Podsevalov, V. (1969). *Fish curing and processing*. Pp. 722. Moscow: MIR Publ.

Zaika, L., Cooke, P. & Dobson, T. (1991). Effect of sodium polyphosphates on ultrastructure of *Listeria monocytogenes.* In: *The 91st general meeting of the American Society*. (Conference proceeding). For microbiology. Pp. 5-9. Dallas, TX.

Zaika, L. L., Scullen, O. J. & Fanelli, J. S. (1997). Growth inhibition of *Listeria monocytogenes* by sodium polyphosphate as affected by polyvalent metal ions. *Journal of Food Science*, **62**, 867–869.

Zakhariya, S. Y., Fotedar, R. & Prangnell, D. (2014). Effects of Refreezing on Microbiological and Physiochemical Properties of Barramundi (*Lates calcarifer*, Bloch) Fillets. *Journal of Food Processing and Preservation*, **38**, 2183–2191.

Zakhariya, S. Y., Fotedar, R. & Prangnell, D. (2015). Effect of Time-Temperature Abuse on Microbiological and Physiochemical Properties of Barramundi (*Lates Calcarifer*, Bloch) Fillets. *Journal of Food Processing and Preservation*, **I**n Press.

Zamir, M., Qasim, R. & Ullah, A. (1998). Changes in physical and chemical constituents of crab meat during storage at refrigerator temperature (7±2°C). *Pak. J. of Pharma. Sci.,* **11**(1), 27-33.

Zhuang, H., Savage, E. M., Smith, D. P. & Berrang, M. E. (2008). Effect of dry-air chilling on Warner–Bratzler shear force and water-holding capacity of broiler breast meat deboned four hours postmortem. *Journal of Food Poultry Science*, **7**(8), 743–748.

Žoldoš, P., Popelka, P., Marcinčák, S., Nagy, J., Mesarčová, L., Pipová, M., Jevinová, P., Nagyová, A. & Mal'a, P. (2011). The effect of glaze on the quality of frozen stored Alaska pollack (*Theragra chalcogramma*) fillets under stable and unstable conditions. *Acta Veterinaria Brno*, **80**(3), 299-304.

Appendix A: LIST OF PUBLICATIONS

- Zakhariya, S.Y., Fotedar R. & Prangnell, D. (2014). Effects Of Refreezing on Microbiological and Physiochemical Properties of Barramundi (*Lates calcarifer*, Bloch) Fillets. Published in Journal of Food Processing and Preservation, DOI: 10.1111/jfpp.12198 (Published).

- Zakhariya, S.Y., Fotedar R. & Prangnell, D. (2014). Effects of Sodium Tripolyphosphate and Various Freeze-Thaw Cycles on Microbiological and Physiochemical Properties of Barramundi (*Lates calcarifer*, Bloch) Fillets, Submitted in International Journal of Food Science and Technology.

- Zakhariya, S.Y., Fotedar R. & Prangnell, D. (2014). Effects of Time-Temperature Abuse on Microbiological and Physiochemical Properties of Barramundi *(Lates calcarifer,* Bloch*)* Fillets. Accepted for publication in Journal of Food Processing and Preservation.

- Zakhariya, S.Y., Fotedar R. & Prangnell, D. (2014). Effect of Two Forms of Ice on Microbiological and Physiochemical Properties of Barramundi *(Lates Calcarifer*, Bloch*)* Fillets. Submitted in Journal of Food Processing and Preservation.

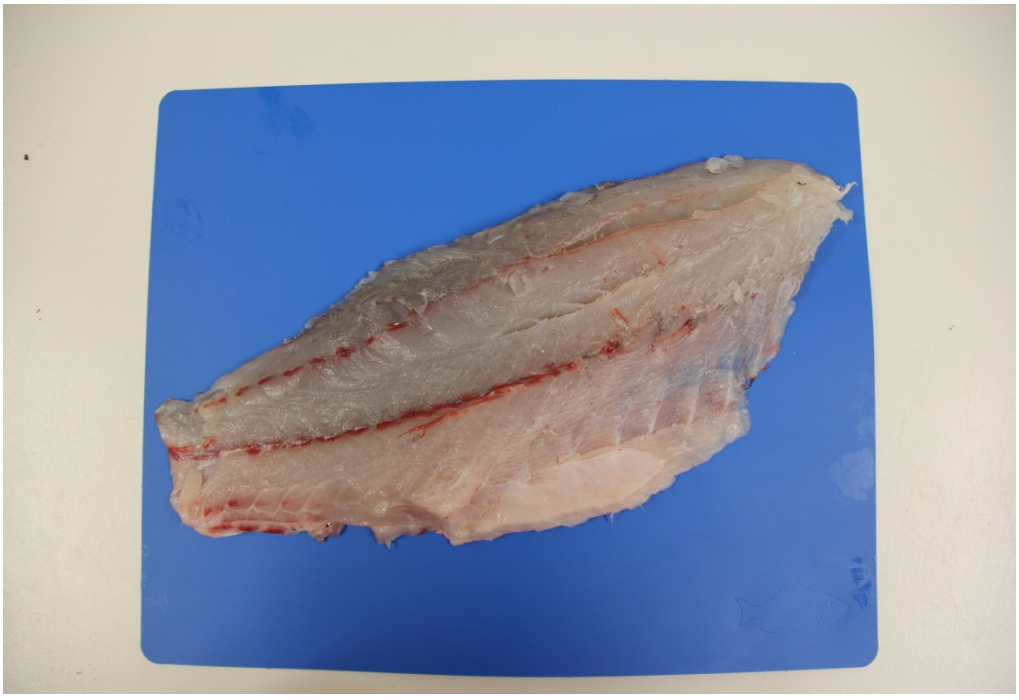
- Zakhariya, S.Y., Fotedar R. and Prangnell, D. (2014). Effect of Glazing, Packaging and Sodium Tripolyphosphate Treatment on Biological and Physiochemical Properties of Barramundi (*Lates Calcarifer,* Bloch) Fillets. (Under Preparation).

Appendix B

Fig. 1: The rheological parameters of barramundi fillets: hardness (firmness), springiness, cohesiveness, gumminess, chewiness and stiffness were measured at the location marked on the fillet.

Shows rheological parameters measured at the location marked

Appendix C

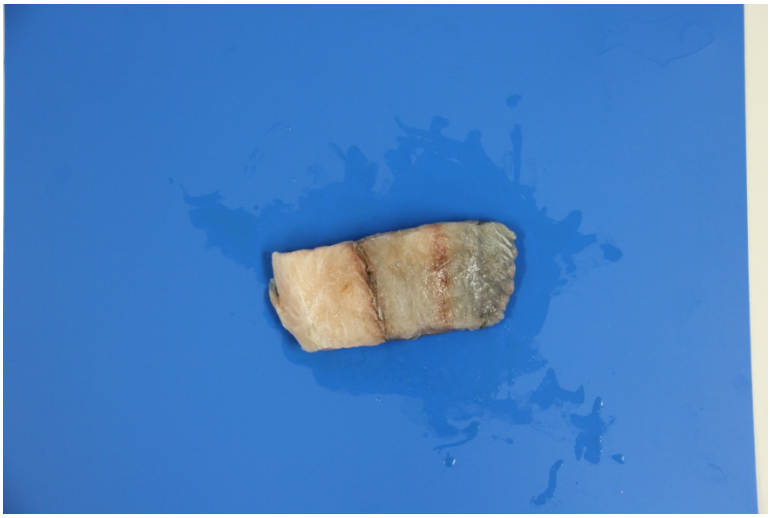


Shows images of a fillet before freezing

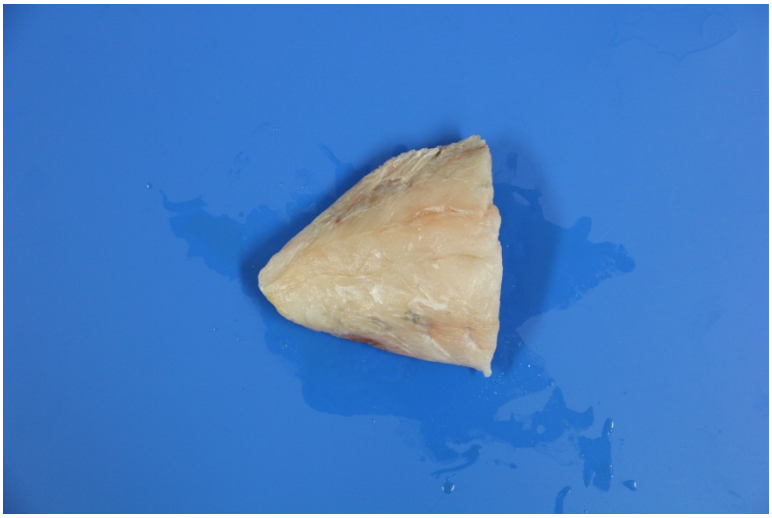
Appendix D

Shows image before and after filleting

Appendix E



A small piece of fillet treated with no chemicals (STPP*)



A piece of fillet treated with chemicals (STPP*)

***STPP-** Sodium Tripolyphosphate

Appendix F

MICROBIOLOGICAL ANALYSIS

TOTAL VIABLE COUNT (TVC)

0.1 ml OF EACH DILUTION PIPETTED ONTO THE SURFACE OF THE PLATE COUNT AGAR

NUMBER OF COLONY FORMING UNITS (C.F.U.) -COUNTED AFTER 48 ± 3 h INCUBATION AT 25°C

MOISTURE CONTENT (%)

OVEN

HOMOGENIZED SAMPLE DETERMINED BY DRYING THE SAMPLE AT 105°C UNTIL A CONSTANT WEIGHT OBTAINED

ASH CONTENT (%)

HEATING THE SAMPLES IN A FURNACE AT 550°C FOR 8–12 h

PROTEIN CONTENT (%)

KJELDAHL MACHINE

FINAL PROTEIN CONTENT IS EXPRESSED ON DRY MATTER BASIS

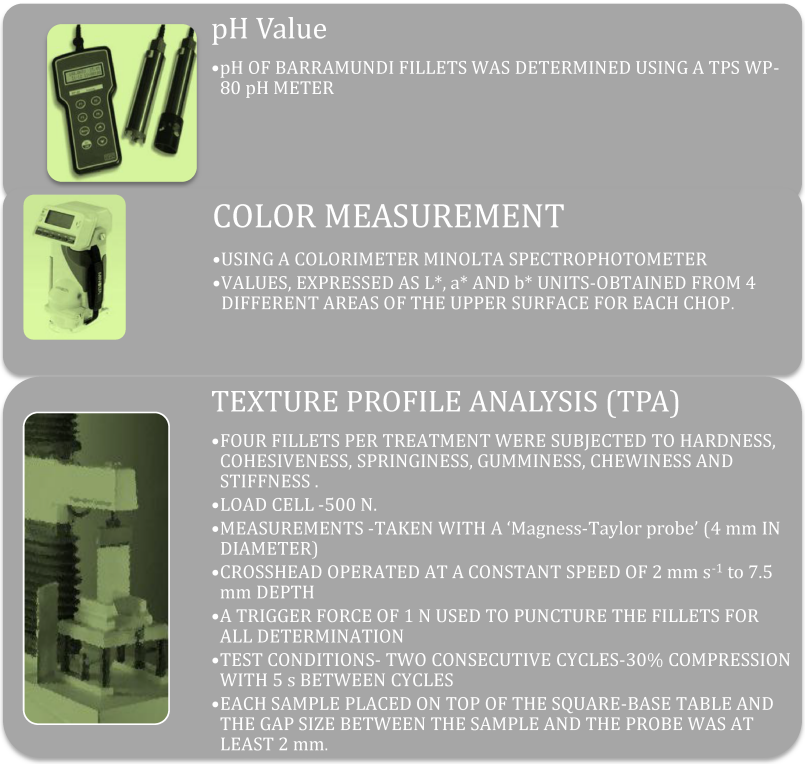
TOTAL VOLATILE BASE NITROGEN (TVBN)

RESULTS WERE EXPRESSED IN mg 100/g OF MUSCLE.

TITRATION WITH 0.1 M SODIUM HYDROXIDE OF A DISTILLATE OF FISH MUSCLE TRITURATE (10 g) IN WATER (300 ML) AND MAGNESIUM OXIDE (2 g).

Equipments used for Analysis (A)

Appendix G



Equipments used for Analysis (B)