Development and application of a best practice supply chain model to 4 species of Australian seafood to define maximum microbial and quality shelflife.

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Abstract

Chilled seafood is considered to have a short quality shelflife when compared to other protein alternatives. Quality deterioration evidenced by high microbial counts, textural degradation and exudates loss affects palatability and subsequently consumer satisfaction and confidence.

Australian produced chilled seafood competes directly for market share with other fresh protein products. Consumer demand, price, repeat purchasing and resultant business viability is based on customer satisfaction achieved at the point of consumption. Barriers to seafood consumption include concerns regarding product origin, freshness and difficulty in evaluating seafood quality. Chilled seafood goes through complex distribution channels before reaching the retail consumer, which can allow quality deterioration to occur. Consequently, one of the most challenging tasks for the seafood industry is controlling product quality and consistency throughout the supply chain.

This research defined best practices for the handling, processing and storage of chilled seafood throughout the supply chain and applied these to 4 Australian species. Flathead (*Platycephalus bassensis*), Atlantic salmon (*Salmo salar*), southern calamari (*Sepioteuthis australis*) and abalone (*Haliotis rubra*) were packed in modified atmospheres comprising primarily CO_2 and in air before storage at temperatures of 0°C and no more than 5°C (maximum regulatory limit).

To date, few published studies specifically related to the species considered in this study or conducted in the Australian context could be located on spoilage rates and quality indicators over time. Consequently, no maximum quality benchmark exists that will enable assessment of supply chain performance. Microbial spoilage and temperature are cited as the most significant factors impacting on seafood shelflife. This study applied the principles of hurdle

technology throughout the supply chain to disrupt microbial growth in order to extend the lag phase and minimise microbial effects on quality shelflife.

The effectiveness of treatments was evaluated by microbial, chemical, textural and sensory parameters from harvest to spoilage. To facilitate comparison between storage temperatures the equivalent days on ice was calculated for each species. The quality shelflife of modified atmosphere packed (MAP) product stored at 0°C was determined as 21 days for flathead, 20 days for Atlantic salmon, 19-20 days for squid and 21 days for abalone. Corresponding microbial total plate counts (TPC) colony forming units per gram (CFU/g) were flathead 1.4 x 10^3 CFU/g, Atlantic salmon 2.9 x 10^4 CFU/g, squid 2.8 x 10^3 CFU/g, abalone 2 x 10^2 CFU/g. It was concluded that microbial spoilage was not the primary factor limiting quality shelflife for any of the species stored in MAP at 0°C. Instead, the primary factors that limited quality shelflife were identified as autolytic spoilage reducing texture, and nucleotide degradation adversely affecting flavour.

The impact on shelflife of storage at temperatures of 0°C and 5°C or less was investigated. The time taken for microbial counts for the 4 species to reach 10^7 CFU/g when air stored at 5°C, or air stored at 0°C or MA packed at 5°C was respectively:

Flathead 9 days: 18 days and 19 to 20 days.

Atlantic salmon: 9 days; 16 days and 17 days.

Southern calamari: 10 days, 17 and 20 days.

Abalone: 10 days, 21 days and 25 days.

Samples held in air at storage temperatures of 5°C or less (mean 4.7°C) had approximately 50% of the microbial shelflife of those stored at 0°C. Samples stored in MAP and held at 0°C

had a shelflife increase of approximately 300% compared to air packed samples stored at 5°C.

This investigation provides new information on spoilage rates, microbial and sensory shelflife and key quality indicators of Flathead, Southern Calamari and Abalone while providing new information on Atlantic salmon in an Australian context. The study also provides practical guidance to industry on best practice applied throughout the supply chain, including harvest, temperature control, cross contamination, processing and packaging. The outcome of this is the establishment of quality benchmarks for each of the species under optimum conditions of MA packed at 0°C. The work conducted on abalone provides industry with key technical information including texture, driploss, microbial and quality shelflife estimations to enable the development of a new chilled MAP packed abalone meat product for the Asian market.

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Abbreviations and Acronyms

ABARE   Australian Bureau of Agriculture and Resource Economics

ATP   adenosine triphosphate

ADP   adenosine diphosphate

AMC   Australian Maritime College

AMP   adenosine monophosphate

ANOVA   analysis of variance

APC   aerobic plate counts

AOAC   association of analytical communities

ASC   acidified sodium chlorite

CFB   *Cytophaga-Flavobacter-Bacteroides*

CFU/g   colony forming units per gram

CO_2 carbon dioxide

CSIRO   Commonwealth Scientific and Industrial Research Organisation

DAD   data acquisition device

Eh   redox potential

EPS   extracellular polymeric substances

G/P   gas product volume ratio

Hx   hypoxanthine

ICMSF   International Commission on Microbiological Specifications for Foods

IMP   inosine monophosphate

Ino   inosine

MA   modified atmosphere

MAP	modified atmosphere packs
O_2	oxygen
NH_3	ammonia
p_d	proportion of distinguishers
pH	acidity
PUFA	polyunsaturated fatty acid
SEM	standard error of the mean
SESSF	south east scalefish and shark fishery
SPSS	statistical package for the social sciences
SSO	specific spoilage organisms
TMA	trimethylamine
TMAO	trimethylamine oxide
TPC	total plate count
TVBN	total volatile base nitrogen
QAC	quaternary ammonium compound

1. Introduction

Consumers make purchase decisions based on the "value proposition", a concept associated with price, perceived quality and related product attributes. In making the purchase decision in relation to seafood the retail customer typically relies on the senses of sight, smell and previous experience to give indications of future eating performance.

The evaluation of quality is subjective. It includes both tangible and intangible aspects, including flavour, texture, odour, appearance, waste, convenience, health perceptions, customer support and ease of preparation. For the product to be successful it must provide the customer with value for money across the full range of tangible and intangible attributes. At some point during chilled storage, the product ceases to represents value at the asking price against alternatives. At this point the products quality shelf life has been exceeded.

In this study quality was defined as an estimated time post harvest after which the product no longer exhibits the necessary edible attributes of the species at the typical retail asking price.

The tangible aspects influencing seafood quality shelflife are complex and are influenced by both extrinsic and intrinsic factors. The factors affecting shelflife judgements are the result of complex interrelationships between microbiological, enzymatic and nucleotide changes occurring pre and post harvest. The rate of change, the nature of the changes and the attributes of the changes are variable. Research has shown that these changes occur in sequence over time but interrelated factors associated with species, extrinsic and intrinsic factors make prediction difficult.

This study investigated the factors affecting chilled seafood shelflife and from results defined best practice models that were applied to the 4 selected species.

1.1   Capture, Harvest and Stress

Studies on the effect of catch method and post hooking soak times in wild fisheries show significant differences in functional measures and shelflife attributable to capture stress (Botta et al., 1987; Cole et al., 2003; Esaiassen et al., 2004; Pankhurst & Sharples, 1992). To date the Australian wild catch sector has made little progress in addressing these capture stress related issues. In contrast the aquaculture sector over the last 15 years has invested substantial research effort and investment into the reduction of harvest stress. The results of this effort have been reductions in the incidence of gaping, enzymatic and nucleotide degradation to maximize shelflife and optimize profitability.

The impacts of stress during harvest on the functional properties of farmed and wild fish post mortem have been widely researched: Atlantic salmon (B. Roth et al., 2009; B. Roth et al., 2006; Sigholt et al., 1997), Cod (Botta et al., 1987; Kristoffersen et al., 2007; G.B. Olsson et al., 2007; Stein et al., 2005), Atlantic halibut (G.B. Olsson et al., 2003), Barramundi (Wilkinson et al., 2008), Haddock (G.B. Olsson et al., 2007), Snapper (Pankhurst & Sharples, 1992), Blue cod (Cole et al., 2003). Other researchers have looked more specifically at the mechanisms of textural degradation post mortem including hoki myocommata (Hallett & Bremner, 1988), cod gaping (Love & Haq, 1970), deterioration of farmed fish connective tissues (Lavety et al., 1988), the role of collagen in fish quality (Sikorski et al., 1984) and post mortem aging in fish (Delbarre-Ladrat et al., 2006). These studies confirmed that the 2 primary extrinsic drivers of post harvest quality were temperature and ante-mortem handling

stress. These extrinsic factors in combination with the intrinsic factors of species, season, size, animal condition have a cumulative effect on enzymes and textural degradation.

The ante mortem catch handling stress affects post mortem sensory quality and the functional properties of seafood. After death, spoilage commences and follows the sequence of rigor mortis and proteolysis, occurring concurrently, followed by microbial degradation (Huss, 1995).

Glycolysis

Stress initiates an endocrine response in fish causing a rapid rise in the animal's metabolic rate. This response results in an increase in plasma levels of catecholamines, adrenaline and noradrenaline (Pankhurst & Sharples, 1992). The effects on the living animal include increased haemoglobin oxygen affinity, increased arterial blood pressure and the release of stored hepatic glycogen to the plasma as glucose (Pankhurst, 2011; Poli et al., 2006; Sigholt et al., 1997).

The biochemical cascade initiated during ante mortem stress utilizes glycogen stored in the muscle and liver. Glycolysis is an anaerobic reaction utilising adenosine triphosphate (ATP), creatine-phosphate (fin fish) and arginine-phosphate (cephalopods), continuing post mortem until all available energy sources are depleted (Sigholt et al., 1997). The result of this process is increased lactic acid and lower muscle pH (Delbarre-Ladrat et al., 2006). The length of time taken to utilize all the stored energy reserves and for the animal to achieve the final pH has an effect on both rigor mortis, proteolysis and in turn quality and functional properties. The key extrinsic mediators to control these biochemical processes are ante mortem stress limitation and reducing temperature post mortem to 0°C as quickly as possible (Huss, 1995; Love & Haq, 1970).

Rigor Mortis

After death the animal progresses through the phases of pre-rigor, rigor mortis and post rigor. These steps are associated with physical changes to the texture of the muscle when the adenosine triphosphate (ATP) concentration decreases below 1-2 μmol/g and the enzyme ATP-ase is activated producing muscle stiffening and rigor mortis (Delbarre-Ladrat et al., 2006; Huss, 1995). Violent rigor contractions associated with stress and high temperatures can result in reduced textural quality due to tearing of the muscle connective tissue and cause gaping to occur (Huss, 1995; Love & Haq, 1970).

In addition, biochemical processes affecting flavour occur in parallel with ATP degrading autolytically to adenosine diphosphate (ADP), adenosine monophosphate (AMP), inosine monophosphate (IMP), inosine (Ino) and hypoxanthine (Hx). An ATP metabolite, Inosine monophosphate (IMP) is the compound associated with desirable fresh fish flavour (Huss, 1995). This compound in turn degrades to inosine (Ino) and hypoxanthine that has been reported as having an undesirable bitter taste or being flavourless (Fletcher et al., 1990; Huss, 1995). When the product reaches this stage it no longer exhibits characteristic flavours of the species and its quality shelflife has been exceeded.

The reduction of capture stress in combination with rapid chilling to 0°C can slow the utilization of adenosine triphosphate (ATP). This positively effects shelflife by slowing onset, increases length, reduces strength and extends the release of rigor mortis (Poli et al., 2006). The longer it takes for the formation of inosine and hypoxanthine to occur, the longer quality shelflife can potentially be maintained. Reducing capture stress and rapid chilling post mortem can also have significant benefits by reducing proteolytic spoilage (Esaiassen et al., 2004; B. Roth et al., 2006) .

Autolysis-Proteolytic Spoilage

While rigor mortis is progressing a parallel autolytic process by enzymes is occurring in the muscle. Essential enzymes in the living animal remain active in the muscle after death. Endogenous proteolytic enzymes degrade muscle proteins, connective tissue and hydrolyse fats (Delbarre-Ladrat et al., 2006) and are located in the intercellular fluids, sarcoplasm or cell organelles (Kolodziejska & Sikorski, 1996).

The proteolytic systems most frequently implicated in chilled fish textural degradation are calpains, cathepsins, and connective tissue hydrolytic enzymes (Delbarre-Ladrat et al., 2006). The combination of the rapid onset of rigor mortis and resultant low post mortem pH can cause lysosome membranes to rupture releasing cathepsins, proteases and other hydrolases (Mukundan et al., 1986). These proteolytic enzymes breakdown the fine collagenous fibrils of the endomysial connective tissue over time and in the most susceptible species including Atlantic salmon and hoki (*Macruronus novaezelandiae*) cause gaping (the detachment of myomeres from one another) (Espe et al., 2004; Hallett & Bremner, 1988; Love & Haq, 1970; Sato et al., 2002). In addition, the lowered pH affects the water-holding capacity of the muscle, resulting in increased exudate loss, which is referred to as driploss (B. Roth et al., 2006). Flesh gaping and driploss are recognized by the industry and consumers as quality defects. Undesirable post mortem flesh quality changes attributable to autolytic spoilage include alteration to flesh colour, textural degradation and gaping, adverse or reduced flavour as well as reduced cohesiveness, elasticity and water holding capacity (Poli et al., 2006).

Temperature and capture stress minimization play important roles in limiting the impact of proteolytic enzymes on quality. For example, the half-life inactivation period for calpain II from tilapia at pH 7.5 and 5.5 is 23 hours and 58 hours respectively at 25°C (Kolodziejska &

Sikorski, 1996). The implications of this are that by keeping pH higher the period in which the enzyme is active will be reduced resulting in less connective tissue damage. Combined with this the more ATP remaining in the muscle post mortem the longer rigor mortis will be delayed and the slower pH reduction will be thereby reducing the potential impact of the enzyme (Huss, 1995). Temperature also plays a vital role in maintaining quality by reducing enzymatic spoilage. At 0°C tilapia calpain II only retains about 20% of the maximum activity at 25°C and pH 7.5 (Kolodziejska & Sikorski, 1996).

The second group of enzymes of concern are the cathepsins. These enzymes are highly active at acid pH and across a broad range of temperatures (-10 to 60°C) (Mukundan et al., 1986). In general cathepsins prefer a pH of less than 5.5, but some remain highly active at pH 6.0-6.5 a range typically found in post mortem seafood. Cathepsin activity can be significantly limited by temperature (Delbarre-Ladrat et al., 2006). Kolodziejska reported that at temperatures of 5°C to 10°C about 15% of cathepsin activity was retained (Kolodziejska & Sikorski, 1996). Again the effects of this group of enzymes on fish quality is limited by minimising capture stress and by reducing post harvest temperature to 0°C as rapidly as possible.

The autolytic process also has a significant role to play in bacterial spoilage by releasing simple sugars, free amino acids and free fatty acids which are ultilised by bacteria (Delbarre-Ladrat et al., 2006). Autolysis also degrades skin and connective tissue making the muscle accessible to bacteria thereby facilitating bacterial spoilage.

In summary, the key harvest and subsequent storage objectives employed in this study were stress minimization by limiting the level and duration of physical activity by euthanasing and by immersing animals immediately in ice slurry post mortem to achieve the maximum

chilling rate to 0°C . The capture/harvest methods employed in this study are representative of best practice methods employed in commercial use.

1.2 Microbial Spoilage

Microorganisms are in the majority of instances the most significant factor in the degradation of chilled seafood quality and associated spoilage (Sivertsvik et al., 2002). The two stages of microbial growth of concern in this study are the lag and logarithmic growth phases.

In the lag phase, microbes adjust to the environment or repair cell damage and numbers do not increase significantly. An underpinning assumption of this study is that by lengthening the lag phase a corresponding increase in shelf life will result. In this study, a number of treatments (hurdles) were applied in order to minimize microbial growth and maximize the lag phase. The benefits of lag phase extension should result in an increase in quality shelf life.

This assumption requires qualification, although bacteria are significant, and in most cases the most significant factor affecting quality shelf life, they are not the only factor. Nucleotide degradation, proteolytic spoilage affecting texture and oxidative rancidity can also affect palatability and limit quality shelflife. The affects of spoilage are not linear and will vary between species due to intrinsic and extrinsic characteristics of the species, individual animals and storage environment (Huss, 1995).

Microbial Spoilage of Seafood in Air

Many of the bacteria found on fish products are gram negative psychrotrophic (Gram & Huss, 1996). These bacteria are capable of growing over a wide temperature range and can grow at temperatures close to or below freezing but have an optimum of 20°C-25°C

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(Hebraud & Potier, 1999; Moyer & Morita, 2007). In temperate waters, these psychrotrophic Gram-negative bacteria species dominate the spoilage flora of fish (H.-C. Chen & Chai, 1982; Chmielewski & Frank, 2003; Huss, 1995; MacCormack & Fraile, 1989; Verran et al., 2008).

Due to high water activity, neutral pH and the presence of autolytic enzymes, seafood is highly perishable when compared to terrestrial mammalian protein sources (Sivertsvik et al., 2002). The susceptibility of finfish to microbial spoilage can be attributed to biochemical composition and the presence of non-protein nitrogenous compounds in the muscle (Botta et al., 1987). These are compounds that can be utilized by bacteria and include free amino acids and volatile nitrogenous bases such as ammonia, trimethylamine oxide, creatine, taurine and others (Huss, 1995). The rate of spoilage is time and temperature dependant with reduced temperatures reducing microbial growth rates (Sivertsvik et al., 2002).

The presence and number of microbes present on or in the flesh can be due to a number of factors. Damage sustained during capture and handling, cross contamination, water quality and time and temperature can all influence the number and species of microbes on freshly caught product. The microbes present have been identified by enumeration techniques as typically of the species *Pseudomonas, Alteromonas, Shewanella, Moraxella, Flavobacterium,* and *Acinetobacter* (Debevere & Boskou, 1996; Gram & Melchiorsen, 1996; Huss, 1995).

Bacteria in cephalopods have been identified as Gram-negative psychrophiles including *Shewanella putrefaciens, Pseudomonas* spp. and *Pseudoalteromonas* spp. (Paarup, Sanchez, Moral, et al., 2002; P. Vaz-Pires et al., 2008). In addition, *Moraxella/Psychrobacter, Vibrio* and *Aeromonas* spp. have also been detected. The production of ammonia (NH_3), a significant spoilage indicator in squid, has been attributed to autolysis over the first 9 days of

storage (Lapa-Guimaraes et al., 2002). The increase in production rate of ammonia past 9 days indicates a bacterial contribution (Paarup, Sanchez, Moral, et al., 2002). The number of microorganisms found in squid at the time of sensory rejection was lower than in fish indicating that autolytic spoilage may be the primary spoilage mechanism in cephalopods (P. Vaz-Pires et al., 2008).

The use of genetic techniques including 16S rDNA-DGGE have identified more diverse microbiota than indicated by traditional enumeration methods (M. B. Hovda et al., 2006; Powell & Tamplin, 2012). A study by Wilson of bacteria found in Atlantic cod slime from the Baltic, Icelandic and North Seas identified a diverse bacterial flora with the dominant species including ϒ-*proteobacteria* and *Cytophaga-Flavobacter-Bacteroides* (CFB). Seasonal variation in bacterial species was also observed in fish from the North Sea (Wilson et al., 2008).

Specific Spoilage Organisms (SSO)

At microbial spoilage, a diverse flora will be present on the seafood (Sivertsvik et al., 2002). Some of the flora is composed of microorganisms that do not contribute to the development of off -odours and flavours (Gram & Huss, 1996). A limited number of species have been identified as being responsible for spoilage in air stored seafood. These species are referred to as Specific Spoilage Organisms (SSO). *Pseudomonas* and *Shewanella putrefaciens* have been identified as the primary spoilage bacteria of marine temperate water fish stored aerobically in ice (Gram & Melchiorsen, 1996). The growth of these species is associated with the production of by-products including biogenic amines, trimethylamine (TMA), and ammonia (NH_3) (Dalgaard, 1995; Emborg et al., 2002) that affect palatability and limit shelflife.

During chilled storage under anaerobic conditions found in modified atmosphere packs without added oxygen, microbial diversity decreases over time. *Shewanella putrefaciens* and *Pseudomonas* spp. are inhibited by dissolved carbon dioxide (Boskou & Debevere, 1997; Dalgaard et al., 1993; Devlieghere & Debevere, 2000; Devlieghere et al., 1998). In MA packs *Photobacterium phosphoreum* has been identified as the SSO responsible for the production of TMA and spoilage (Dalgaard et al., 1997; Emborg et al., 2002; Gram & Huss, 1996).

Temperature and Spoilage

The effect of time and temperature on seafood quality shelflife is cumulative (Huss, 1995). Microbial growth is the result of a sequence of interrelated chemical reactions. Both bacteria and enzymes remain active at typical chill supply chain temperatures. To limit the actions of both bacteria and enzymes temperature needs to be maintained at or close to 0°C until consumption. Bacterial growth rates decline as product temperatures decrease towards 0°C (Gram & Huss, 1996; Huss, 1995). The relationship between spoilage and temperature has been studied extensively (H.A. Bremner et al., 1987; McMeekin & Ross, 1996; Ratkowsky et al., 1982; Sivertsvik et al., 2002). The effects of temperature on growth can be expressed by the Arrhenius equation (Hebraud & Potier, 1999). These and other growth models have identified relationships between bacterial growth rates as being highly temperature dependant with reduced temperatures resulting in reduced growth (Koutsoumanis et al., 2000; Zwietering et al., 1990).

Microbial Growth at Chill Temperatures

The population of organisms on seafood will generally consist of mesophiles, psychrotrophs and psychrophiles (Huss, 1995). The species, numbers and proportion is dependent on a number of factors including the natural environmental flora, water temperature and cross

contamination from other sources throughout the chain (Huis in't Veld, 1996). This in turn means the stress responses to low temperatures of less than 5°C will be different and varied depending on the species breakdown.

Microorganisms have been shown to exhibit a cold shock response when exposed to reduced temperatures. This extends the lag phase while the organism readjusts to the altered conditions. The temperature reduction required to stimulate a cold shock response is organism-specific as is the reaction. To date, no singular molecular determinant of cold temperature adaptation has been identified (Beales, 2004; Berry & Foegeding, 1997). To achieve growth at low temperatures all aspects of the organism's metabolism must be functional. For growth to occur at low temperatures proteins, ribosomes and membranes must be maintained and regulated in order for protein synthesis and nutrient uptake to occur (Berry & Foegeding, 1997). In bacteria, reactions include adaptive changes in solubility, reaction kinetics, membrane fluidity, protein conformation and stability and changes in gene expression (Hebraud & Potier, 1999). In terms of adaptation, the energy requirements for the individual species will be different and may range from having little effect for cold adapted psychrophiles to metabolic exhaustion and death for organisms with a dependence on high temperatures. This selectivity results in reduced species diversity in the product over time.

1.3   Chilling Methods

Seawater ice slurry is the most effective chilling method generally employed in the commercial sector (Huss, 1995; Margeirsson et al., 2010). This method has a number of advantages including 100% contact between the coolant and product. Seawater ice slurry is simple to prepare and easy to monitor. The presence of ice on top of the mix indicates that the slurry is at 0°C. The fish farming sector have developed an optimized system where product

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is transferred to the slurry within 10 seconds of stunning and bleeding. This method of chilling is also commonly used in the wild catch sector with line caught fish.

1.4   Cross-contamination

Cross contamination can occur at any point in the supply chain. In Australian studies, Tamplin identified filleting benches that used trough sinks as being responsible for a 20-fold increase in pink ling total plate counts (TPC) during processing (Tamplin, 2008). While Slattery identified cutting tables as a source of cross contamination due to a buildup over time of fish based material and subsequent bacterial load (S. Slattery et al., 1998). Gillespie observed an increase in the number of bacteria on Queensland fish during handling and processing (Gillespie & Macrae, 1975).

The surfaces of fish bins and cutting boards are continuously exposed to fish-based organic material while in use, and can develop high microbial loads dominated by psychrotrophic gram-negative bacteria (Gram & Huss, 1996). Chen and Chai found bacterial levels in melt water from iced lizard fish, black croakers, cuttlefish and nemipterids stored in plastic bins for 10-15 days, ranged from 2.1 x10^7 to 2.2 x 10^9/ ml. (H.-C. Chen & Chai, 1982).

Damaged or poorly maintained food contact surfaces have been implicated as contributors to cross contamination (Tebbutt et al., 2007). Rough surfaces, including cracks, cuts and scoring, found on fish bins, cutting boards and chiller surfaces provide a surface topography that aids microbial retention (Chmielewski & Frank, 2003; Tang et al., 2011; Verran et al., 2008). Fish-based material becomes embedded in these surface defects. This provides a source of nutrition for microbes and a place of refuge that is not exposed to surface abrasion and is difficult to clean and sanitise (Reynisson, Guobjornsdottir, et al., 2009). This provides a source of viable cells with biotransfer potential to processed fish on the resumption of

processing (Verran et al., 2008). Factors affecting microbial retention include properties associated with the microorganism, the surface and presence of organic or inorganic material (Verran et al., 2008).

In the case of processing facility cutting board temperatures are generally near optimum for psychrotrophs to grow. Tang found the transfer rate of *Campylobacter jejuni* in chicken significantly higher for surface-scored compared to unmarked polyethylene cutting boards (Plate: 1.1) (Tang et al., 2011). Surfaces in good condition provide a less favourable environment to microorganisms due to more severe dehydration when not in use, better exposure to detergents and sanitisers and lower nutrient levels (Verran et al., 2008).

Plate 1.1: Cutting board showing damage and discolouration due to embedded material

The use of running water to clean cutting boards and prevent the build-up of fish based material is widely practiced internationally with studies by Seafish in the UK confirming the advantage of running water in reducing the buildup of microbes during processing (SEAFISH, 1999).

Microbial Communities and Cross Contamination

Food contact surfaces are generally described as being open or closed (Verran et al., 2008). In fish processing plants not many truly closed systems exist. What does occur are hybrid environments that have characteristics of both open and closed systems. These environments are provided by mechanical processing equipment including filleting machines, portion cutters, conveyor belts, skinners and mincers or any surface that is continuously exposed to fish based material and water. If not cleaned regularly or with poor maintenance microbial communities can become established. While the many of these communities are not true biofilms they do produce slime at high microbial numbers. This slime is composed of extracellular polymeric substances (EPS) that may contain polysaccharides, proteins and phospholipids (Chmielewski & Frank, 2003). The EPS provides significant benefits to the cells within the structure by forming a barrier against physical and chemical threats, concentrating nutrients, reducing dehydration and preventing access to biocides. Bacteria in biofilms can be up to 1000 times more resistant to disinfectants than free living forms (Verran et al., 2008). The environments in many Australia processing plants are suited to the potential development of biofilms or microbial communities with high moisture levels and readily available food in the form of fish-based organic material.

1.5 Development of the Optimised Handling Model

Resistance of Bacteria to Quaternary Ammonium Compounds

Quaternary Ammonium Compounds (QAC) were used as the primary facility and equipment sanitiser in this study. They are more stable in the presence of organic matter than chlorine and iodine based sanitisers. They form a bacteriostatic film after application to surfaces

giving a residual effect and can be used in water with a hardness of 500-1000 ppm (Marriott, 1999).

While this type of sanitiser has a number of advantages, a major disadvantage has been reported as the limited effectiveness against most gram-negative microorganisms (Marriott, 1999). The finding that *Pseudomonas* spp. may have certain resistance mechanisms to quaternary ammonium compounds has been presented by other authors (Langsrud et al., 2003). This is a major concern given *Pseudomonas* spp. have been identified as an SSO responsible for spoilage in fruits, vegetables, meats, low acid dairy products as well as seafood (Verran et al., 2008). This finding is contradicted by other authors who reported that QAC are effective against gram positive and gram negative bacteria, yeasts and moulds (Chmielewski & Frank, 2003; Riley et al., 2007; Tamplin, 2008).

1.6   Acidified Sodium Chlorite (ASC)

Sanitisers have been shown to be effective in the reduction of organisms post harvest and in the inhibition of organisms for extended periods post application. To further reduce the initial microbial load on processed seafood prior to packing and as an additional surface sanitiser in light of some identified issues with QAC, an alternative sanitiser was investigated. This product was required to be certified by regulators as suitable for direct application to food, cost effective, simple to mix and apply and safe to handle and store. Ozone was found in trials to be limited by water turbidity and the gas emitted from treated water was unacceptable in the processing environment. ASC is a broad spectrum bactericide, fungicide, virucide and algaecide food surface treatment and is intended for use as a spray or dip for poultry, meat, vegetables, fruits and seafood (Midgley & Small, 2006). Reported advantages include; not affected by organic load, no toxic residues, classified as no rinse food grade

sanitiser by regulators. The antimicrobial activity of acidified sodium chlorite is the result of the oxidative effect of chlorous acid. This acid is derived from the conversion of chlorite ions into its acid form under acidic conditions.

Studies on the application of ASC found it significantly reduced the number of spoilage micro-organisms on food surfaces, which helped to extend shelf life (Midgley & Small, 2006). Acidified sodium chlorite has been used successfully to reduce microbial loads in beef (Bosilevac et al., 2004; Midgley & Small, 2006) and chicken (EFSA, 2005; Oyarzabal et al., 2004). After being applied to parsley for 5 minutes it resulted in a TVC 1 log reduction (Tamplin, 2008). Tamplin also investigated the effect of rinsing fish fillets for 1 minute to reduce the bacterial loads. The results gave a 0.5-1.5 log reduction (Tamplin, 2008) but the method was not considered to be appropriate for commercial applications due to the inconsistency of bacterial reduction and application logistics. Based on the work of Tamplin (Tamplin, 2008) and Riley (Riley et al., 2007) ASC was selected for evaluation as a surface spray for direct application to seafood.

1.7   Hurdles in Maintaining Food Quality

By manipulating food preservation parameters microbial growth can be adjusted, restrict or prevented (Leistner, 2000). Up to 40 so called hurdles have been identified including time, temperature, hygiene, sanitation, water activity, acidity (pH), redox potential (Eh), modified atmosphere, O_2 packaging (Venugopal, 2006). Rapid chilling, sanitary handling practices, the application of sanitisers and modified atmosphere packaging have all been shown individually or in limited combinations to have significant impacts on the numbers of microorganisms present, their growth and the shelflife of chilled seafood products (Leistner & Gould, 2002).

In this study the preservation methods used are designed to not cause changes in consumer perception from untreated product. Hurdles used in combination can have an additive or synergistic effect (Leistner & Gould, 2002). Hurdles investigated in this study included low temperatures, gas atmospheres, direct product sanitation and pH reduction resulting from CO_2 absorption into the raw material and sanitary handling practices.

Basic Aspects

To be effective hurdles need to inactivate or inhibit microbial growth. Microbes react to hurdles and adjust to the altered environment as demonstrated during the lag phase of microbial growth. Individual microbes have a limited ability to make significant adjustments to the environment that is often highly variable. In large groups microbes can form biofilms that have the ability to moderate the effects of changes in the external environment. Microbes have evolved homeostatic mechanisms to help cater with major environmental extremes (Leistner, 1999). Homeostatic mechanisms in the microorganism act to ensure that key physiological activities and parameters are maintained resulting in survival and growth. Homeostatic mechanisms can be active and the cell must expend energy to counter the environmental stress. The application of hurdles and the impact on microbial homeostasis can result in metabolic exhaustion. In this situation microbe energy levels may be depleted in an attempt to maintain homeostasis as a result of the application of a hurdle. The next hurdle may exceed the microbes ability to maintain homeostasis resulting in death.

1.8   Modified Atmosphere Packing (MAP)

Despite the volume of research and publications pertaining to the use of modified atmospheres to extend the shelflife of seafood products, there is still no clear agreement on the shelflife of most seafood species. Shelflife extensions of 50-400% have been quoted for

carbon dioxide based chilled packs (Farber, 1991; Phillips, 1996; Reddy et al., 1992; Sivertsvik et al., 2002). Estimates of shelflife for even well studied species such as Atlantic Salmon (*Salmo salar*) and Cod (*Gadus morhua*) are highly variable (Reddy et al., 1992; Sivertsvik et al., 2002; Skura, 1991).

The basis of this review is to identify and explain potential sources of variability. In turn these provided justification for the method used in this study and placed the results into context.

A possible explanation for this variability in results is the number and difficulty in controlling, standardising and measuring factors that can indicate and influence product shelflife. The range of variables identified that affect chilled modified atmosphere (MA) packed products include: species, product form, initial quality and condition of the raw materials, carbon dioxide percentage and partial pressure in the pack, accumulated temperature history of the product in chain, microbial counts and species composition at the time of packaging, product: gas volume ratio and the barrier properties of the packaging materials (Devlieghere et al., 1998; Dixon & Kell, 1989; Sivertsvik, 2007; Sivertsvik et al., 2002; Sivertsvik et al., 2002). In addition different researchers may use different indicators and methods to measure shelflife.

There is no standard definition of shelflife which is a subjective estimate, or consistent method that has been applied to these studies. Many factors may be considered to form the basis of the shelflife estimate. These can be grouped as microbial, physical and chemical indicators including gross microbial counts (TPC), Specific Spoilage Organisms (SSO's) like *P.phosphoreum*, chemical indicators - TMA and TMAO, TVBN, NH_3, drip loss, texture,

sensory evaluations using a variety of scales and measurable attributes (Boskou & Debevere, 1998; Dalgaard et al., 1993; Lawless & Heymann, 1999).

Given the volume of publications there are few that give clear guidelines on how to pack seafood product to achieve shelflife optimisation. The exception to this is the online Seafood Spoilage Predictor (Dalgaard et al., 2002). This is in contrast to other sectors such as horticulture where detailed tables of key pack parameters for shelflife optimisation of fruits and vegetables are published (Faber et al., 2003). There is limited published information available on the application of MAP to Australian fish species with Statham examining morwong (*Nemadactylus macropterus*) and trevalla (*Hyperoglyphe porposa*) in 1985 (J.A. Statham, 1984; J.A. Statham & Bremner, 1985; J.A. Statham et al., 1985) and Seafood Services Australia having published a serial "Seafood Packing Technologies" designed to provide guidance to seafood processors. In addition equipment manufacturers recommend suitable gas mixtures but there is little evidence of how these figures were derived.

The Action of Carbon Dioxide in Seafood

Carbon dioxide is the basis for modified atmosphere food preservation because of its ability to inhibit the growth of a wide variety of microorganisms (Dixon & Kell, 1989; Faber et al., 2003; Venugopal, 2006). Other gases frequently used in conjunction with carbon dioxide include nitrogen and oxygen. Nitrogen is inert and has no influence on microbial growth and is included to prevent pack shrinkage (Sivertsvik et al., 2002) . Oxygen is generally added to alter the microbial flora of products, prevent the reduction of TMAO, and potentially prevent the growth of anaerobic pathogens, particularly *Clostridium botulinum* (Dixon & Kell, 1989; Sivertsvik, 2007).

The level of dissolved CO_2 is subject to Henry's Law. Absorption rates for fish are slightly lower than for water due to moisture levels of 77.55% in squid flesh, 72.4 % water and 7.2% oil in Atlantic salmon fillet while the moisture range of fish fillets generally is between 66-81% (Huss, 1995; Rotabakk et al., 2007; Vlieg, 1988). After the pack is initially flushed, CO_2 starts to be absorbed into the flesh and fat. When CO_2 dissolves in food it is hydrated and carbonic acid is formed. This will dissociate to form a bicarbonate ion (Devlieghere et al., 1998; Dixon & Kell, 1989). This process depletes the gas in the package headspace. The process of absorption generally lasts between 2-4 days when the flesh is saturated and equilibrium has been reached. The full preservation effect of CO_2 will only be achieved if the CO_2 in the headspace exceeds the amount required to saturate the flesh. (Jakobsen & Bertelsen, 2004b).

The concentration of CO_2 dissolved in the flesh determines the level of microbial growth inhibition in a modified atmosphere (Debevere & Boskou, 1996; Devlieghere & Debevere, 2000). The volume of CO_2 dissolved in flesh is determined by independent groups of factors. These include the product associated factors of initial pH, water activity, fat content, fat type and the processing related factors of partial pressure and concentration of CO_2 in the pack headspace, pack permeability, the gas/product volume ratio (*g/p*) and temperature (Boskou & Debevere, 1997; Devlieghere et al., 1998; Sivertsvik et al., 2002; Stiles, 1991). Carbon dioxide is highly soluble in water and fat with greater solubility occurring as temperatures decrease towards 0°C (Devlieghere et al., 1998; Gill & Tan, 1979; Rotabakk et al., 2007; Simpson et al., 2009). The increased solubility at lower temperatures results in increased microbial inhibition. This implies the gas effectiveness is conditional on storage temperature.

In modified atmosphere packaging, air in the pack is replaced with an alternative gas mixture. After the introduction of the alternative gas mixture no further controls are performed but

changes in composition are likely to occur through respiration and absorption of the food product. CO_2 is the most effective gas used in the inhibition of spoilage bacteria growth (Devlieghere & Debevere, 2000). It has both bacteriostatic and fungistatic properties but the action of carbon dioxide on microbes is not well understood (Simpson et al., 2009). It has been suggested that the mechanisms acting on microbes may include:

Alteration of the functioning of the cell membrane including nutrient uptake

Inhibition of metabolic processes and disruption of enzyme activity

Penetration of bacterial membranes resulting in intracellular pH alteration

Changes in the physico-chemical properties of proteins

(Dixon & Kell, 1989; Farber, 1991; Sivertsvik et al., 2002 ; Stiles, 1991).

Atmospheric levels of carbon dioxide in excess of 30% pack volume has been shown to extend shelflife by retarding microbial growth (Sivertsvik, 2007; Stiles, 1991). At lower temperatures higher levels of absorbed gas into the aqueous component of the flesh will be more effective at inhibiting bacterial growth (Sivertsvik et al., 2002). Bacterial levels in raw materials also have a significant effect on the shelflife of MA packs (Sivertsvik et al., 2002; S. L. Slattery, 2000). Only fish of good microbial quality should be packed in MA containing CO_2 (preferably less than 10^4) to achieve significant shelf life extension (Sivertsvik et al., 2002; S. Slattery et al., 1998).

CO_2 ratios and volumes within the pack headspace are critical indications as to potential bacterial survival. Bacterial numbers are increasingly inhibited as levels of dissolved CO_2 rise to the point of saturation (Dixon & Kell, 1989; Faber et al., 2003). Headspace gas levels need to be measured to indicate the levels of dissolved CO_2 in the product. Without initial

measurements the level of gas dissolved in the flesh is not known and the comparison of results between various studies is difficult.

The absorption of CO_2 into the flesh results in a lowering of the pH. Changes in headspace gases occur during storage. These changes occur due to muscle respiration, microbial metabolism, gas absorption and permeability of the packaging material (Zhao, 1994).

Studies on Modified Atmosphere Packaging of Seafood Products

The effect of Modified Atmospheres on the shelflife of foods has been extensively reviewed by Statham 1984, Farber 1990, Skura 1991, Reddy 1992, Davies 1995, Phillips 1996, Sivertsvik, 2002 and Venugopal, 2006 (Davies, 1995; Farber, 1991; Phillips, 1996; Sivertsvik et al., 2002; Skura, 1991; J.A. Statham, 1984; Venugopal, 2006).

These reviewers summarized the outcomes of a number of seafood related studies investigating the shelflife extension attributable to modified atmosphere packing including: Statham (1984) 14 studies; Reddy (1992) 33 studies; Venugopal (2006) 17 studies; Sivertsvik (2002) 31studies and Skura (1991) 29 studies including Perch *Meronia americanus*, Croaker *Micropogon undulates*, Morwong, *Nemadactylus maropterus*, Trevalla, *Hyperoglyphe porosa*, Flathead, *Platycephalus bassensis*, Greenland halibut *Reinhardtius hippoglossoides*, Hamachi *Seriola aurvittata*, Broadbill Swordfish, *Xiphias gladius*, Channel catfish *Ictalurus punctatus*, Rockfish *Sebastes* spp, White hake, *Urophycis tenuis*, Herring and Sardines *Sardinops melanostictus*, Mackerel *Trachurus declivis*, *Scomber scombrus*, Scallops, *Pecten alba*, *Pecten maximus* Prawns *Macrobrachium rosenbergii*, *Pandalus platyceros*, Cooked freshwater crayfish *Pacifastacus leniusculus*, *Procambaris clarkia*, Crab *Cancer pagurus*.

The list of seafood species treated with carbon dioxide as a preservative and subject to research also include; Atlantic Salmon (*Salmo salar*) (Emborg et al., 2002; Fernandez et al., 2009; Sivertsvik et al., 2003), King salmon (*Oncorhynchus tshawytscha*) (Fletcher et al., 2002),Spotted Wolf-fish (*Anarhichas minor*) (Rosnes et al., 2006), Broadbill Swordfish (*Xiphias gladius*) (Pantazi et al., 2008), Cod (*Gadus morhua*) (Boskou & Debevere, 1998; Debevere & Boskou, 1996; Sivertsvik, 2007; T. Wang et al., 2008), Halibut (*Hippoglossus hippoglossus*) (M. B. Hovda et al., 2006), Eel (*Anguilla anguilla*) (Arkoudelos et al., 2007), Rock Cod (*Sebastes spp*.) (M. Y. Wang & Ogrydziak, 1986), Prawns and Cooked Shrimp (*Pandalus Borealis*) (Sivertsvik et al., 1997), Chinese Shrimp (*Fenneropenaeus chinensis*) (Shengmin, 2008), Brown Shrimp (*Panaeus aztecus*) (Lannelongue et al., 1982), Scallops (*Argopecten purpuratus*) (Simpson et al., 2006), Sea Bass (*Dicentrarchus labrax*) (Poli et al., 2006), Mussels (*Mytilus galloprovincialis*) (Goulas, 2008), Red Claw Crayfish (*Cherax quadricarinatus*) (G. Chen & Xiong, 2008), Rainbow Trout (*Salmo gairdneri*) (Randell et al., 1997), Hake (*Merluccius merluccius*) (Pastoriza et al., 1996b) and others.

While extensive work has been conducted it is difficult to directly compare the results. The significant outcome of research to date has been products packed in carbon dioxide atmospheres greater than 30%, at product gas ratios of 1:2 or higher have extended microbial shelflife (Sivertsvik et al., 2002).

2   Justification, Problem Statement, Need

2.1   Supply Chain Suboptimisation

Chilled seafood is considered to have a short quality shelflife when compared to terrestrial protein alternatives. Quality deterioration evidenced by high microbial counts, textural degradation and exudate loss impacts on flavour, odour, appearance, mouth feel and consumer satisfaction. Consequently one of the most challenging tasks for the seafood industry is controlling product quality throughout the supply chain.

It is suspected that Australian fresh chilled seafood supply chains are not functioning at optimal levels. The 2007 study of the South East Scalefish and Shark Fishery (SESSF) Seafood Supply Chain identified up to 8 transactions in the chain between catch and retail sale (Anon, 2007). The complexity of the chain suggests there is significant scope for suboptimisation to occur. This suboptimisation impacts both consumers and the sector negatively.

The main factors contributing to product sub-optimisation include time, temperature, effects of handling and cross contamination. This is evidenced as reduced shelflife and quality, product inconsistency, poor customer value, discounting and discarding. Anecdotal evidence supplied by industry indicates issues with handling practices resulting in variable in-chain temperatures and microbial cross contamination of product (Sedman, T. 2011, pers. comm., 7 June), (Boulter, M. 2009, pers. comm., 5 October). This evidence is supported by limited published Australian studies. Gillespie and Macrae observed increasing bacterial numbers during product handling, Slattery observed high and variable numbers of microbes in processing facilities and product prior to packing while Powell found high microbial species variability between commercial packs of Atlantic salmon (*Salmo salar*) (Gillespie & Macrae,

1975; Powell & Tamplin, 2012; S. Slattery et al., 1998). The impacts of this sub-optimisation ultimately affect profitability throughout the chain (Sparrow, A. 2009, pers. comm., 8 April) (Sedman, T. 2011, pers. comm., 7 June). Currently no quality shelflife benchmark exists to act as a basis for the evaluation of retail seafood supply chain performance.

2.2 Consumer Behaviour

Australian produced chilled seafood competes directly for market share with other fresh protein products. Consumer demand, price, repeat purchasing and resultant business viability is based on customer satisfaction achieved at the point of consumption. In making the purchase decision the retail customer typically relies on sight and smell to give indications of future eating performance (quality).

The perception of quality is individual and subjective. The weighting attributed to both tangible and intangible aspects of quality alters with the individual. At some point during chilled storage, the product ceases to represent value at the asking price against alternatives. At this point the products quality shelf life has been exceeded.

Seafood deteriorates rapidly when compared with terrestrial meat products resulting in a comparatively short shelf life (Emborg et al., 2002). Currently seafood suppliers are being encouraged by the 2 leading retail chains to further increase the shelflife of modified atmosphere (MA) packed chilled salmon products (Sedman, T. 2011, pers. comm., 7 June). Sub optimal practices including microbial contamination and temperatures exceeding 0°C are cumulative (Gram & Huss, 1996; Huss, 1995). Maximum microbial guidelines of 10^7 (ICMSF., 1986) may be exceeded after 14 days for chilled Atlantic salmon (*Salmo salar*) in modified atmosphere packs (MAP) (Sedman, T. 2011, pers. comm., 7 June).

In this study an optimized shelflife model for chilled seafood products was developed and applied to 4 representative species to determine the maximum microbial and quality shelflife. These benchmarks will provide a basis for comparison of supply chain performance. Commercial operators in the sector may use this information to make value judgments on the cost benefits of addressing supply chain deficiencies and investing in technology to extend shelf life.

3   Theoretical Framework

3.1   Species Selection

Three species were selected as being broadly representative of species consumed in fresh chilled form by Australian consumers and a fourth as an example of a chilled high value export product. The group included two fish species, a cephalopod and a mollusc. Flathead (*Platycephalus bassensis*) are an example of wild catch, whitefish species with a broad distribution having relatively low fat levels. Atlantic salmon (*Salmo salar*) is an example of an aquaculture produced fish with national retail distribution and relatively high levels of oil in the flesh. Southern calamari (*Sepioteuthis australis*) is a species with a broad distribution and represent wild catch cephalopods, a group with increasing consumer acceptance, and abalone (*Haliotis rubra*) is an example of a high value mollusc primarily exported to Asia.

3.2   Developing the Best Practice Method

In order to develop an optimized shelflife model for chilled seafood, it was necessary to identify and evaluate all steps, processes, and materials typically used in the chain.

The term shelflife in this study is defined as a product having the edible attributes of a good example of the species as judged by a sensory panel. The term "good example" is referenced by the notion of representing acceptable quality typical of the species as judged by an expert panel.

Quality was defined in terms of microbial total plate counts, driploss, pH, texture and palatability. While it is recognized that quality is associated with autolytic spoilage and nucleotide breakdown this was not measured due to a lack of resources and exceeded the scope of the study.

This study adopted the following criteria as the basis for the selection of methodologies:

Improved shelflife by inactivating enzymes and spoilage microorganisms

No changes in organoleptic and nutritional attributes

No residues left on food

Convenient to apply

Cheap

Practical

No objections from consumers and legislators

(Rajkovic et al., 2010).

Driploss is considered to be an indicator of textural degradation while water loss and accumulated exudate are of economic and presentational significance (G.B. Olsson et al., 2003). In addition it has been suggested that driploss is indicative of the degradation of myofibrillar structures and connective tissue over time post mortem (Love & Haq, 1970).

3.3   Microbial Shelf Life Extension Principles

- Reduce the potential for microbial growth by applying best practice chilling methods and packing technologies.

- Prevent cross contamination by applying best practice sanitation and hygiene practices

- Maximise the generation interval and lag phase at 0°C and 5°C

3.4   Research Aims

The purpose of this research was to describe the seafood supply chain, ascertain best practice for each of the steps in the chain and practically apply these in order to define the maximum quality and microbial shelflife for the 4 species under investigation.

3.5   Objectives

- To determine the maximum quality shelf life of the 4 species by applying best practice handling, processing and preservation methods during the study

- To assess microbial shelflife for the 4 species at 0°C and 5°C temperatures under different atmospheres.

3.6   Product Form

Fish

A number of factors were taken into consideration when selecting the product forms. For the fish species skinless fillets were selected based on:

- Consumer preference for ready to prepare products with no waste as identified in consumer studies by Ruello and Associates (Anon, 2002).

- Recognition that this product form represents a typical method of processing when compared to whole or gilled and gutted fish.

- This method should represent the greatest potential for shelflife extension if all aspects of the production and distribution are controlled due to fish muscle being sterile. By eliminating the high bacterial areas of the fish that can't be sanitised –skin, gut and gills the sterile muscle should be left with low microbial numbers.

Cephalopod

In line with consumer preference for ready to eat products with little waste, the product form selected for this species was cleaned skinless tubes. Cephalopods are generally presented for retail sale as chilled whole unprocessed animals at fish mongers or as imported, defrosted, nib off cleaned skinless tubes.

Abalone

Processed abalone meat is typically sold frozen in vacuum packs. Abalone was shucked from the shell, the viscera and mouth parts removed before scrubbing or tumbling to remove black pigment.

4 General Materials and Methods

4.1 Raw Material: Flathead

Flathead *(Platycephalus bassensis)* were caught in June, 2009, April, June, 2010 and February 2013 for microbiological, taste panel, drip loss, pH, acidified sodium chlorite (ASC) and texture testing. Fish (n= 80, whole weight: 78-400 g, mean weight 167 g, SEM ± 7.5 g) were line caught in 7-12 meters of water in the Tamar river estuary, Tasmania (latitude 41.15°, longitude 146.85°). Fish used for textural analysis (n=10, whole weight 141-477 g, mean weight 207.3 grams, ± 31.8 g) were line caught off West Head, Tasmania (latitude 41.06°, longitude146.71°) in 19-21 meters of water.

Capture/Harvest Handling

The line capture method resulted in fish struggling for less than 30 seconds before being euthanased and immediately transferred to prepared ice slurry. Slurried fish were processed, treated and stored within 5 hours of capture at the Australian Maritime College (AMC), Beauty Point seafood laboratory.

4.2 Raw Material: Atlantic Salmon

Atlantic salmon *(Salmo salar)* were purchased on 4 occasions in December 2009, June 2010, September 2010 and December 2012 from Van Diemen Aquaculture, Tamar River, Tasmania for microbiological, taste panel, drip loss, pH, acidified sodium chlorite (ASC) and texture testing. In total 30 fish with whole weights ranging from 3.14-5.1 kg, mean weight 4.12 kg, ± 229 g were analysed.

Capture/Harvest Handling

Atlantic salmon were collected directly from the harvest line after stunning and bleeding and immediately transferred to an ice slurry as per industry practice. The fish were transported for 20 minutes in the slurry to the Australian Maritime College, Beauty Point seafood laboratory and immediately processed.

4.3   Raw Material: Southern Calamari

Southern Calamari (*Sepioteuthis australis*) were line caught by a professional fisher on 3 occasions on December 2010, October 2011 and December 2012 at Greens Beach (latitude 41.05, longitude 146.73), and Ringarooma Bay (latitude 40.70, longitude 147.76) northern Tasmania. On capture, whole calamari were immediately placed in a seawater ice slurry where they remained for 3-8 hours before transfer to the AMC Beauty Point facility for processing. The whole calamari were processed to cleaned, skinned tubes before portioning treatment, packing and storage. In total, 27 calamari weighing between 112 and 2850 grams with a mean weight of 834 ± 127 g were processed for microbiological, taste panel, drip loss, pH, and texture testing.

4.4   Raw Material: Black Lip Abalone

Abalone (*Haliotis rubra*) were harvested live by diver on 3 occasions from Eddystone Point area (April 2010, latitude 40.987, longitude 148.338) and Fortescue Bay, Tasmania (March and August 2012, latitude 43.136, longitude 147.956) with whole weights ranging from 178-660 grams, mean weight 373.8 grams and ± 27.4 g. In all trials harvested abalone were transported live to the AMC Beauty Point facility where the animals were removed from the shell and meat separated from gut and radula. Shucked meat was scrubbed with ASC at 300

ppm to remove black pigment from the frill and foot areas, allocated to treatments before packing and storage. Samples were removed at regular intervals for microbial enumeration, textural, pH, driploss and sensory analysis.

4.5   Facility Preparation

In preparation for each species, all contact surfaces, processing facilities and equipment, including the ice slurry container, ice box, cutting boards, trays, scales, benches and knives, were sprayed with detergent ((Tiger Plus, Applied Chemicals-Foaming alkaline, chlorinated detergent (Potassium hydroxide 1-5%, Sodium hypochlorite 1-5%)), scrubbed, rinsed and then sanitized using a commercially available Quaternary Ammonium Compound (FS Formula 7000, Calman Australia) applied at a dilution of 500:1 as recommended by the manufacturer. Thirty minutes before equipment use Acidified Sodium Chlorite (Vibrex Foodplus Sanitiser and Vibrex Activator C10^{TM} , Technica Pty. Ltd, Bayswater, Victoria) was applied as a spray at a concentration of 500 ppm.

4.6   Time Temperature

Ice or ice slurry was used for temperature control throughout the full chain from capture / harvest to processing, packing and storage. After each step in the process the product was returned to temperature control to avoid product temperatures exceeding 7°C for greater than 1 hour. The storage temperatures selected were based on 5°C representing the maximum storage temperature for chilled seafood products under Australian food legislation while 0°C is considered best practice in Australian chill supply chains.

Maximum storage temperature samples of less than 5 °C were placed in an air blast chiller set at 4°C ± 0.5°C and held until microbial counts exceeded 10^7 CFU/g. This chiller was used for

general seafood storage and has an air temperature range (3.2°C to 14°C) and variability more indicative of commercial applications.

Samples prepared for holding at 0 °C were placed in ice within an insulated container, which was located in the air blast chiller set at 4°C. The environment in this container remained very stable at a mean temperature of 0.1°C for all trials with standard error of the mean SEM ± for all readings ranging from 0 (flathead, salmon), 0.005 (abalone) 0.14 (squid). Temperatures were recorded using Hobo Onset UA 002-64 data loggers calibrated in fresh water ice slurry at sea level.

4.7   Storage Times-Equivalent Days on Ice

Time is expressed using both the traditional method of days and as a calculated Equivalent Days on Ice. This method developed by CSIRO, Division of Food Research, (H.A. Bremner et al., 1987) uses a relationship linking time, temperature and spoilage rate in relation to a constant spoilage rate at 0°C. This method takes into consideration the effects on spoilage of variable temperature environments (commercial chillers) and provides a mechanism for comparing studies conducted at differing temperatures. Temperature logger data was recorded every 30 minutes in each storage environment and a mean temperature for each 24 hour period calculated. These mean temperatures were converted to equivalent days on ice times using the method outlined by Bremner. The additive equivalent days on ice times allow direct comparison between samples held at various temperatures over time.

Equivalent days on ice calculates the rate of spoilage relative to 0°C using the simplified formula:

$r = (0.1\,t + 1)^2$

where *r* is the relative rate of spoilage and *t* is temperature (°C) (H.A. Bremner et al., 1987).

Equivalent days on ice was calculated for both control and MAP at 0 and 5°C for all species.

4.8   Microbial Analysis

Pour Plates

A total aerobic plate count (TPC) method was used as detailed in AOAC Official Method 966.23 (AOAC, 2000) with some alterations. These alterations included an incubation temperature of 25°C as recommended by the ICMSF for aerobic plate counts (APC) when analysing seafood products (ICMSF., 1986). Additionally, water and sample volumes were reduced proportionately due to the capacity of the stomacher. Approximately 20 gram samples of each treatment, except squid were aseptically transferred to a stomacher bag and weighed. 180 mL of sterile water was added to the bag and the contents stomached for 1 minute (Colworth 400, Seward, London, UK). In the case of squid the sample weight was 10 grams and the volume of water was 90 mL. A decimal dilution series was prepared in Macartney bottles containing 10 mL of sterile 0.1% peptone water according to estimated contamination. Duplicate 1 mL samples of each dilution were transferred to 90 mm Petri dishes.

Plate Count Agar (Merck) was prepared according to the manufacturer's instructions with the addition of 1% NaCl. The agar was tempered in a water bath at 45°C until required. The poured plates were incubated for 72 hours at 25°C or until colonies could be distinguished in sufficient detail to count. In some instances, incubation times were extended to 120 hours in order for colonies to grow sufficiently to allow counting. Mean results of duplicate measurements are presented as colony-forming units per gram of muscle (CFU/g).

Swab Method

Cutting boards were assessed preprocessing to determine the effectiveness of the cleaning and sanitation methods and post processing to assess the effectiveness of water rinsing in removing contamination during processing. A 50 x 50 mm metal template (25 cm^2) was used as a guide. Swabs were moistened in sterile water and rubbed on the cutting board surface within the template boundaries. Swab tips were cut off into Macartney bottles containing 10 mL of sterile 0.1% peptone water. The tips were left to soak for 15 minutes before being shaken vigorously to remove bacteria. Total viable counts were determined by a dilution series of pour plates prepared with plate count agar (Merck) containing 1% NaCl, incubated at 25°C for 72 hours and expressed as CFU/ cm^2.

The indicative microbial maximum for this study was set at 10^7 CFU/g by total plate count method. When samples had exceeded this number it was assumed their microbial shelflife had been exceeded and no further readings were taken. This number (10^7 CFU/g) is the maximum number of microorganisms allowed to be present on a single sample of seafood product when measured by Total Plate Count (TPC) as part of a 5 sample plan (ICMSF., 1986). Microbial lag phase was defined as mean count within a range of 1 log above the initial count.

4.9 MAP Pouch Gas Ratios, Volume, Sealing and Transpiration Trials

Two trials were conducted to assess pouch and packing equipment accuracy, reliability, repeatability and pouch gas transpiration rates. All MAP samples were packed in 2 layer co-extruded barrier (Nylon and Linear Low Density Polyethylene) pouches with an oxygen transmission rate of 50-55cc m^{-2} 24 hr ATM using a model Compaq vacuum packer (Kramer + Grebe, Biedenkopf, DE) with a Witt gas mixer. All pouches were double heat sealed.

The first trial was to ascertain gas mixer accuracy, volumes, consistency and gas transmission rates over time. Two replicate batches of pouches (n=5) were flushed with an indicative gas ratio of 50% CO_2 and 50% O_2, (BOC gases, Launceston, Tasmania). Pouch gas volumes and ratios were measured on day 1 and 8 after storage in ice at a mean temperature of 0.1°C, using a Checkmate II Gas Analyser (PBI-Dansensor, Ringsted, Denmark) and by manual extraction with a 60 mL syringe inserted through a rubber septum. The gas mixer delivered gas volumes ranging from 300 - 400 mL per pouch at a mean ratio of 55% O_2 ± 0.015 and 45% CO_2 SEM ± 0.014 on day 1. The replicate 5 pouches at day 8 had a mean ratio of 55% O_2 ± 0.19 and 43% CO_2 ± 0.26.

In the second trial, pouches (n=10) were flushed with 100% CO_2 (gas purity 98% BOC) and measured after 8 days storage to determine CO_2 transpiration rates. At day 8, CO_2 percentages ranged from a minimum of 93% to a maximum 97.5% ± 0.5 and O_2 percentages ranged from a minimum of 0.8% to a maximum of 6% ± 0.6.

4.10 Modified Atmosphere Packaging (MAP)

Gas to product (*g:p*) and volume to weight ratios typically exceeded 4:1 (v/w) and were never less than the critical 2:1 for all species and for all product forms (Sivertsvik, 2007).

Gas mixes selected for flathead, were oxygen and carbon dioxide at a ratio of 55% 0_2: 45% CO_2.

All other species were packed in 100% CO_2.

Control Samples

Control samples were packed in gas permeable low density polyethylene bags (36μm), tied at the top to prevent contamination before storage at 0°C or less than 5°C.

4.11 Quality Attribute Assessment

Table 4.1: Experimental Summary

Test area	Experimental aim	Storage characteristics	Test detail	Experimental design
Microbial	Assess microbial growth rate over time, temperature and treatment	0°C and 5°C in sealed gas impermeable bags 100% CO_2 for all species except flathead 45 CO_2: 55 O_2 and in unsealed gas permeable plastic bags	TPC (AOAC 2000) with modifications- incubation temperature (25°C)	Control v treatment at 0°C and 5°C
pH, driploss	Monitor the effects of controls and treatments over time and temperature	As above	pH meter, tip inserted in the front upper muscle block (Thomas 1999) driploss method (Santos 1990)	As above
Texture	Monitor textural degradation over time for all variants	As above	Shear method (Chamberlain 1993)	As above
Sensory	Compare the edible attributes of control and treatment to define the maximum quality shelflife	Control (fresh chill retail purchased) and Stored MAP at 0°C	Acceptance test 9 point Hedonic scale, Triangle difference test and preference tests	Control v Treatment

pH

The pH of samples were measured using a pH meter (WTW pH 330, Weilheim Germany) in combination with internal (WTW Sentix SP) electrode. Flesh pH was measured by completely inserting the glass tip of the WTW Sentix SP electrode into the muscle as described by (Thomas et al., 1999).

Drip Loss

Drip loss was determined by recording initial sample weights at day 0 prior to treatment and packing. Samples were subsequently randomly allocated to treatments, packed and stored. Treated sealed pouches were removed from their storage environments at designated time points and patted dry with absorbent paper. The sample was transferred to scales (AND HF 3000 G Japan error= 0.01 g) and weighed. The data was recorded and the sample repacked and returned to the storage environment. Drip loss was calculated using the following formula:

% drip = [(Initial wt of fish- Final wt of fish) / Initial wt of fish] x 100 (Santos & Regenstein, 1990).

Texture

The raw flesh of flathead, Atlantic salmon, squid and abalone were measured over time to monitor the rate and magnitude of textural degradation. Abalone texture was measured using an Agro Technologie (Forges Les Eaux, France) Agrosta 100 Statistic Durometer with modified tips. Squid texture was measured by both puncture and shear methods.

The texture of the 2 fish species and squid was measured using a fish shear (Chamberlain et al., 1993), load cell, data acquisition device, signal conditioner and computer software (Fish shear fabricated by AMC technical staff to the specifications cited in Chamberlain with a 20 mm x 10 mm sample opening, Xtran S1W Load cell 450 N Applied Measurements, Melbourne, Australia. NI USB-6008 12-Bit, 10 KS/s data acquisition device (DAD), National Instruments, Australia. PT100LC Load cell conditioner/transmitter, Auckland, NZ and LabVIEW Signal Express 2009 software, National Instruments, Australia).

The connective tissue alignment in relation to the shear blade is addressed in the individual species materials and methods but for the fish species (flathead, Atlantic salmon) the shear blade was aligned across the connective tissue at approximately 90° (refer plate 4.1).
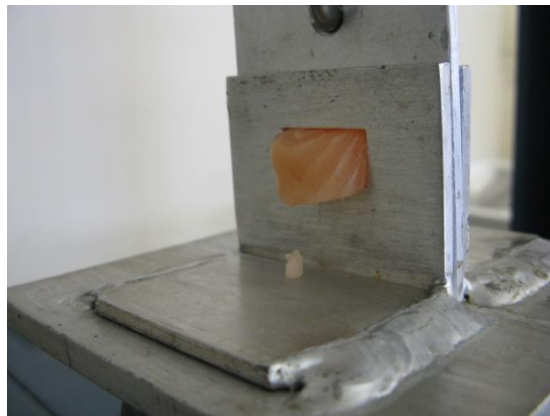
Plate 4.1: Fish shear with Salmon sample in position for texture measurement

4.12 Taste Panels

Expert Panels

The four species were initially assessed over storage time by 3 person expert panels consisting of Australian Maritime College staff. The objective of using expert panels was to

reduce the logistical and organizational requirements and therefore risk of variability associated with conducting multiple panels of more than 23 participants.

The expert panels were selected based on experience and knowledge derived from previous panel participation and being regular consumers of the species (while not a requirement all tasters had greater than 15 years experience). The controls and treatments were prepared according to the same methods defined in the individual species materials and methods. Tastings were conducted individually with tasters required to compare 2 samples (treatment and control). Tasting notes were discussed and based on these outcomes the decision to run a full panel to verify the results was made.

Large Scale Panels

Human ethics approval H11114 was received from the University of Tasmania to conduct sensory evaluation. All tasters were invited to participate and instructed as to the objectives of the panel prior to commencement (Appendix 2). The Triangle test was selected based on its efficiency and simplicity with the objective of reducing the risk of taster availability and variability associated with trained panels.

Discrimination, preference and acceptance tests were used either individually or in combination. Salmon, squid and abalone were assessed by Triangle discrimination tests and preference tests while flathead was assessed by a 9 point Hedonic scale acceptance test (Lawless & Heymann, 1999; Meilgaard et al., 2007).

Triangle test panel size was calculated according to the test sensitivity parameters defined in (Meilgaard et al., 2007). Values for $\grave{\alpha}$ Type I error and β type II error were set at 0.05 with a

p_d (proportion of distinguishers) set at 50%. At these levels of sensitivity 23 or more

respondents were required for each panel.

4.13 Statistical Analysis

Statistical analysis was carried out using SPSS 19.0 for windows. Results were tested for

homogeneity of variance and when violated log 10 transformed or non parametric tests

applied. In all experiments graphical presentation of untransformed data is shown as mean

values ± standard error mean (SEM). Differences were considered to be significant at p

<0.05.

5   Determination of Flathead Shelflife

5.1   Introduction

White Fish

Sand flathead (*Platycephalus bassensis*) were selected for this study as being broadly representative of white fleshed teleost marine ground fish. This group represents a significant proportion of edible marine fish consumed in Australia. Of the family *Platycephalidae*, 14 species are regularly caught during fishing activities. Flathead are found right around the Australian coastline in a variety of habitats including rivers, estuaries, bays and offshore waters. They form a valuable commercial fishery in southern Australia with an annual catch of 4127 tonnes valued at $20 434 000 (ABARE, 2008). The recreational catch weight is estimated at 2300 tonnes from all Australian states (Henry & Lyle, 2003).

Flathead are a well known and highly regarded table fish in Australia, with firm white and flaky flesh (Yearsley et al., 1999). Skinless boneless fillets are highly valued and achieve prices as high as $40 per kilogram at retail. Sand flathead (*Platycephalus bassensis*), line caught in the Tamar River, Tasmania were primarily used for experiments.

Experimental Objectives

- To determine the maximum quality shelflife of sand flathead (*Platycephalus bassensis*) by applying best practice handling, processing and preservation methods.

- To assess microbial shelflife at 0°C and 5°C temperatures under different atmospheres.

5.2   Methods

The methods are as listed in the General Materials and Methods section with the following specific details and/or modifications.

Microbiology

Flathead samples (n=2 of each treatment) were analysed for microbial counts (TPC CFU/g) on days 0, 2, 4, 7, 9, 11, 13, 15, 18, 21, 25, 32, or until microbial counts exceeded 10^7 per gram (ICMSF., 1986).

pH

At day 0, four whole fish with a mean weight of 103 ± 14 g, (range 78-147 g) were randomly selected and the flesh pH measured. The individual fillets (n=8) from these fish were allocated to replicate treatments of MAP and control at 0°C and 5°C for further pH measurement on days 5, 12, 19, 30 or until spoiled.

Drip Loss

The drip loss of flathead was determined for 8 fillets with a mean weight of 90.5 ± 2.5 g, (range of 81-104 g). Replicated treatments of MAP and control at 0°C and 5°C were assessed for driploss on days 0, 5, 12, 19, 30 or until spoiled.

Texture

Texture was determined by fish shear for 20 fillets that were randomly allocated to 4 treatments (MAP, Control at 0°C and 5°C) and was measured on days 0, 3, 7, 10, 14, 17 and

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21 or until spoiled. All measurements were conducted on similar cross sections of the fillet at each time point commencing at day 0 forward of the tail at a section of the fillet offering a greater than 20 mm x 10 mm cross section and working forward over time. Samples were aligned so that the connective tissue was across shear blade. The blade passed through the connective tissue joining the muscle blocks at angles approaching right angles. After each reading the remaining fish was repacked and returned to storage for further readings.

Sensory Analysis

Control and treated flathead fillets were compared by taste panel. Prior to full panel assessment, treated samples and controls were assessed for general acceptability in a blind tasting by an expert panel of 3. The control and treated samples were considered by the expert panel to have good eating attributes before full panel assessment. Microbial TPC's were conducted on the treated and control samples. This was to ensure panel safety and to determine if the microbial growth characteristics were the same as in the microbiological growth assessment over time trial.

Test Method Flathead

Because of insufficient sample material, 2 other flathead species were included in the sensory assessment. This prevented the use of direct comparison methods and so an Acceptance test based on a 9 point Hedonic scale was used to assess product flavour acceptability. The Hedonic scale assumes that consumer preferences exist on a continuum and that preference can be categorised by responses based on likes and dislikes.

The *Platycephalus bassensis* samples held for 21 days in a MA (mean 45 CO_2: 55 O_2) at 0°C were compared with skinless boneless tiger flathead (*Neoplatycephalus aurimaculatus & N.*

richardsoni) fillets purchased fresh chilled from a local fish monger. All species were presented as cooked skinless boneless fillet pieces of 20-30 g. Fish were dry fried in pans using commercially available spray-on canola oil to prevent adhesion of fish to the pan. Two replicate pans, hotplates and temperature settings were used to prepare pairs of samples in batches. Cooked samples were presented to panellists on a plate and identified by individual codes (Appendix 2).

Panel

Twenty eight tasters participated in the panel. All tasters were invited to participate and instructed as to the objectives of the panel prior to commencement (Appendix 2). The panellists were recruited from a diverse group of seafood consumers and included undergraduate students enrolled in a seafood-handling elective, academic and technical staff from the AMC's Beauty Point campus and mature age students from the maritime sector. Panelist ages ranged between 20 and 54 years. The majority of panellists were male. All panellists were screened as being regular seafood consumers before selection for the panel. Some AMC staff are experienced seafood and flathead tasters having regularly participated in panels. The students were regular seafood consumers and were aware of panel procedures.

Statistical Analysis

Taste Panel

The 9 point Hedonic scale is considered to have equal interval properties that allows the use of parametric statistics for data analysis (Lawless & Heymann, 1999). Summary statistics of means and standard deviations were prepared. An Independent Groups t-Test and a Kruskal

Wallis one way ANOVA were used to evaluate if the treated sample varied from the retail purchased flathead fillet control (SPSS Version 19).

pH, Driploss and Texture

Repeated measures analysis of variance (ANOVA) was used to statistically analyse pH, driploss and texture data. If Mauchly's test indicated that the assumption of homogeneity was violated then the Greenhouse-Geisser correction was used.

5.3 Results

Storage Temperature

Samples stored at 5°C had a mean temperature of $5.0 \pm <0.1$°C over the 21 day trial. Samples stored at 0°C had a mean temperature of 0.1 ± 0°C over the 24 days logged.

Equivalent Days on Ice

Equivalent days on ice factor calculations were performed on the logged temperature data and are listed in the table below. A mean equivalent days on ice was calculated at 2.2 ± 0.03 on logged (5°C or less) data. It can be seen in Table 5.1 that storage at approximately 5°C more than doubled the equivalent days on ice, whereas storage at slightly above 0°C produced virtually no increase.

Table 5.1: Calculated equivalent days on ice for flathead stored at 0°C and 5°C in comparison to actual days.

Days	2	4	7	9	11	13	15	18	21	24
Calculated DOI 0°C	2.09	4.13	7.19	9.23	11.27	13.31	15.35	18.41	21.47	24.98
Calculated DOI 5°C	4.8	9.26	15.89	20.29	24.69	29.09	33.43	39.97	46.6	

Microbial

The initial number of bacteria present on flathead portions pre-packing averaged 1×10^3 CFU/g (TPC), ranging from $6.8 - 13.0 \times 10^2$ CFU/g.

Lag and Logarithmic Growth Phase

The mean counts of the controls indicated that the samples remained within the lag phase for 4 days at 5°C and for 10 days at 0°C. The day 15 microbial count (1.7×10^6 CFU/g) of control at 0°C indicated the standard was exceeded on days 16-17 while the control held at 5°C (1.5×10^7 CFU/g) exceeded it at 9 days post harvest. The microbial counts of the MAP samples at 5°C and 0°C indicated a lag phase lasting for between 11-12 days at 5°C and 32 days at 0°C (Figure 5.1).
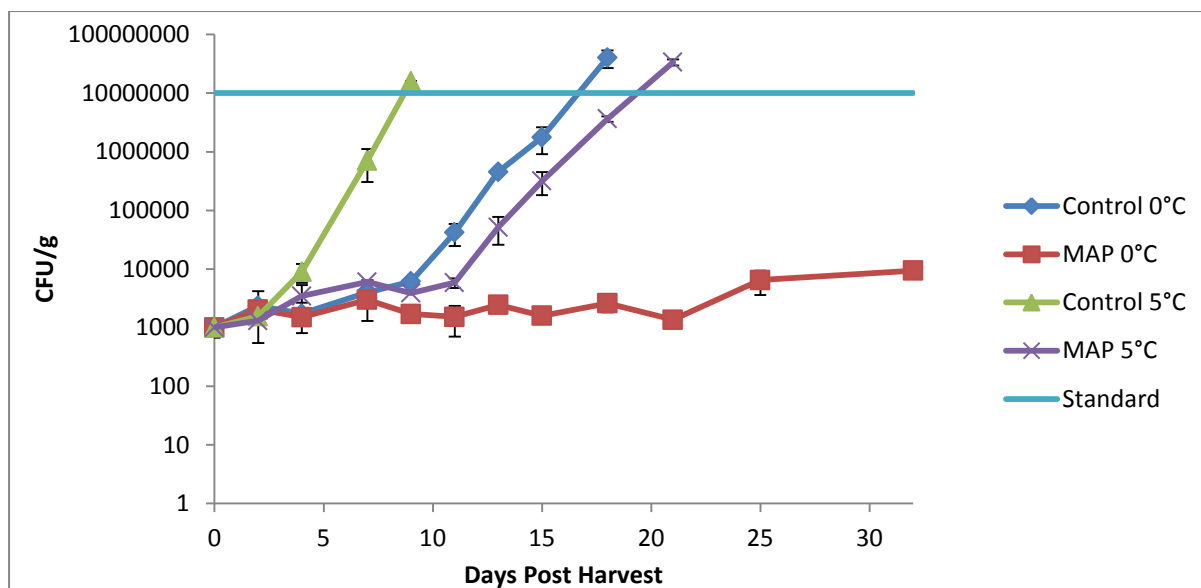
Figure 5.1: Mean (n=2) TPC (±SEM) for skinless flathead portions, control and MAP (45:55 (CO_2 O_2)) at 0°C and 5°C, sampled at 0, 2, 4, 7, 9, 11, 13, 15, 18, 21, 25 and 32 days or until spoiled. ICMSF (1986) standard of 10^7 CFU/g is indicated by the pale blue line.

The microbial count (1 x 10^5) of MAP 0°C did not exceed the standard at day 40 (not graphed) when samples ran out, while MAP held at 5°C (3.6 x 10^6 CFU/g) exceeded it after 18 days post harvest.

pH
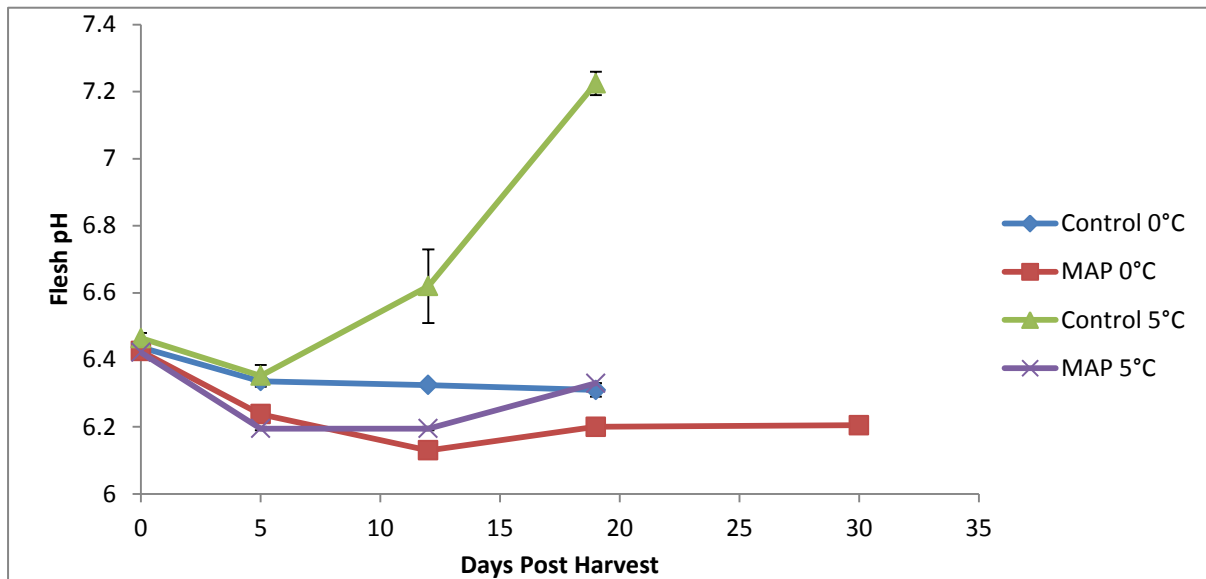
Figure 5.2: Mean (n=2) muscle pH (± SEM) for flathead fillets stored as controls in gas permeable plastic bags and MA packs (gas ratio of 45:55 CO_2: O_2) at temperatures of 5°C and 0°C sampled at 0, 5, 12, 19 and 30 days or until spoiled.

An initial pH measurement was conducted 4 hours post capture and ranged from a minimum of 6.41 to a maximum of 6.48 with a mean of 6.44 ± 0.008. The repeated measures ANOVA indicated that pH differed statistically significantly between MAP treatments and controls over time, F (3, 12) = 41.043, $p<0.05$ partial η^2 =.969. A series of pairwise comparisons adjusted using the Bonferroni correction revealed that MAP treatments at 0°C and 5°C were not significantly different from each other, but were different to the controls. The control at 5°C was significantly higher than all other treatments from day 12 onwards and reached 7.2 by day 20 indicating microbial spoilage. In comparison the other treatments did not exceed the initial pH of 6.4 at day 19 (Figure 5.2).

Driploss
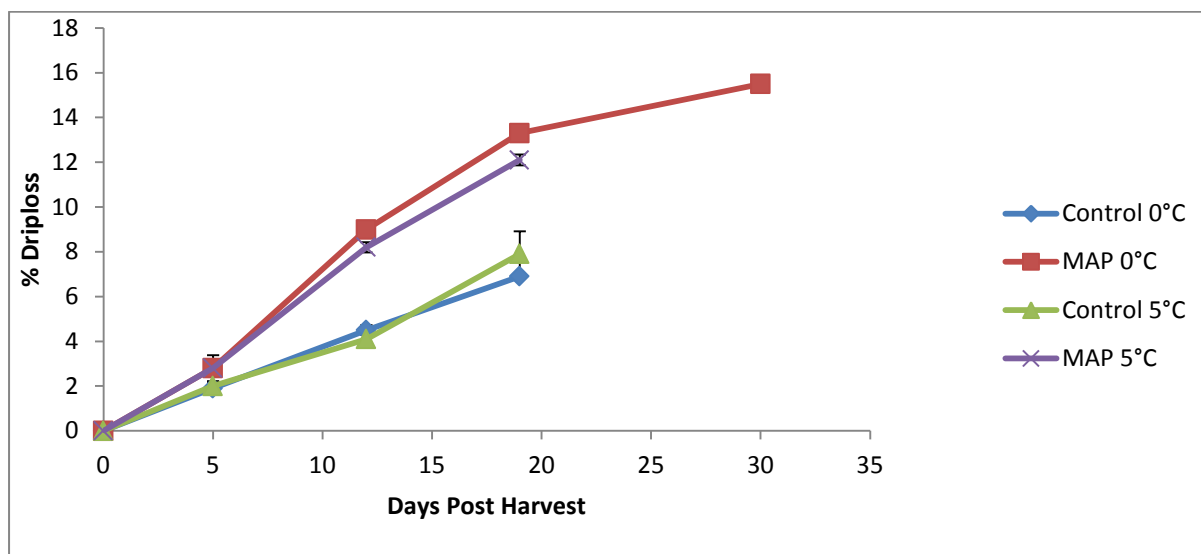
Figure 5.3: Mean (n=2) driploss (± SEM) for flathead fillets stored as controls in gas permeable plastic bags and MA packs (gas ratio of 45:55 ($CO_2 O_2$)) at temperatures of 5°C and 0°C sampled at 0, 5, 12, 19 and 30 days or until spoiled.

Driploss was generally linear for all treatments over the first 19 days of storage. By day 19 mean losses over initial weights were control 0°C = 7%, control 5°C = 8%, MAP 0°C = 13% and MAP 5°C = 12% (Figure 5.3). The results indicated that driploss as a percentage of original fillet weight was statistically larger in MA treated packs than in controls at both 0°C and 5°C, $F = (3,12) = 23.185$, sig .000, $p<0.05$. Pairwise comparisons with Bonferroni correction indicated MAP at 0°C (M=93.75) and MAP at 5°C (M=94.375) were not significantly different from each other at a significance of ($p = 0.138$) but were significantly different from controls at 0°C and 5°C (M=96.5 and M=96.65) which in turn were similar to each other ($p = 0.934$).

Texture
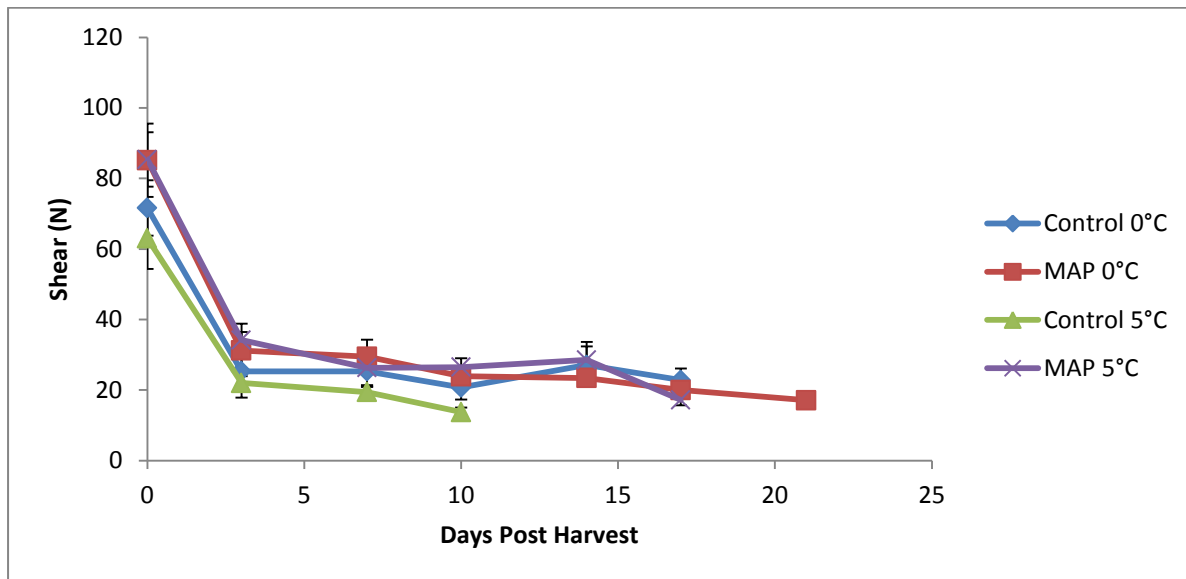
Figure 5.4: Mean (n=5) texture of (± SEM) flathead fillets stored as controls in gas permeable plastic bags and MA packs (gas ratio of (45:55 $CO_2 O_2$)) at temperatures of 0°C and 5°C sampled at days 0, 3, 7, 10, 14, 17 or until spoiled.

As determined by the shear texture method, a marked decrease in texture was observed at day 3 from initial pre-rigor readings. The residual texture remaining at day 3 for each of the controls and temperature treatments was in the range 32 – 40 % of the initial texture with little difference between controls and MAP treatments. The control at 5 C° was judged to be spoiled at day 10 and no further readings were undertaken. All other treatments at 0°C and 5 C° temperatures continued to gradually decline with the residual texture remaining at day 17 being control at 0°C = 32%, MAP at 0° C = 25% and MAP at 5°C = 20% of initial day 0 readings. The trend of slow decline from day 3 levels continued for MAP 0 C°, the only remaining treatment, which retained 20% of its original texture at day 21 (Figure 5.4). An analysis of days 0 to 10 data by repeated measures ANOVA indicated that there was no

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significant difference in texture attributable to either treatment or temperature (control or MAP at 0 C° and 5 C°) over time.

Taste Panel

A 9 point Hedonic scale acceptance test was applied to 3 species of flathead (*Platycephalus bassensis* and *Neoplatycephalus aurimaculatus* and *N. richardsoni*) with 28 tasters participating. The mean score for the treated samples (*Platycephalus bassensis*) was 7.11 ± 0.24 and the retail control (*Neoplatycephalus aurimaculatus* or *N. richardsoni*) 7.14 ± 0.22. Twelve tasters preferred the treated samples while 9 preferred the control and 7 had no preference of one sample over the other. A Kruskal-Wallis ANOVA indicated there were no significant differences ($p < 0.05$) between the treated flathead (MAP 21 day, 45:55 $CO_2:O_2$ ratio) *Mean Rank* 28.55 and the control (purchased fresh from a local fish monger) *Mean Rank* 28.45. χ^2 (corrected for ties) = 0.001, $df = 1$, $N = 28$, $p = 0.979$. Samples of the control (retail purchased) and treated raw material were taken before tasting and analysed by total plate count (TPC). The results were a mean ($n=3$) of 1.2×10^5 CFU/g for the treated flathead (MAP 21 days at 0°C) and 1.2×10^6 CFU/g for the retail purchased flathead.

5.4 Discussion

The concept of quality and freshness in relation to seafood has been discussed by a number of authors including Bremner and Nielsen (H.A. Bremner, 2000; H.A. Bremner & Sakaguchi, 2000; Nielsen et al., 2002). Businesses define functional properties for produce they procure as product specifications where parameters are defined and attributed ratings. To be accepted, a product must achieve a certain level of performance against the stated criteria. International supermarkets (Waitrose) and food retail outlets McDonalds, Captain D's and Red Lobster for

example have product specifications and rigorously assess incoming product against these standards. In fresh-cut produce there are generic quality, packing and preservation guidelines for producers (Faber et al., 2003).

In contrast, the Australian chilled seafood sector has no indicative standard for quality shelflife. The generally accepted benchmark of 5 x 10^6 CFU/g (AQIS) to 10^7 CFU/g (ICMSF) (superseded food standards) is based on maximum microbiological standards that were set by regulators in the past as indicators of product safety. These regulatory maximum numbers of organisms have been adopted as a *defacto* standard by industry. However, microbial numbers are not always good indicators of eating quality and a difference exists between shelflife based on microbial and sensory criteria (Huss, 1995; Liu et al., 2006). As a general indication, microbial counts of 10^7 and greater can produce metabolites including TMA-associated off odours and flavours, H_2S-related off odours and flavours and IMP, which is broken down by bacteria to form hypoxanthine (Dalgaard et al., 1993; Gram & Huss, 1996; Huss, 1995; Jorgensen & Gibson, 1988; McMeekin & Ross, 1996). In addition, other volatiles including, sulphur compounds, aldehydes, ketones and esters attributable to bacteria develop (Gram & Huss, 1996). The production of these metabolites results in reduced palatability and the product being no longer acceptable or representing value for money at the retail asking price. Therefore maximum quality shelflife occurs some time before these products are detected.

Best Practice Effect on Raw Material

One of the key assumptions of this study was that best practice methods applied throughout the supply chain would result in extended lag phases and maximised microbial shelflife. The microbiological flora of seafood products consists of those naturally on the animal at harvest

and those acquired as contaminants during handling and processing (Huss, 1995; McMeekin & Ross, 1996). The progression of organisms in seafood during storage is influenced by initial numbers and species composition in combination with factors they are exposed to during storage (McMeekin & Ross, 1996). In order to limit microbial numbers and species diversity on processed fish, best practice temperature, hygiene and sanitation practices were applied.

Flathead were immediately stored post capture in undamaged, washed and sanitised containers of ice slurry and processed under hygienic conditions with benches rinsed with town water during processing. The effectiveness of this method was confirmed by both pre and post processing microbial assessments of cutting boards with colony numbers for all sites less than the minimum levels of detection of 25 CFU/cm^2 (AOAC, 2000). Pre-processing (TPC) 62% of sites recorded 0 colonies while post processing only 9 of the 13 sites (69%) recorded any colonies.

The application of best practice methods resulted in pre-packing microbial (TPC) numbers ranging from 6.8 – 13.0 x 10^2 CFU/g with a mean of 1 x 10^{3}CFU/g. These counts are significantly less than those reported in other Australian studies by McMeekin for frozen defrosted flathead of 4.46 x 10^4 CFU/g (McMeekin et al., 1982), Statham 10^4 CFU/g for trevalla (*Hyperoglyphe porosa*) (J.A. Statham & Bremner, 1985), while Tamplin reported counts (CFU/g) of 5.2 x 10^5 in pink ling, 4.2 x 10^4 Goulds squid, 5.7 x 10^5 banana prawns and 4.1 x 10^4 saucer scallops (Tamplin, 2008). Gillespie noted that bacterial counts increased from 10^5 post filleting to 10^7 at retail shops (Gillespie & Macrae, 1975) while Slattery found counts of 10^5 in fish processing and packing areas (S. Slattery et al., 1998). The implications of initial product contamination at 10^4- 10^5 CFU/g on shelflife are reduction or elimination of

the lag phase and negation of the benefits of MAP (S. Slattery et al., 1998). From the current study it can be estimated

that to reach 10^4- 10^5 CFU/g the flathead control at 0°C took 9-11 days, MAP at 5°C, 11-13 days and MAP at 0°C did not reach these levels after 32 days storage.

Microbial Shelflife

Air Stored at 5°C

The primary spoilage mechanism in this study of air-stored flathead at 0°C and 5°C was determined to be microbial. Flathead at 5°C or less achieved a TPC of 1.5 x 10^7 on day 9 post harvest (Figure 5.1). At this point the product had reached its microbial shelflife and was considered to have exceeded its quality shelflife. It is concluded that the maximum quality shelflife for flathead fillets handled throughout the supply chain under best practice conditions is 6-7 days at 5°C. This conclusion is based on an initial microbial lag phase of 4 days and 2-3 days of logarithmic growth resulting in a microbial TPC of less than 10^6 CFU/g at 6-7 days. This conclusion is supported by the taste panel data where the retail purchased control had a TPC of 1.2 x 10^6 CFU/g and was considered to be of good eating quality. At 6-7 days pH had increased from a mean of 6.35 at day 5 to 6.5 at day 7, driploss had risen from 2% at day 5 to an estimated 3% at day 7 and texture declined from 19 N on day 7 to 14 N on day 10 (Figures: 5.2. 5.3, 5.4), all indicative of increasing spoilage (Sivertsvik et al., 2002). In practical terms, a maximum shelflife of 7 days post harvest for flathead at 5°C or less would only allow at best 4 days for retail sale. This scenario assumes a vessel landing catch daily with processing and distribution over the subsequent 2 days.

Air Stored at 0°C

The indicator of maximum microbial shelflife, TPC 10^7 CFU/g (ICMSF., 1986) was reached in 18 days for flathead stored at 0°C. This occurred typically 3 days later than the upper shelflife estimates for cod, haddock, hake and lean fish (Dalgaard, 1995; Huss, 1995; Sivertsvik et al., 2002), which shows the benefit of best practice methods applied throughout the supply chain.

The maximum quality shelflife for flathead at 0°C was estimated at 13-14 days post harvest. This estimate represents a 100% increase in quality shelflife over flathead fillets stored at 5°C and correlates well with the equivalent days on ice multiplier of 2.2. At 13-14 days, pH was slightly lower at 6.31 compared to 6.33 at day 3 indicating microbial numbers were not excessive. Microbial counts were less than 10^6 CFU/g, driploss was 4% and texture was little altered from day 3 levels decreasing only slightly from 25 N to 23 N (Figures: 5.1, 5.2, 5.3, 5.4).

MAP 5°C

Microbial spoilage was confirmed as the limiting factor in the shelflife of MAP flathead fillets at 5°C (gas ratio of 45:55 CO_2:O_2). Maximum microbial shelflife was estimated to occur at 19-20 days post harvest. These conclusions are supported by microbial counts of 3.6 x 10^6 CFU/g on day 18 and 3.3 x 10^7 on day 21, increasing pH which rose from 6.2 on day 12 to 6.33 on day 19 and the decline in shear strength from 29 N at day to 14 to17 N at day 17. In addition driploss increased from 8.2% on day 11 to 12.1% at day 19.

From the results it was concluded that the maximum quality shelflife should be estimated at 15 days post harvest. At this time bacterial counts are less than 10^6, driploss would be more

acceptable at less than 10%, pH would be less than 6.3 and texture only 15% down from day 3 levels (Figures: 5.1, 5.2, 5.3, 5.4).

MAP 0°C

The quality shelflife of flathead packed in MA at 0°C was estimated to be 21 days. By comparison the shelflife of lean fish in MAP was estimated at 7-21 days (Dalgaard, 1995), cod under MAP, 14-20 days at 0-3°C (Sivertsvik, 2007) and superchilled cod (-1.5°C) in excess of 24 days (T. Wang et al., 2008). The shelflife limitations of MA packed white fish have been generally considered to be due to microbial spoilage by the specific spoilage organism (SSO) *Photobacterium phosphoreum* (Dalgaard et al., 1993; Debevere & Boskou, 1996; Devlieghere & Debevere, 2000; Gram & Huss, 1996). Spoilage due to *P. phosphoreum* is characterised by the production of TMA and associated "fishy" off-odours and flavours (Dalgaard et al., 1993; Gram & Huss, 1996). The presence of off-odours were not detected after 30 days in MA packed flathead at 0°C indicating that *P. phosphoreum* may not have been present. This result is supported by a study by Powell on MA packed Atlantic salmon that failed to detect photobacteria using either culture-based or DNA-based methods (Powell & Tamplin, 2012).

Optimised Shelflife

At day 21, microbial counts of flathead packed in MA at 0°C were in the range of 10^3 CFU/g. Other indicators: pH remained at 6.2, driploss had increased to 13.5% and texture declined from 31 N on day 3 to 17 N at day 21 while the expert panel identified a slight loss of sweetness and flavour intensity. The taste panel did not significantly identify a difference between the treatment and control. The control was considered by the expert panel to be

nearing the end of its quality shelflife based on microbial counts of over 10^6 and the presence

of slight odour prior to cooking. Based on these factors it was considered that the product

packed in MAP and stored at 0°C was reaching its shelflife maximum primarily due to

autolytic spoilage as indicated by flavour, decreased shear strength and the high level of

exudates. This conclusion is supported by Fletcher who attributed the spoilage of King

salmon in MAP to gradual autolytic deterioration while counts remained below 10^7 CFU/g

(Fletcher et al., 2002).

Gas Mix

While high CO_2 concentrations are effective at inhibiting micro-organisms they also have a

number of negative impacts including increased driploss and organoleptic implications

(Dixon & Kell, 1989; Rosnes et al., 2006; Sivertsvik, 2007). To reduce the negative impacts

of high CO_2 levels a number of studies were conducted to determine the optimum gas mix for

the packing of whitefish. The use of O_2 with whitefish was found to have positive benefits

while reducing the negative benefits. The gas ratios used in this study (45:55% CO_2 O_2) were

based on the findings of Boskou, Sivertsvik and Hovda who reported atmospheres with high

concentrations of CO_2 and O_2 inhibited the reduction of TMAO to TMA or TVB-N in lean

fish (Boskou & Debevere, 1997, 1998; M. B. Hovda et al., 2006; Sivertsvik, 2007). A range

of CO_2, O_2 gas ratios have been reported to inhibit psychrotrophic and H_2S-producing

bacteria ranging from 60-70 ml CO_2 and 30-40 ml O_2, 50: 50 CO_2, O_2 and an optimised ratio

for cod of 37% CO_2 and 63% O_2. (Boskou & Debevere, 1998; M. B. Hovda et al., 2006;

Sivertsvik, 2007). In contrast Dalgaard found *P. phosphoreum* as the limiting factor in Danish

cod fillets packed in 60: 40 CO_2 and O_2 mix (Dalgaard et al., 1997).

pH

The pH of MAP packed at 0°C flathead portions decreased from 6.4 at day 0 to 6.2 at day 5. The pH remained at 6.2 until the end of monitoring at day 30. This initial decrease is in line with the effects of CO_2 applied to fish tissue. The CO_2 gas in the pack head space is dissolved in the liquid phase of the flesh primarily as dissolved CO_2 gas which forms carbonic acid. At pH >6.0 carbonic acid will dissociate to form bicarbonate and hydrogen ions which causes a drop in flesh pH (<0.1 pH unit) (Farber, 1991). The pH of flathead portions packed in MAP at 0°C gave no indication of microbial spoilage.

Driploss

The mean level of driploss for flathead was found to be significantly higher in MAP than in the air packed control of 13.3% and 6.9% respectively at day 19. This finding is supported by other studies on white fish including cod and spotted wolf-fish. Cod packed in 100% CO_2 were reported to have 13 to 17% driploss, while cod packed at low CO_2 levels had 6-7% (Sivertsvik, 2007). Higher driploss levels were found in spotted wolf-fish packed in CO_2, N_2 (ratio 60: 40) at 4°C than in air stored. Additionally, MAP driploss levels were higher at 4°C than at -1.0°C indicating a temperature effect (Rosnes et al., 2006). This current study did not identify a significant temperature effect at 0°C and 5°C but did confirm a significant treatment effect impacting on driploss.

Texture

Texture is one of the most important quality factors of fish (Hyldig & Nielsen, 2001). It has been reported that softening of fish flesh occurs within 24 hours post mortem at 0°C (Kagawa et al., 2002). The loss of shear strength found between initial readings at day 0 and day 3 is

likely to be associated with rigor mortis and is similar to textural degradation profiles of other species including Atlantic salmon, gilthead seabream and arrow squid (Ando et al., 1999; Huidobro et al., 2001; B. Roth et al., 2002). Comparatively, raw flathead muscle is initially stronger and maintains more residual strength post rigor than raw Atlantic salmon. Flathead had a mean (n=4) shear strength at day 0 of 76.25 N while Atlantic salmon had a mean (n=4) strength of 31.85 N. Shear strength declined post rigor for flathead and Atlantic salmon to 28 N and 16.25 N respectively. In comparison, flathead stored in MAP at 0°C by day 21 had a mean shear strength of 17 N. This indicates that flathead is firm in texture compared to Atlantic salmon and that the mean shear strength of flathead at day 21 is similar to Atlantic salmon at day 3. This is supported by the taste panel that did not statistically differentiate between the control or MAP treatment at 0°C.

Taste Panel

The inability of the taste panel to determine any preference between retail purchased flathead fillets and the 21 day old MAP stored at 0°C product lends support to the case for 21 days to be established as the maximum quality shelflife. This result was supported by the expert panel assessment that the retail fish and treated fish represented good edible attributes.

5.5   Conclusion

The results of the tests conducted during this study indicated that 21 days (equivalent time on ice of 21.47 days) is the maximum quality shelflife for skinless flathead fillets packed in a MA at 45:55 CO_2, O_2, ratio stored at a mean temperature of 0.1°C. This result is supported by taste panel, pH and microbial results. While driploss for MA packed fillets is higher than

60

controls the difference is considered not to be sufficiently large to represent a limitation to the use of MAP.

Typically flathead is air packed for retail distribution. The application of MA packing and best practice handling throughout the supply chain could double the current commercial shelflife.

6 Determination of Atlantic Salmon Shelflife

6.1 Introduction

Fatty Fish

Atlantic salmon (*Salmo salar*) available in the Australian fresh chilled retail sector are farmed in Tasmania. Aquaculture grown salmon have a moderate oil content but high levels of polyunsaturated fatty acids (PUFA) (Yearsley et al., 1999). Salmonids represent a significant proportion of the aquaculture produced fish consumed in Australia.

This species has a national distribution in retail and food service applications with the majority of Australian production being consumed domestically. Production figures in 2009-10 were 31 915 tonnes valued at 369 million dollars. In world terms the production of Atlantic salmon is over 1 400 000 tonnes (2010) and represents greater than 90% of the farmed salmon market and greater than 50% of the total global salmon market (FAO, 2011). All salmon used in this study were produced by Van Diemen Aquaculture, Rowella, Tasmania.

Experimental Objectives

- To determine the maximum quality shelf life of Atlantic salmon (*Salmo salar*) by applying best practice handling, processing and preservation methods during the study

- To assess microbial shelflife at 0°C and 5°C temperatures under different atmospheres.

6.2 Methods

The methods are as listed in the General Materials and Methods section with the following specific details and/or modifications.

Microbiology

Atlantic salmon samples were analysed for microbial counts (TPC CFU/g) on days 0, 5, 8, 11, 14, 17, 20, 23, 26, 29, 31 or until microbial counts exceeded 10^7 CFU/g (ICMSF., 1986). Logged chiller air temperatures for control and MAP at 5°C or less had a mean temperature of 4.7°C ± <0.1°C (range of 3.2°C to 14°C).

pH

At day 0, four fish with weights ranging from 3.1 to 3.56 kg and mean weight of 3.39 ± 0.1 kg were randomly selected and the flesh pH measured (general materials and methods). Individual fillets (n=8) from these fish were allocated to replicated treatments of MAP and control at 0°C and 5°C for pH measurement on days 0, 6, 9, 12, 16, 19 and 22 or until spoiled. Logged chiller temperatures for control and MAP at less than 5°C samples had a mean of 4.7°C ± <0.1°C (range 3.8 to 8.1°C).

Driploss

Fillets were portioned and the upper loin removed (Plate: 6.1). Driploss was determined for fillet portions (n = 8), with mean weights of 126 ± 12.5 g, (range of 61-176 g). Replicated treatments of MAP and control at 0°C and 5°C were measured for driploss on days 0, 6, 9, 12, 16, 19 and 22 or until spoiled.

Texture

Texture was determined by fish shear for 5 fish with a mean weight 3 kg ± 0.15 kg (range of 2.69-3.55 kg). Fish were initially filleted, skinned and loins removed (Plate: 6.1). The upper loins were selected to provide textural consistency due to increasing shear strength towards the tail (Ashton et al., 2010; Casas et al., 2006; Jonsson et al., 2001). Loins were cut length wise into 4 portions and allocated to treatments of MAP and control at 0°C and 5°C (Plate: 6.2). This provided 10 replicates of each treatment and temperature. Raw texture was measured on days 0, 1, 8, 13 and 20 or until spoiled. All measurements were conducted on similar cross sections of the fillet at each time point commencing at the rear of the portion on day 0 and working forward (Plate: 6.1). Samples were aligned so that the shear blade passed through the connective tissue joining the muscle blocks (Plate: 6.2). Logged chiller air temperatures for control and MAP at less than 5°C samples had a mean 4.7 ± 0.02°C (range 3.7 to 8.1°C).

Plate 6.1: Atlantic salmon fillet with upper portion removed for pH, driploss and texture assessment
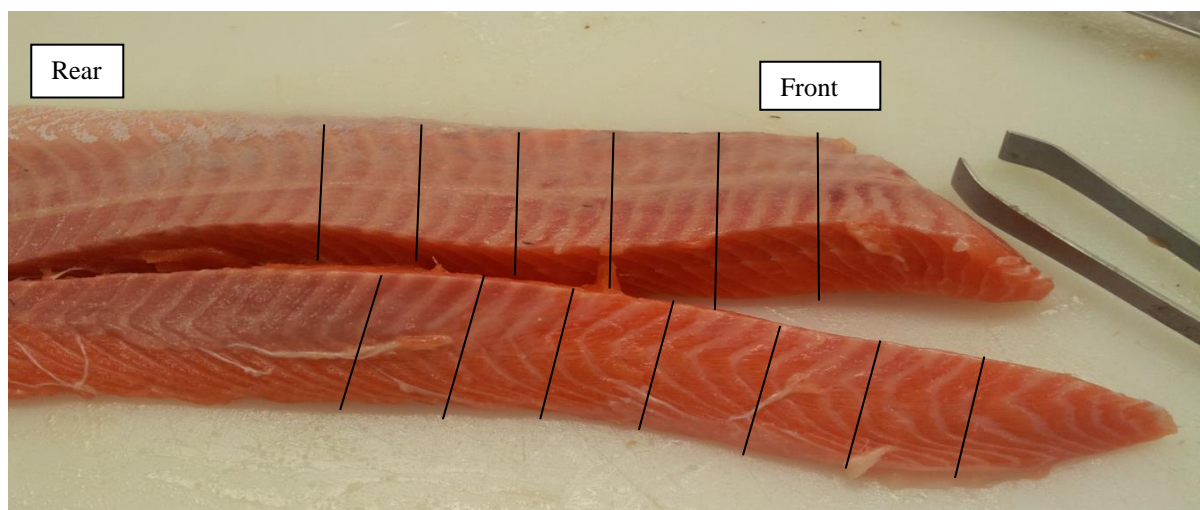
Plate 6.2: Strips of Atlantic salmon from the upper portion of the fillet (Plate 6.1) used in texture assessment. Black lines indicate >20 mm x 10 mm blocks which were removed from rear to front and trimmed for texture shear testing.

Sensory Analysis

Control and treated Atlantic salmon fillets were compared by taste panel. Prior to assessment treated samples and controls were assessed for general acceptability in a blind tasting by an expert panel of 3. The control and treated samples were considered by the expert panel to exhibit good edibility attributes and representative of the species. The expert panel assessed the untreated control material as having acceptable edible attributes after 11 equivalent days on ice. Microbial TPC's at the time of sensory testing were control mean 1.3×10^5 CFU/g and MAP 2.9×10^4 CFU/g.

Test Method Atlantic Salmon

A discrimination test was selected as the most suitable method to achieve the test objectives of determining if any difference could be detected in cooked skinless Atlantic salmon portions stored at 0°C for 11 days in air and treated samples MA packed in 100% CO_2 for 20

days at 0°C. An expert panel of 3 assessed the 11 day control and 20 day old treatment fillet as being of good eating quality. Of the range of discrimination tests available, the Triangle Test was selected as the most suitable. This was based on the statistical efficiency of the test in reducing number of tasters required to achieve a significant result.

Treated and control samples were frozen as fillets in sealed plastic pouches for 4 weeks at -25°C and were water thawed in the pouch at 17°C prior to portioning and cooking.

Fillets were prepared for portioning by removing the belly flap and discarding. The fillet was subsequently cut in half from front to rear. Portions of 40-50 grams were cut from the half fillet blocks. This process resulted in portions of similar dimensions reducing the potential for cooking variability. Samples were cooked in replicate frypans at 180°C for 1 minute on each side. Commercially available Canola oil spray was used to lubricate the frypans. Serving plates were marked with 3 randomly selected numbers according to the Serving Order for Triangle Test, Balanced Order (Appendix 2) (Meilgaard et al., 2007) .

Panel

A panel of 24 was used to determine if a difference in cooked taste existed between the 11 day on ice equivalent untreated control and the treated sample. The ages of panel members ranged from 18 to 60 years. The panel was heavily male dominated with 19 males to 5 females. The panellists were all regular seafood consumers. Panellist experience with the test species ranged from highly experienced to aware of the species. An orientation session was conducted prior to the test (Appendix 2).

6.3   Results

Storage Temperature

Samples stored at 5°C had a mean temperature of 4.7 ± <0.1°C over the 23 days logged.

Samples stored at 0°C had a mean temperature of 0.1 ± 0°C over the 29 days logged.

Equivalent Days on Ice

Equivalent days on ice calculations were performed on the logged temperature data and are listed in the table below. A mean equivalent days on ice multiplier factor was calculated at 2.13 ± <0.1 on logged 5°C or less data. It can be seen in Table 6.1 that storage at approximately 5°C more than doubled the equivalent days on ice, whereas storage at temperatures slightly above 0°C produced virtually no increase.

Table 6.1: Calculated equivalent days on ice for Atlantic salmon stored at 0°C and 5°C or less in comparison to actual days.

Days	5	8	11	14	17	20	23	26	29
Calculated DOI 0°C	5.15	8.24	11.33	14.42	17.5	20.6	23.69	26.78	29.87
Calculated DOI 5°C	10.16	16.56	24.46	30.49	36.85	42.88	49		

Microbial counts

The initial number of bacteria present on Atlantic salmon portions pre-packing averaged 3.5 x 10^3 CFU/g (TPC), ranging from 1.6 – 5.5 x 10^3 CFU/g.
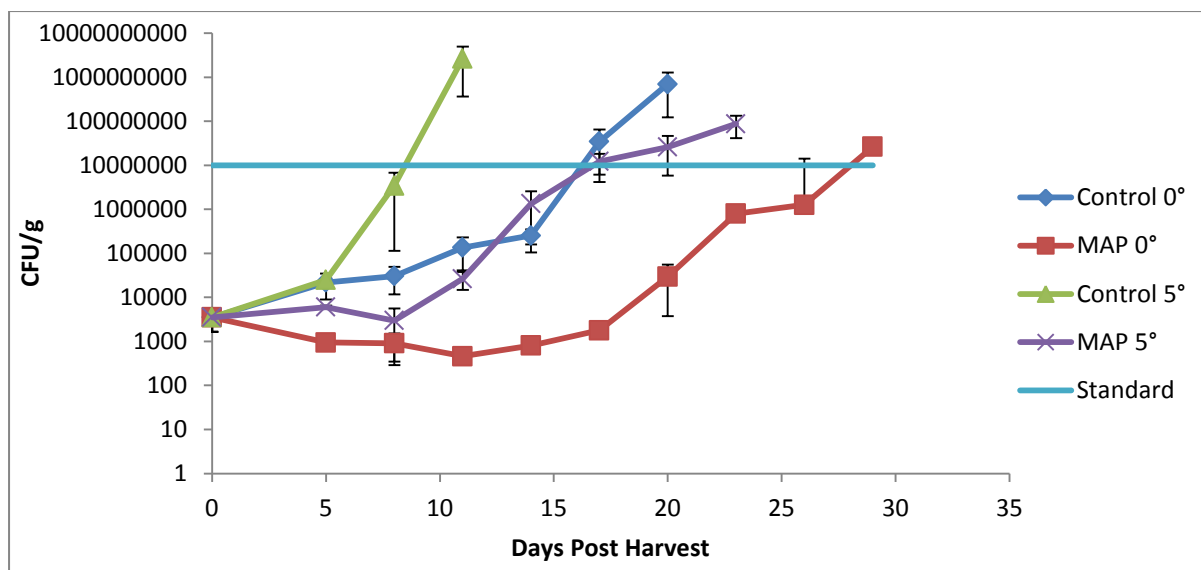
Figure 6.1: Mean (n=2) TPC (SEM ±) for skinless Atlantic salmon portions, control and MAP (100% CO_2) at 0°C and 5°C, sampled at 0, 5, 8, 11, 14, 17, 20, 23, 26 and 29 days or until spoiled. ICMSF (1986) standard of 10^7 CFU/g is indicated by the pale blue line.

Lag and Logarithmic Growth Phases

The mean counts of the controls indicated that the samples remained within the lag phase for a maximum of 4 days at 5°C and 8 days at 0°C. The microbial TPC (3.4 x 10^7 CFU/g) of control at 0°C had exceeded the standard on day 17, while the control held at 5°C (3.4 x 10^6 CFU/g at day 8) exceeded it after 9 days post harvest. The microbial counts of the MAP samples at 5°C and 0°C indicated a lag phase lasting just under 10 days at 5°C and about 17 days at 0°C. The microbial count (2.6 x 10^7) of MAP 0°C had exceeded the standard at day 29, while MAP held at 5°C (1.2 x 10^7 CFU/g) exceeded it after 17 days post harvest.

In a replicated study as part of this research the initial number of bacteria present on Atlantic salmon portions pre-packing (TPC) averaged 4 x 10^3 CFU/g. By day 5 counts of MA packed Atlantic salmon at 0°C had declined and ranged from 5 x 10^1 to 10^2 CFU/g (Figure 10.2).

The mean counts of samples packed in MA indicated that they remained within the lag phase for a maximum of 14 days at 5°C and 23 days at 0°C. The maximum microbial TPC (1.8×10^6 CFU/g) of control at 0°C exceeded the standard (10^7 CFU/g) after day 32, while the control held at 5°C (7.3×10^6 CFU/g) exceeded it after 20 days post harvest (Figure 10.2).

pH
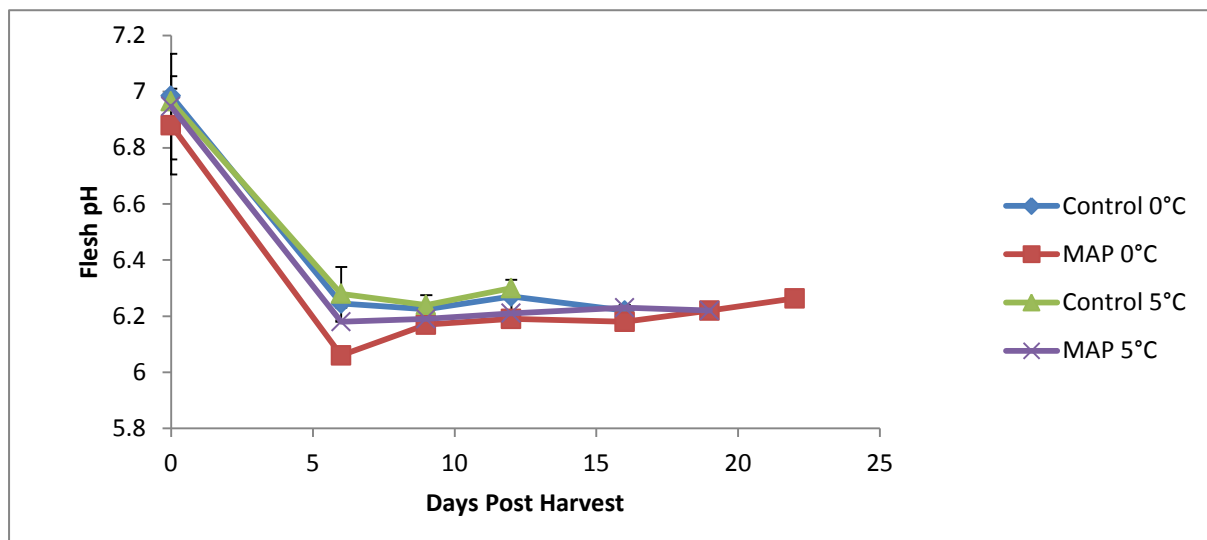
Figure 6.2: Mean (n=2 control, n=3 MAP) muscle pH (±SEM) for Atlantic salmon loins stored as controls in gas permeable plastic bags and MA packs (gas 100% CO_2) at temperatures of 5C° and 0C° sampled at 0,6,9,12,16, 19 and 22 days or until spoiled.

An initial pH measurement was conducted 2 hours post capture and ranged from a minimum of 6.96 to a maximum of 7.01 with a mean of 6.98 ± 0.01. The pH declined for all samples from day 0 readings with the 2 MAP treatments reaching pH 6.06 and 6.18 and controls 6.25 and 6.28 on day 6 (Figure 6.2).

The pH of the 5°C control did not increase correspondingly with increasing TPC over time. The pH changed little from 6.25 on day 6 to 6.3 on day 12, with corresponding microbial counts (TPC) increasing to 10^9 CFU/g at day 12 at which time the flesh was considered

spoiled. This trend was also observed for controls stored at 0°C with a pH of 6.28 at day 6 decreasing to 6.22 on day 16 and an estimated TPC of 10^7 CFU/ g indicating the end of microbiological shelflife.

The pH of the MAP samples at 5°C also changed little form the day 6 reading of pH 6.18 reaching 6.22 on day 19. This corresponded to microbial counts (TPC) reaching 2.5 x 10^7 CFU/g at day 20 at which time the flesh was considered spoiled. MAP stored at 0°C had a day 6 reading of pH 6.06 which increased to pH 6.26 and an estimated TPC of 10^5 at day 22.

A repeated measures ANOVA was used to analyse the pH data for all samples from days 0-12. The data failed Mauchly's test for sphericity and so the Greenhouse-Geisser correction was applied. The ANOVA did not identify any significant difference in pH between treatments or storage temperatures (F (1.09, 4.35) = 2.18 sig. 0.211 and F (1.09, 4.35) = 0. 42 sig. 0.564 respectively.

Driploss

The results (Figure: 6.3) indicate that driploss as a percentage of original fillet weight was statistically larger in MA treated packs than in controls at both 0°C and 5°C, (F = (3, 12) = 7.9 sig. 0.004, p< 0.05). Driploss was approximately linear over time and at day 16 mean losses over initial weights were control 0°C = 5.1%, control 5°C (day 12) = 3.5%, MAP 0°C = 6.3% and MAP 5°C= 6.65 %. Drip loss continued to increase over time and at day 19 mean drip of MAP 0°C was 7.2% and MAP 5°C 9% (Figure: 6.3).
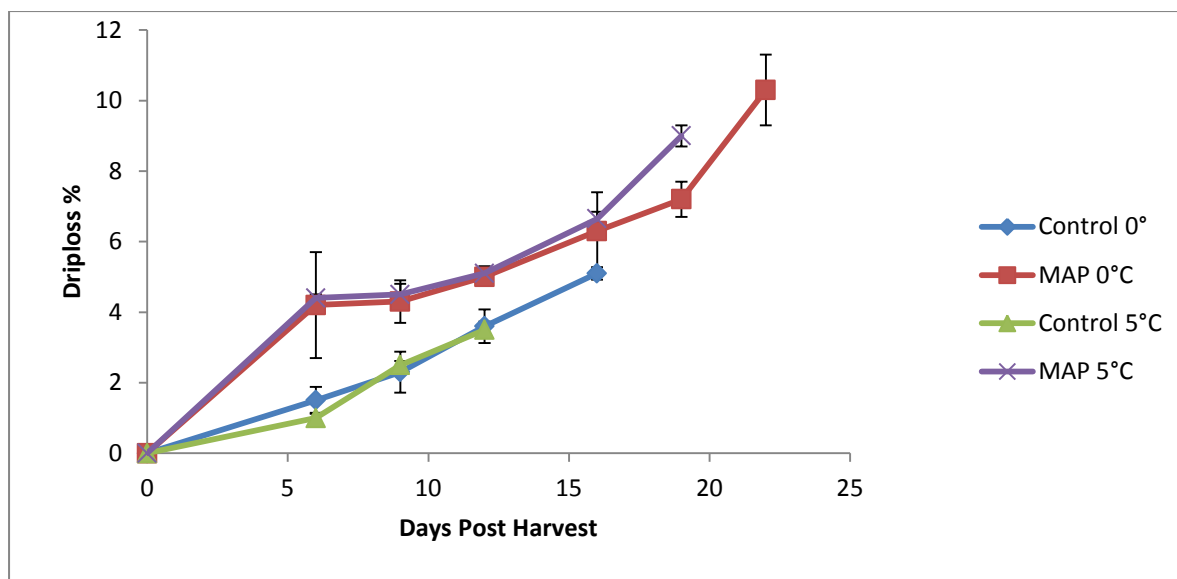
Figure 6.3: Mean (n=2) driploss (±SEM) for Atlantic salmon loins stored as controls in gas permeable plastic bags and MA packs (gas ratio 100% CO_2) at temperatures of 5°C and 0°C sampled at 0, 6, 9, 12, 16, 19 and 22 days or until spoiled.

Texture

The most significant shear strength decline occurred between day 0 (prerigor) and day 1 (in rigor). Shear percentages decreased for control at 0°C and 5°C by 40% and 43% respectively and MAP at 0°C and 5°C by 57% and 69% respectively from initial day 0 figures (Figure: 6.4). Shear strength data for control 0°C, MAP 0°C and MAP 5°C was assessed to determine if a significant difference attributable to treatment or temperature over time could be detected.
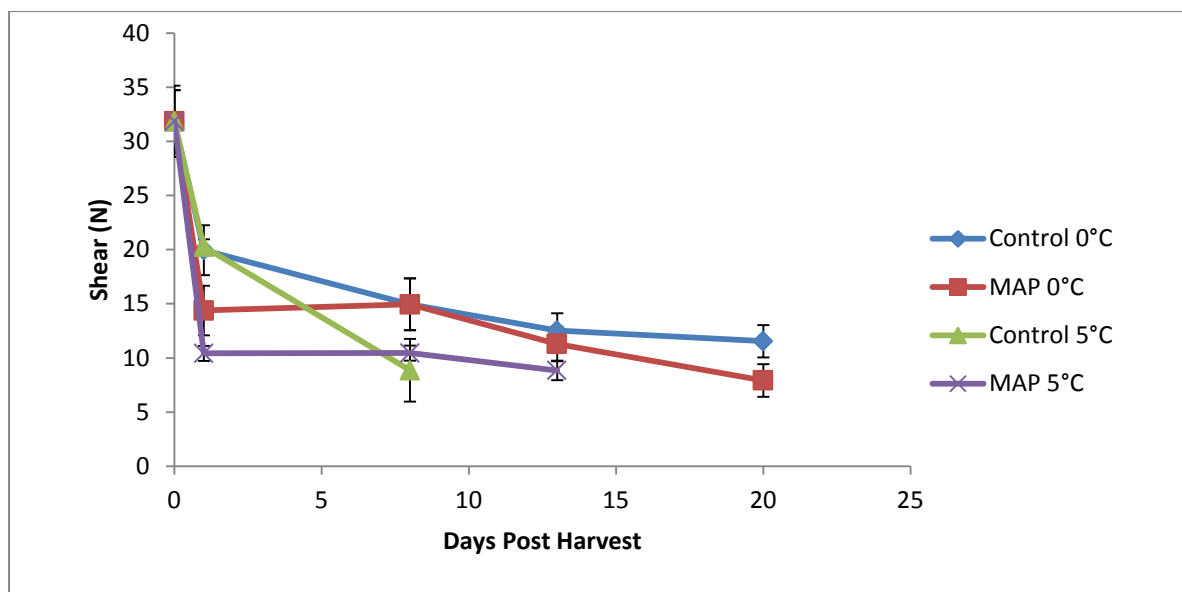
Figure 6.4: Mean (n=10) shear strength (±SEM) for Atlantic salmon loins stored as controls in gas permeable plastic bags and in MA packs (gas ratio 100% CO_2) at temperatures of 5°C and 0°C sampled at 0,1,8,13 and 20 days or until spoiled.

A repeated measures ANOVA and pairwise comparison with Bonferroni correction was used to analyse the data from days 0 to 13 (Table 6.2). Mauchly's test indicated that sphericity had been violated and so the Greenhouse-Geisser adjustment was applied. Atlantic salmon packed in a MA of 100% CO_2 and stored at 5°C or less had significantly ($F =, (2, 27) = 11.72$ sig. 0.00, $p < 0.05$) less shear strength than the control and MAP at 0°C.

Table 6.2: Pairwise comparison identifying treatment 3, MAP at 5°C, as being significantly different from control and MAP at 0°C

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.[a]	95% Confidence Interval for Difference[a]	
					Lower Bound	Upper Bound
1.00	2.00	2.289	1.565	.465	-1.706	6.285
	3.00	7.400[*]	1.565	.000	3.405	11.396
2.00	1.00	-2.289	1.565	.465	-6.285	1.706
	3.00	5.111[*]	1.565	.009	1.116	9.106
3.00	1.00	-7.400[*]	1.565	.000	-11.396	-3.405
	2.00	-5.111[*]	1.565	.009	-9.106	-1.116

Based on estimated marginal means

a. Adjustment for multiple comparisons: Bonferroni.

*. The mean difference is significant at the .05 level.

Sensory Analysis

A triangle discrimination test was conducted with 24 tasters in the panel. Of the 24 tasters, 10 identified the odd sample correctly. This level of identification was not significant at 0.05, 5% level based on the Critical Number of Correct Responses in a Triangle Test tables (Meilgaard et al., 2007).

This means that there is no reason to reject the null hypothesis of no difference between the control and MAP treatment.

6.4   Discussion

Atlantic salmon has a national distribution through major supermarkets. Given the length of the supply chain, shelflife becomes a key economic driver. Profitability is reduced by discounting and finally disposal due to quality deterioration. There have been no published maximum microbial or quality shelflife estimates for Atlantic salmon in the Australian context. Given the demands by retailers for extended product shelflife, a lack of a comparative basis makes supply chain optimisation by industry difficult. The estimates derived in this study were achieved by the application of best practice procedures. These procedures should be replicable in commercial applications..

There have been a number of studies investigating the spoilage process of both King and Atlantic salmon packed under varying conditions (Fletcher et al., 2002; Mace et al., 2012; Pastoriza et al., 1996a; Powell & Tamplin, 2012; Sivertsvik et al., 2003). The time, temperature and handling history of raw material in studies is often unknown or poorly described resulting in initial microbial numbers ranging from < 25 CFU/g (Sivertsvik et al., 2003), 10^3 CFU/g (Fletcher et al., 2002), 10^4 CFU/g (Fernandez et al., 2009) and 10^3-10^5 CFU/g (Mace et al., 2012). Initial contamination is a crucial factor affecting shelflife (Mace et al., 2012). The microbial flora present on Atlantic salmon were identified by genetic techniques and indicate far more complex and variable communities than previously reported, possibly due to contamination within the chain (Powell & Tamplin, 2012). Evidence exists that this contamination may occur on individual fish up to the time of packing resulting in shelflife variability within the same batch (Powell & Tamplin, 2012).

Shelflife is generally quoted in terms of storage time in days to sensory rejection, or to specific microbial or chemical parameters or combinations of these. This, in combination

with variability in gas mixes and gas product ratios as well as a lack of information on relative spoilage rates at 0°C makes direct comparative exercises difficult. These issues may partially explain the large shelflife variations quoted for salmon (Reddy et al., 1992; Sivertsvik et al., 2002)

Microbial Shelflife

In this study, it was found that microbial growth was always higher at the same time and temperature in air-stored Atlantic salmon compared to MA-packed salmon. This result is in agreement with the findings of Sivertsvik and confirms the benefit to shelflife of packing in a modified atmosphere containing carbon dioxide (Sivertsvik et al., 2003). The primary limiting factor of air packed Atlantic salmon at 0°C and 5°C and MAP stored at 5°C was microbial. MAP stored at 0°C was considered to be spoiled primarily by autolytic factors as indicated by high levels of driploss and declining shear strength , conforming the results of Fletcher on King salmon (Fletcher et al., 2002).

Specific Spoilage Organism (SSO)

The spoilage of chilled fish is characterised by the decrease in microbial diversity over time and the emergence of a Specific Spoilage Organism (SSO). In air packed Atlantic salmon the main SSO's are reported to be *Shewanella putrefaciens* and *Pseudomonas* spp. (Gram & Melchiorsen, 1996; Mace et al., 2012; Powell & Tamplin, 2012; Reynisson, Lauzon, et al., 2009; Sivertsvik et al., 2002). While in MA packs *Photobacterium phosphoreum* is the SSO identified as being responsible for the production of TMA and spoilage (Dalgaard et al., 1997; Emborg et al., 2002; Gram & Huss, 1996). It has been reported that SSO's have to be present in numbers of at least 10^7 CFU/g to produce sufficient metabolites to cause spoilage (Dalgaard et al., 1997; Mace et al., 2012). Packs with counts of 10^7 CFU/g *Photobacterium*

phosphoreum had correspondingly similar numbers of non classified organisms (Dalgaard et al., 1997). In this study the maximum microbial shelflife was set at 10^7 CFU/g and the maximum quality shelflife was found to occur at a TPC of less than 10^6 CFU/g. In MAP at 0°C counts did not reach 10^7 CFU/g until after 26 days at which time the product had been judged to have exceeded its maximum quality shelflife of 20 days due to non microbial factors. In 2 independent studies on Atlantic salmon conducted by Powell using 16S rRNA techniques no *Photobacterium phosphoreum* were identified (Powell & Tamplin, 2012). The second study is in draft awaiting publication (Powell, S. 2013, pers. comm, 24 April). It is concluded that the SSO, *Photobacterium phosphoreum* did not significantly limit MAP shelflife at 0°C in Atlantic salmon or any other species in this study.

Air Stored at 5°C

The maximum microbial shelflife for air stored Atlantic salmon at 5°C was estimated to be 9 days based on microbial count (TPC) of 3.4 x 10^6 CFU/g on day 8 rising to 10^8 CFU/g on day 11 (Figure: 6.1). The day 11 count is nearing the maximal microbial value for Atlantic salmon of $10^{8.5}$ CFU/g (Mace et al., 2012). Correspondingly drip loss rose from 1% at day 6 to an estimated 2.2% at day 7 and texture declined from 32 N on day 0 to 9 N on day 8 (Figure: 6.3, 6.4), indicating increasing spoilage (Sivertsvik et al., 2002).

The quality shelflife for Atlantic salmon handled throughout the supply chain under best practice conditions was found to be 6-7 days at 5°C. This conclusion is based on an initial microbial lag phase of 4 days and 2-3 days of logarithmic growth resulting in a microbial TPC of no more than 10^6 CFU/g at 6-7 days. The count of 10^6 CFU/g as the upper microbial limit of quality shelflife in Atlantic salmon was supported by Sivertsvik who reported a good correlation between microbial count and sensory spoilage (Sivertsvik et al., 2003). This result

is similar to the 7 day shelflife estimate for Atlantic salmon packed in air at 4.4°C (Sivertsvik et al., 2003).

Air Stored at 0°C

The lag phase of 8 days for air packed Atlantic salmon at 0°C is longer than the published figure for king salmon of 4 days at 0°C (Fletcher et al., 2002). Maximum microbial shelflife was estimated to be 16 days with TPC's rising from 2.5×10^5 CFU/g on day 14 to 3.4×10^7 CFU/g by day 17. This figure compares to a shelflife estimate of 11 days at 2°C based on 10^6 CFU/g as the upper acceptable limit (Fernandez et al., 2009), 18 days for king salmon based on 3×10^8 CFU/g (Fletcher et al., 2002), 14 days based on 10^6 CFU/g at 0°C (Rosnes et al., 2003) and 10 days at 2°C TPC 7.4×10^7 CFU/g (Pastoriza et al., 1996a).

The maximum quality shelflife for Atlantic salmon at 0°C was estimated at 13-14 days post harvest. This estimate represents a 100% increase in quality shelflife over Atlantic salmon fillets stored at 5°C and correlates well with the equivalent days on ice multiplier of 2.2. The basis for the 13-14 days estimate was a mean microbial total plate count of 2.5×10^5 CFU/g, driploss of 4% and texture of 13 N having decreased only gradually after the initial rapid drop from day 0 to day 1 (Figure: 6.1, 6.2, 6.3, 6.4). This estimate is similar to the literature where 14 days shelflife was estimated for Atlantic salmon at 0°C (Sivertsvik et al., 2003), 15 days for 25% of a sensory panel to gave a fail score (Fletcher et al., 2002) and 14 days (Emborg et al., 2002).

MAP at 5°C

Maximum microbial shelflife was achieved at day 17 post harvest with a TPC of 1.2 x 10^7 CFU/g by comparison the microbial shelflife of commercial MA packed Atlantic salmon is reported to be 13 days based on microbial TPC data (Sedman, T. 2011, pers. comm, 7 June). This estimate of general commercial shelflife performance is supported by the TPC of 1.2 x 10^7 found by Powell at day 15 on commercially packed Australian produced Atlantic salmon (Powell, S.M. 2011 pers. comm.6 November).

Microbial spoilage was confirmed as the limiting factor in the shelflife of MAP at 5°C (gas 100% CO_2) product with a TPC of 1.2 x 10^7 CFU/g at day 17 post harvest. Other parameters at this time were pH which had changed little from 6.21 on day 12 to 6.23 on day 16 and a shear strength not significantly different from day 1 to day 13 readings. In addition driploss increased from 5.1% on day 12 to 6.65% at day 16.

Maximum quality shelflife was estimated at 12 days post harvest. At this time bacterial counts were less than 10^6 CFU/g, driploss was 5%, pH was 6.21 and texture was only slightly down on day 1 measurements (Figure: 6.1, 6.2, 6.3, 6.4). By comparison, MA packed Atlantic salmon stored at 4.4°C had a sensory shelflife of 10 days (Sivertsvik et al., 2003).

MAP 0°C

In MAP at 0°C, bacterial growth was markedly inhibited by high CO_2 levels and low temperatures. In this study, the lag phase was increased to 17 days and the growth rate of bacteria reduced in line with previous research (Dalgaard et al., 1993; Pastoriza et al., 1996a; Reddy et al., 1992; Sivertsvik et al., 2003). For example, Fletcher in a study of King salmon reported a lag phase of 18 days for MAP at 0°C (Fletcher et al., 2002).

The decrease in MA packed Atlantic salmon microbial TPC numbers at 0°C from 3.5 x 10^3 at day 0 to 9 x 10^2 at day 8 (Figure 6.1) has been reported by other researchers. Fletcher documented microbial counts decreasing until day 8 after packing in MAP while Fernandez described initial numbers decreasing until day 5 in MAP at 2°C (Fernandez et al., 2009; Fletcher et al., 2002).

The maximum microbial shelflife (TPC 10^7 CFU/g) was estimated at 28 days post harvest with counts (TPC) increasing from 1.2 x 10^6 CFU/g on day 26 to 2.6 x 10^7 CFU/g on day 29.

The maximum quality shelfife was calculated to be 20 days. At this time TPC was 2.9 x 10^4 CFU/g, the pH was 6.22, driploss was 8% and texture 8 N. Reduced texture and a slight deterioration of flavour as a result of autolytic spoilage was considered by the expert panel to be the main limiting factor. The taste panel did not record a significant response at $p = 0.05$. It was considered that while the result was not significant at 0.05 the product was nearing its quality shelflife limit based on high driploss, softening texture and no further panel assessment was performed.

Other studies on Atlantic salmon give support to the quality shelflife conclusions with microbial counts in MAP at 2°C of 10^6 CFU/g at 24 days being documented (Fernandez et al., 2009). Shelflife based on sensory assessment was determined to be in excess of 14 days (Emborg et al., 2002), 21 days (Fletcher et al., 2002) and 20 days (Pastoriza et al., 1996a). Superchilled (-1.9°C) salmon had a sensory shelflife (rated as good quality) of 24 days (Sivertsvik et al., 2003).

pH

The mean (n= 10) initial pH of Atlantic salmon was found to be 6.98. This figure compares to 7.63 and 7.08 reported for rested anesthetized fish (Fletcher et al., 2003), pH 7.5 for rested fish decreasing to 6.67 and 6.4 on day 4 post harvest (Erikson et al., 2011) and pH 7.0 unstressed and pH 6.7 stressed fish (Sigholt et al., 1997). This result indicates the current harvest practices of the farm from which the fish were purchased are in line with best commercial practice.

The decrease in pH observed between days 0 and 6 is indicative of fish attaining minimum pH with the resolution of rigor mortis. Controls at 0°C and 5°C attained mean (n= 2) pH of 6.25 and 6.28 respectively (Figure: 6.2). These results are similar to those reported by Siversvik of 6.2-6.3 and higher than for King salmon, pH 6.12 (Fletcher et al., 2002; Sivertsvik et al., 2003).

The air packed fish had a higher pH than those packed in MA at 0°C and 5°C which had pH values of 6.06 and 6.18 respectively (Figure: 6.2). This decrease in pH for MAP packed samples is typical and caused by the creation of carbonic acid, a mild acid formed by the disassociation of CO_2 into the water phase of the flesh (Dalgaard et al., 1993; Dixon & Kell, 1989; Farber, 1991). These results are similar to the reported pH 6.1 for MA packed Atlantic salmon (Hansen et al., 2009).

Driploss

The effects of temperature on driploss remain unresolved after this study. The results indicated a significant effect associated with treatment over time in line with the findings of (Dalgaard et al., 1993). MAP at both 0°C and 5°C had higher levels of driploss at 16 days

post harvest than controls at 0°C and 5°C. This result was different to that reported by Sivertsvik who documented a significant increase in driploss associated with temperature and a temperature, MAP interaction (Sivertsvik et al., 2003). In contrast, no significant difference between the control and CO_2 treated samples was determined by Pastoriza (Pastoriza et al., 1996a) while Fletcher found all treatments (MAP and air stored control) gave linear increases in driploss over time at the temperature of melting ice (Fletcher et al., 2002).

The driploss figures determined for Atlantic salmon in the current study at 16 days were higher than those found by other researchers in both MAP and air stored environments. All exudates were initially clear, becoming increasingly cloudy in appearance over time. MAP at 0°C and 5°C reached 6.3% and 6.65% driploss respectively while the air stored control at 0°C and 5°C (day 12) had 5% and 3.5% driploss respectively (Figure: 6.3). These results compare to 2% driploss for air stored at 0°C after 14 days (Rosnes et al., 2003), 3% for MAP at 2° and 4-5% in air stored at 4°C (Sivertsvik et al., 2003) and 2.9% at day 22 for air stored at 0°C (Fletcher et al., 2003). The possible cause of these elevated driploss percentages compared to other studies is not known but may be attributed to the high CO_2 levels used in the MA packs. The levels of driploss observed in this study in MA packs after 16 days may be of concern to industry and constitute a shelflife limitation. This aspect of the study in the future could be repeated to clarify the findings.

Texture

The magnitude of the decline in shear strength observed from pre-rigor to the fish entering rigor mortis was large (40-69%). After day 1, all samples commenced a gradual decline in texture until the conclusion of the trial (Figure: 6.4). The implications of this result are

significant for the local aquaculture Atlantic salmon industry where textural deterioration and resultant gaping is a significant cost (Maynard, D. 2012, pers. comm., 15 October). While in Europe the incidence of textural downgrading is reported as being up to 40% due to soft flesh or gaping before retail distribution (Ashton et al., 2010; Michie, 2001). The practice of pre-rigor processing could have significant benefits over the traditional practice of holding fish for 3 days until rigor mortis resolves before processing.

Textural degradation of Atlantic salmon over time in the supply chain is also considered to be a significant quality limitation. Fernandez concluded texture was one of the keys to sensory rejection with texture declining after 18 days and limiting shelflife to 26 days (Fernandez et al., 2009). Despite the concerns with texture being a limiting edibility factor the taste panel did not significantly identify a difference between control and treatment.

Likewise the results (Figure: 6.4) seem to indicate that MAP treatments were different from the control at 0°C, but this was not supported by the statistics. A larger number of sample points may have provided a more definitive result. This issue possibly requires further investigation due to Atlantic salmon already having recognised textural problems. Given the majority of chilled retail Atlantic salmon is MA packed an optimised gas mix may offer textural and therefore financial benefit.

This is possibly due to changes in texture attributable to cooking. The cooking process causes changes and interactions between the myofibrillar, connective tissue (mainly collagen) and sarcoplasmic proteins which affect shear strength. Heating of these proteins causes denaturation, dissociation and shrinkage resulting in increases of fibre diameter, decreases in sarcomere length and shrinkage of the muscle. During cooking the connective tissue (mainly

collagen) which is responsible for shear strength in raw fish is solubilised while myofibrillar protein denatures and shrinks altering shear strength (Kong et al., 2008).

6.5   Conclusion

Atlantic salmon is one of the most researched species of commercial fish. The majority of the research is related to aquaculture production. The methods used in this study are designed to be commercially applied. The current study has proposed 20 days as the maximum quality shelflife for this species when optimum conditions are applied throughout the chain and fish are packed in MAP at 0°C. This represents a significant gain over current commercial shelflife estimates.

The rapid decrease in textural strength occurring after the fish enters rigor mortis has implications for how processing is approached with clear benefits to be gained from pre-rigor processing. Associated with this are harvest methods that minimise stress and maximise the time pre-rigor.

The study again highlights the negative impact of 5°C, the chilled temperature maximum, on quality and shelflife. The benefits of MAP can be largely negated by poor temperature procedures occurring throughout the chain.

7 Determination of Southern Calamari Shelflife

7.1 Introduction

Cephalopod

There are about 500 species of squid worldwide with 6 main commercial squid and calamari species found in Australia. Southern Calamari (*Sepioteuthis australis*) investigated in this study are found in estuarine and near coastal waters around southern Australia. The species is important for both the commercial and recreational sectors in New South Wales, Tasmania and South Australia with commercial catches of approximately 500 tonnes per year (ABARE, 2008). Squid species are caught in all states of Australia and this species, southern calamari (*Sepioteuthis australis*) was selected as being representative of cephalopods generally. This group is becoming increasingly significant for human consumption. To date no research has been done on the spoilage rates or shelflife characteristics of this species.

Experimental Objectives

- To determine the maximum quality shelf life of southern calamari (*Sepioteuthis australis*) by applying best practice handling, processing and preservation methods developed during the study.

- To assess microbial shelflife at 0°C and 5°C temperatures under different atmospheres.

7.2   Methods

The methods are as listed in the General Materials and Methods section with the following
specific details and/or modifications.

Equivalent Days on Ice

Equivalent days on ice was calculated for both control and MAP at 0°C and 5°C.

Gas Ratio

Squid were packed in a MA in 100% CO_2.

Microbiology

At day 0, eight whole Southern Calamari (*Sepioteuthis australis*) with a mean weight of 452
$\pm$ 51 g (range 322-694 g) were randomly selected and allocated to replicated treatments (n=2)
of control and MAP at 0°C and 5°C for microbiological, pH and driploss assessment.
Calamari were analysed for microbial counts (TPC CFU/g) on days 0, 4, 7, 10, 13, 16, 19,
22, 27 days or until microbial counts exceeded 10^7 per gram (ICMSF., 1986).

pH

The skinless calamari tubes (control and MAP at 0°C and 5°C) were measured for pH on
days 0, 4, 7, 10, 13, 19 or until spoiled.

Driploss

The drip loss was determined for 8 mantle portions with a mean weight of 115 ± 12.3 g, (range of 61-176 g). Replicated treatments of MAP and control at 0°C and 5°C were assessed for driploss on days 0, 5, 8, 11, 15, 18 or until spoiled.

Texture

Texture was determined by fish shear for 4 whole calamari with a mean weight of 538 ± 69 g, (range of 364-702 g). Skinless mantles with a mean weight of 184 ± 45 g, (range of 129-237 g) were cut in half length wise and replicates allocated to 2 treatments (MAP, Control at 0°C). Texture was measured on days 0, 5, 10, 14, and 19 or until spoiled. Rings of mantle 3 cm wide were taken 2 cm back from the head end of whole skinless tubes. Portions were placed in the shear inner tunic side down and aligned with the blade in a longitudinal direction as per the method outlined by (Kuo et al., 1991).

Sensory Analysis

Two taste panel assessments, a Preference ranking test and a Triangle difference test were conducted (Lawless & Heymann, 1999; Meilgaard et al., 2007). For assessments 15 whole calamari mean weight 834 g ± 114 g (range 112-2850 g) were processed to cleaned, skinless mantles before random allocation to either a control or treatment. Prior to assessment, treated samples and controls were assessed for general acceptability in a blind tasting by an expert panel of 3. The control and treated samples were considered by the expert panel to have good edibility attributes and representative of the species. At 7 days controls were removed from ice, repacked, and sealed in laminate bags before freezing and holding at -25°C for 3 weeks. At 14 and 17 days MAP samples were removed from ice and frozen at -25°C for

approximately 2 weeks. A retail sample for use in the Preference test was purchased from a retail supplier as cleaned, skinned, frozen, tubes.

Sample Preparation

All samples except the commercial nib off tubes (air thawed at 4°C) were water thawed (while remaining in the bag) at 17°C prior to testing. Thawed tubes were sliced down the centre and cut into 4-7 gram pieces of similar area for cooking. For the preference test four batches of 10 pieces were placed in ceramic containers and sealed with cling film before cooking in a Sharp brand, model Carousel microwave oven (1100 watts) on high setting. Pieces were cooked for a total of 2 minutes. After each minutes cooking the oven was stopped, the product agitated and the oven reset for a further minute to ensure an even cook. For the triangle test cooking time was reduced to 1 min 30 sec due to the smaller mass of the 3 lots of 10 portions being cooked per batch. This length of time was judged to produce an acceptable cook with all protein denatured.

After cooking, samples were assigned to number coded plates before transfer to individual booths for assessment. The tasters were instructed prior to the activity on the objectives and method of completing the Preference or Triangle test (Appendix 2).

Test Method

Preference Ranking Test

A total of 22 panellists participated in the Preference test. This test was used to rank 4 cephalopod examples for eating quality. The material being assessed included the 7 day post harvest air stored control, MA packed treatments at 14 and 17 day post harvest and defrosted

retail tubes. Panellists were instructed to rank the 4 samples from 1, most preferred, to 4, leastpreferred based on personal preference. Samples were presented to panellists in a balanced random order.

Triangle Discrimination Test

In total 25 tasters participated in the tasting and completed the response sheet. The Triangle test was conducted to determine if a difference could be detected between southern calamari (*Sepioteuthis australis*) portions stored for 7 days post harvest in air and MA packed in 100% CO_2 for 17 days.

Panel Demographics

The panellists were recruited from a diverse group of seafood consumers and included Indonesian academics from Jakarta Fisheries University and academic and technical staff from the AMC's Beauty Point campus. The panellist ages ranged between 25 and 55 years of age. The sex ratio was split 60: 40 male: female. All panellists were screened as being regular seafood consumers before being selected to participate in the panel.

Statistical Analysis

Taste Panel

Preference Test

For the preference test a randomised complete block design was applied. The data obtained were ordinal and therefore the rank values are not independently distributed indicating the need for a non parametric test. A Friedman's test was conducted to determine if there was

any significant difference between treatments at 95% confidence limits. This was followed by a Wilcox signed rank test to identify where differences might occur. All statistics were analysed using SPSS version 19.

Triangle Discrimination Test

The panellists were scheduled into groups of 5 to ensure randomisation within groups. Significance of a difference was determined at a level of 95% from tables in (Meilgaard et al., 2007).

pH, Driploss and Texture

Repeated measures analyses of variance (ANOVA) were used to statistically analyse pH, driploss and texture data. If Mauchly's test indicated that the assumption of homogeneity was violated then the Greenhouse-Geisser correction was used.

7.3   Results

Storage Temperature

Samples stored at 5°C had a mean temperature of 4.6 ± <0.1°C over the 22 days logged. Samples stored at 0°C had a mean temperature of 0.2 ± <0.1°C over the 27 days logged.

Equivalent Days on Ice

Equivalent days on ice calculations were performed on the logged temperature data and are listed in the table below. A mean equivalent days on ice was calculated at 2.09 ± 0.01 on

logged (5°C or less) data. It can be seen in Table 7.1 that storage at approximately 5°C more than doubled the equivalent days on ice, whereas storage at 0°C added 1 day over 27 days.

Table 7.1: Calculated equivalent days on ice for southern calamari stored at 0°C and 5°C in comparison to actual days.

Days	4	7	10	13	16	19	22	27
Calculated DOI 0°C	4.48	7.61	10.89	14.02	17.11	20.19	23.25	28.1
Calculated DOI 5°C	8.9	15.11	21.31	27.59	33.79	40.01	46.19	

Microbiology

The initial number of bacteria present on southern calamari portions pre-packing averaged 1.7 x 10^3 CFU/g (TPC), ranging from 1.4 – 2.08 x 10^3 CFU/g.
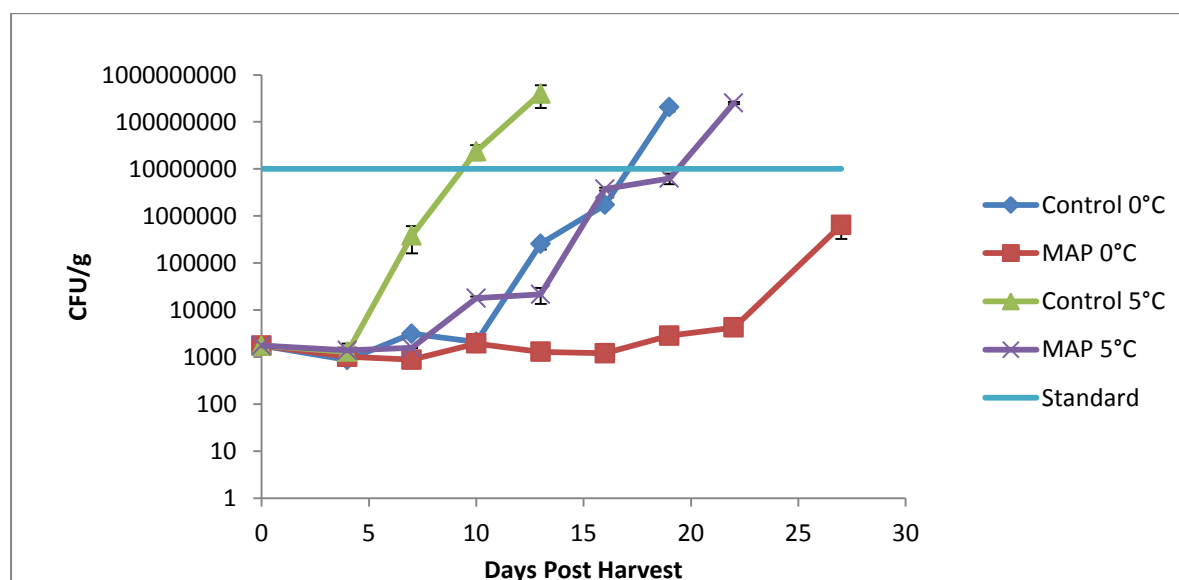
Figure 7.1: Mean (n=2) TPC (SEM ±) for skinless southern calamari portions, control and MAP (100% CO_2) at 0°C and 5°C sampled on days 0, 4, 7, 10, 13, 16, 19, 22, 27 or until spoiled. ICMSF (1986) standard of 10^7 CFU/g is indicated by the pale blue line.

Lag and Logarithmic Phases

The mean counts of the controls indicated that the samples remained within the lag phase for just over 4 days at 5°C and for 10 days at 0°C. The microbial counts of the MAP samples at 5°C and 0°C indicated a lag phase lasting for 7 days at 5°C and over 22 days at 0°C (Figure: 7.1).

Shelflife

The microbial count on day 16 (1.7×10^6 CFU/g) for control at 0°C suggested the ICMSF (1986) standard of 10^7 CFU/g was exceeded on day 17 while the control held at 5°C (2.3×10^7 CFU/g) had exceeded the standard after 10 days, rising to 3.9×10^8 CFU/g on day 13. The microbial count (6.4×10^5) of MAP 0°C did not exceed the standard at day 27 when samples ran out, while MAP held at 5°C rose from 2.1×10^4 CFU/g on day 13 to 6.3×10^6 CFU/g on day 19 indicating a maximum microbial shelflife of 20 days post harvest (Figure: 7.1).

pH

An initial pH measurement was conducted on 2 calamari at 8 hours post capture and a mean pH of 6.43 ± 0.0 was recorded.

For the control at 5°C, pH increased over 13 days from pH 6.43 on day 0 to pH 7.0 on day 10, at which time it was judged to be spoiled, and pH 7.7 at day 13.

The pH of control 0°C commenced a gradual increase from day 0 levels of pH 6.43 to pH 7.1 on day 19. The corresponding microbial TPC reached 2×10^8 CFU/g at day 19 (Figure: 7.1).
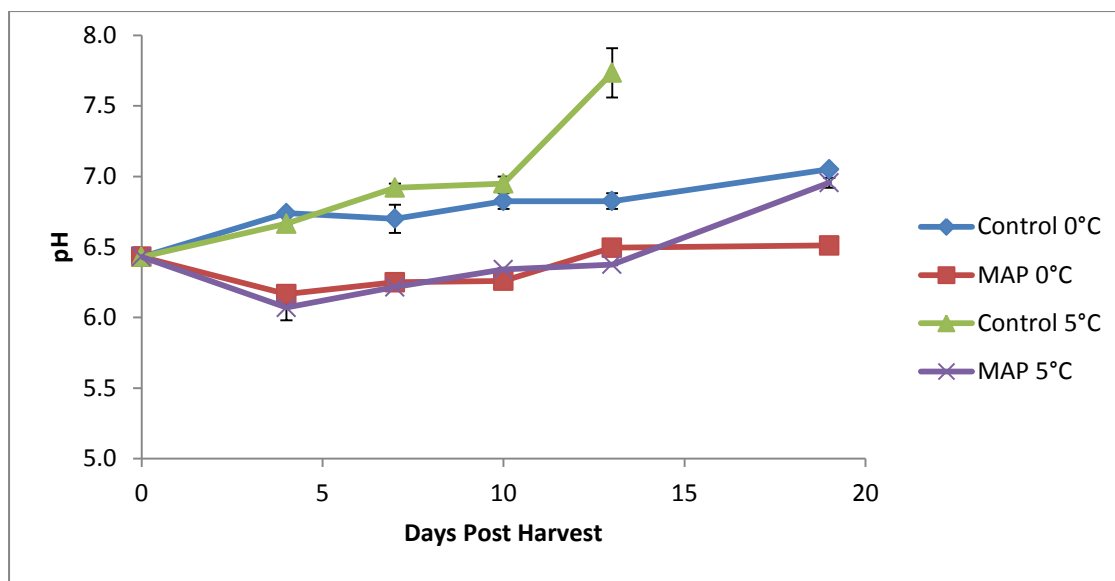
Figure 7.2: Mean (n=2) muscle pH (± SEM) for southern calamari portions stored as controls in gas permeable plastic bags and MA packs (gas ratio 100% CO_2) at temperatures of 5°C and 0°C sampled at days 0, 4, 7, 10, 13 and 19 or until spoiled.

A decrease in pH for both MAP samples after packing in CO_2 was observed with mean (n=2) decline from pH 6.43 pre-packing to pH 6.2 MAP 0°C and pH 6.1 MAP 5°C on day 4. The pH of both MAP samples increased gradually over the next 9 days reaching pH 6.5 and pH 6.4 respectively at day 13 before MAP 5°C rapidly rose to pH 7.0 on day 19 with a corresponding microbial count of 6.3 x 10^6 CFU/g.

The repeated measures ANOVA on days 0-13 data indicated that pH differed significantly between MAP treatments and controls, $F (3, 15) = 182.52$, sig. 0.000, $p<0.05$. A series of pairwise comparisons adjusted using the Bonferroni correction revealed that MAP treatments at 0°C and 5°C were not significantly different from each other but different to both controls at 0°C and 5°C . The control at 5°C was different from all other treatments.

Table 7.2: Statistical output from SPSS version 19 for pH indicating the relationship between treatments: 1; Control 0°C, 2; MAP 0°C, 3; Control 5°C, 4; MAP 5°C.

Pairwise Comparisons

(I) Treat	(J) Treat	Mean Difference (I-J)	Std. Error	Sig.[a]	95% Confidence Interval for Difference[a] Lower Bound	Upper Bound
1	2	.384[*]	.031	.000	.253	.515
	3	-.236[*]	.041	.014	-.410	-.062
	4	.418[*]	.030	.000	.289	.547
2	1	-.384[*]	.031	.000	-.515	-.253
	3	-.620[*]	.028	.000	-.736	-.504
	4	.034	.020	.914	-.051	.119
3	1	.236[*]	.041	.014	.062	.410
	2	.620[*]	.028	.000	.504	.736
	4	.654[*]	.042	.000	.476	.832
4	1	-.418[*]	.030	.000	-.547	-.289
	2	-.034	.020	.914	-.119	.051
	3	-.654[*]	.042	.000	-.832	-.476

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

a. Adjustment for multiple comparisons: Bonferroni.

Driploss

The driploss of calamari samples stored at 0°C was relatively linear for the duration of the trial (Figure 7.3). In contrast both MAP and controls at 5°C exhibited increases after 11 and 5 days respectively with control at 5°C having a total driploss of 7.7% by weight at day 11. By comparison driploss for MAP 0°C, 5°C and control 0°C were at similar driploss levels of 3.25%, 3.65% and 3.7% respectively at day 11. After day 11 the rate of driploss increased for MAP 5°C compared to all remaining treatments concluding at 6.75% by weight at day 15. The driploss of control and MAP 0°C continued to be measured until day 18 at which time total losses were 6.5% and 5.6% respectively.
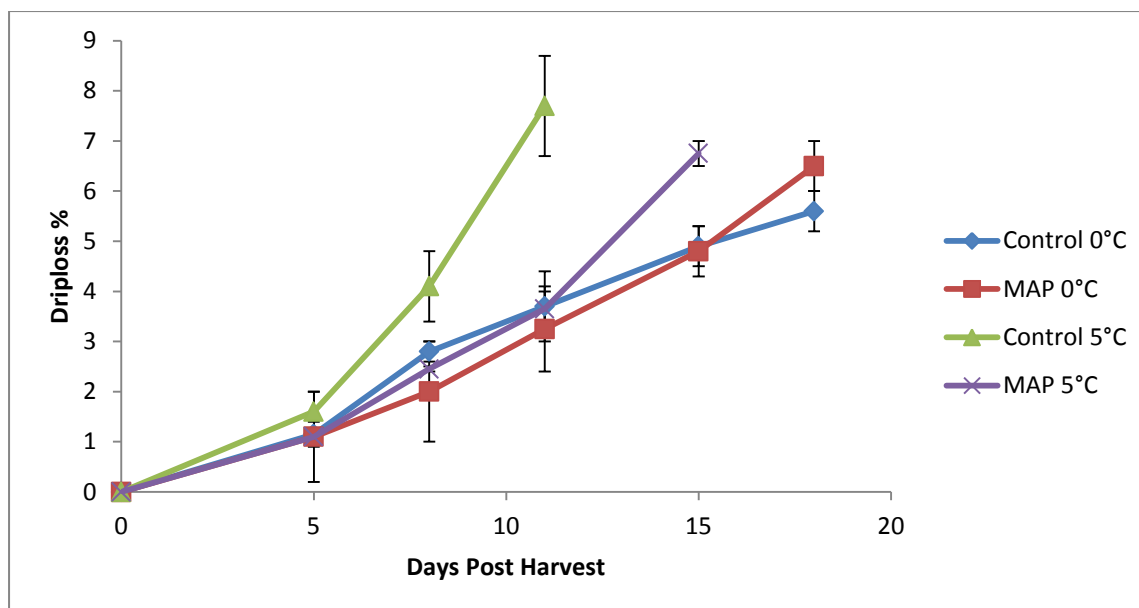
Figure 7.3: Mean (n=2) driploss (SEM ±) for southern calamari portions stored as controls in gas permeable plastic bags and MA packs (gas ratio of 100% CO_2) at temperatures of 5°C and 0°C sampled at days 0, 5, 8, 11, 15, 18 or until spoiled.

The repeated measures ANOVA on days 0 to 11 data indicated that a significant difference in driploss existed between MAP treatments and controls, $F(1.2, 4.8) = 9.19$, sig. 0.028, $p<0.05$. A series of pairwise comparisons adjusted using the Bonferroni correction identified the difference occurring between the control at 5°C and other treatments.

Texture

Texture increased between days 0 and 5 for both control and MAP from an initial reading of 48 N to 67 and 98 N respectively. After day 5 texture generally declined over the rest of the storage period to 44 and 45 N respectively on day 19. The repeated measures ANOVA indicated that calamari texture differed significantly between untreated controls and treatment (MAP 100% CO_2), ($F = (1,15) = 5.34$, sig. 0.036, $p<0.05$) with the MAP treatment exhibiting a higher shear strength over time.

94
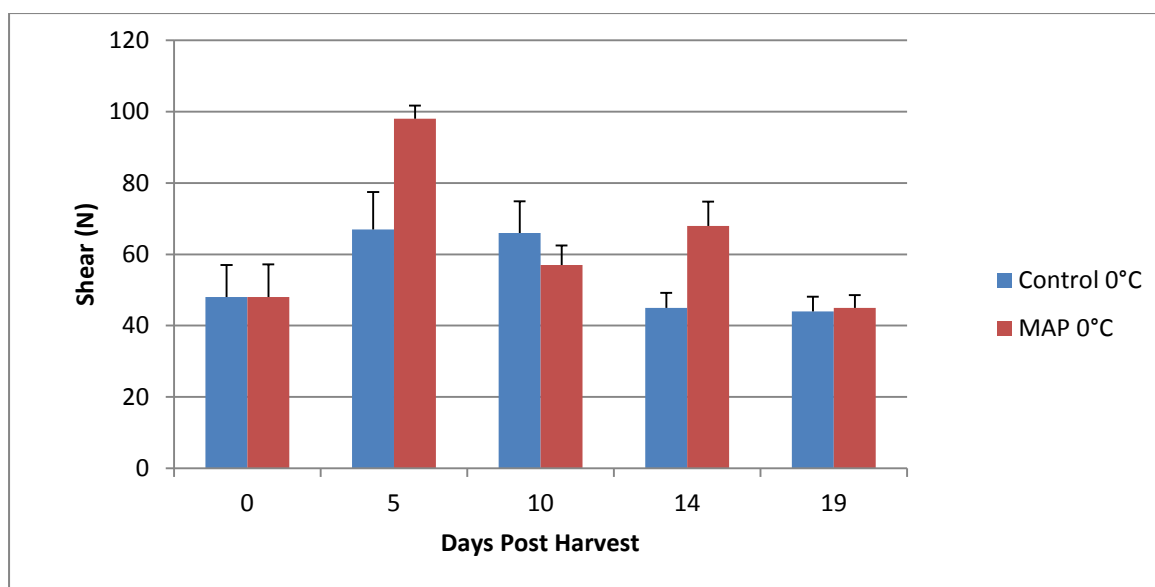
Figure 7.4: Mean (n=4) texture of (SEM ±) southern calamari portions stored as controls in gas permeable plastic bags and MA packs (gas ratio of 100% CO_2) at temperatures of 0°C and 5°C sampled at days 0, 5, 10, 14, 19 or until spoiled.

Sensory Analysis

Triangle Difference Test

A Triangle test was used to test the hypothesis that no difference existed between control and MAP treated southern calamari mantle stored at 0°C over 7 and 17 days respectively. Of the 25 participants only 10 panellists were correct in their response. This was well short of the 13 correct responses required for significance at $p=< 0.05$. The test failed to provide any reason to reject the null hypothesis that no difference in eating quality existed between control and treatment.

Preference Test

A Preference test was used to provide detail as to the relationship between trial samples and commercially available squid tubes. The outputs of the test indicated the retail product (*Mean Rank*=3.86) was significantly different to all other treatments (MAP 17 days, MAP 14

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days and control 7 days). No significant differences at $p<0.05$ could be detected between MAP 14 day (*Mean Rank*=1.86), MAP 17 day (*Mean Rank*=2.09), and control 7 day (*Mean Rank*=2.18) samples.

A Friedman ANOVA indicated that preference rankings of calamari varied significantly $\chi^2 =$ 33.9 (corrected for ties), $df = 3$, N-ties = 22, $p = 0.000$. Follow up pairwise comparisons were conducted using the Wilcox Signed Rank test with a Bonferroni adjusted α of .0083. To assess the size of the effects the z statistic was converted to r by applying the equation:

$$r = z/\sqrt{N}$$

The degree of the preference for the treatments compared to the retail product is in all cases described as "large", MAP 17 days (*p=.000, r=.85*), MAP 14 days (*p=000, r=0.82*), and control 7 days (*p=.000, r=0.85*) (Table 7.3).

Table 7.3: Summary Statistics Treatments v Retail Product

Treatment	Mean Rank	T	z	N	p	r	Effect size
MAP 17 days	2.09	5	-4.001	22	.000	0.85	large
MAP 14 days	1.86	10	-3.839	22	.000	0.82	large
Control 7 days	2.18	6	-3.985	22	.000	0.85	large
Retail product	3.86			22			

7.4 Discussion

The texture of squid is cited as the dominant quality attribute which influences demand for squid on Japanese domestic and international markets (Kagawa et al., 2002). Squid quality deteriorates very rapidly after capture (Ando et al., 1999). As squid spoils texture becomes

soft and flabby (Lapa-Guimaraes et al., 2002). Delayed icing and subsequent temperature abuse in the first 6 hours post capture was found to reduce shelflife by up to 5 days (Ganesan et al., 2006). Calamari in this study were caught by jig and immediately transferred to ice slurry after capture.

Cephalopod shelflife is generally reported as being less than fish species with the primary spoilage mechanism being enzymatic (Lapa-Guimaraes et al., 2002; Sykes et al., 2009; P. Vaz-Pires, Seixas, P., Mota, M., Lapa-Guimaraes, J., Pickova, J., Lindo, A. & Silva, T., 2008). Endogenous enzymes have been determined as being responsible for volatile basic nitrogen (VBN) changes and rapid putrefaction (P. Vaz-Pires et al., 2008). Paarup concluded the spoilage mechanism of squid was a combination of autolytic and bacterial attributed changes. At day 13 sensory rejection, biogenic amines, agmatine, cadaverine, tyramine and putresine were present in squid mantle (Paarup, Sanchez, Pelaez, et al., 2002) while agmatine and putresine were dominant at day 7 spoilage (Sykes et al., 2009). Paarup attributed TMA-N and NH$_3$ increases from days 9 to13

sensory rejection, as indicative of bacterial spoilage (Paarup, Sanchez, Moral, et al., 2002). These spoilage indicators were reflected in sensory assessments and a shelflife of generally less than 2 weeks (Paarup, Sanchez, Moral, et al., 2002; P. Vaz-Pires, Seixas, P., Mota, M., Lapa-Guimaraes, J., Pickova, J., Lindo, A. & Silva, T., 2008).

Microbial Shelflife

Initial TPC's of 1.7 x 10^3 CFU/g were found in this study. Initial counts cited in the literature range widely from 8 x 10^2 CFU/g and 1 x 10^4 CFU/g (Lapa-Guimaraes et al., 2002), 10^2 CFU/ cm^2 (P. Vaz-Pires et al., 2008), 3.9 x 10^3 CFU/g (Gou et al., 2010), 10^4 CFU/g (Paarup, Sanchez, Pelaez, et al., 2002) and 10^6 CFU/g (Jeyasekaran et al., 2010). In all of these cases

the initial raw material histories were lacking details of time, temperature, and handling. The absence of initial post harvest details must cast some doubt on the relevance of estimates cited in these studies for comparative purposes.

The primary spoilage mechanism in this study of air stored southern calamari at 0°C and 5°C or less was determined to be microbial. The literature on the rate of cephalopod microbial spoilage in air and at chill temperatures varies widely. It has been reported that for *Loligo plei* held on ice, microbes were 5 x 10^6 CFU/g after 12 days rising to greater than 10^8 CFU/g at day 17 (Lapa-Guimaraes et al., 2002). This corresponds with VBN and TMA-N levels that rose exponentially from day 12 -17. This result gives support to the Paarup finding of squid spoilage being a combination of microbial and autolytic processes as TMA-N is associated with microbial action (Dalgaard et al., 1993; Gram & Melchiorsen, 1996). Paarup reported in 2 studies sensory rejection of cooked mantles (*Todaropsis eblanae*) and aerobic plate counts of 5 x 10^7 CFU/g occurring after 10-12 days storage at 0.3 and -0.1°C (Paarup, Sanchez, Moral, et al., 2002). In the second study the initial microbial counts of squid mantle held at 4°C were 10^4 CFU/g, rising to 10^7 on day 7 and subsequent sensory rejection (Paarup, Sanchez, Pelaez, et al., 2002). In contrast Vaz-Pires reported TPC's of only 10^3 CFU/cm^2 at sensory rejection at 9-10 days for cuttlefish *Sepia officinalis* and broadtail shortfin squid *Illex illecebrosu,* with unpleasant odours being detectable at 6 days

(P. Vaz-Pires, Seixas, P., Mota, M., Lapa-Guimaraes, J., Pickova, J., Lindo, A. & Silva, T., 2008).

Air Stored at 5°C

Calamari at 5°C or less achieved a mean (n=2) TPC of 2.3 x 10^7 CFU/g and a pH of 7.0 on day 10 post harvest (Figure: 7.1). At this point the product was considered to have reached

its maximum microbial shelflife and exceeded its quality shelflife. This result is similar to that of gutted squid tubes at rejection having reached pH 7.3 and TPC of 5 x 10^7 CFU/g (Paarup, Sanchez, Moral, et al., 2002).

It is suggested that the maximum quality shelflife for calamari handled throughout the supply chain under best practice conditions is 7 days at 5°C or less. This conclusion is based on an initial microbial lag phase of 4 days and 3 days of logarithmic growth resulting in a microbial TPC of 10^5 CFU/g at 7 days. At these microbial counts metabolites should not be evident. At 7 days pH had increased from a mean of 6.66 at day 4 to 6.92 (Figure: 7.2), driploss had risen from 1.6% at day 5 to an estimated 4.1% at day 8 all indicating increasing spoilage (Figure: 7.3).

In practical terms a maximum shelflife of 7 days post harvest for calamari would only allow at best 4 days for retail sale. This scenario assumes a daily catch landing with processing and distribution over the subsequent 2 days.

Air Stored at 0°C

The indicator of maximum microbial shelflife, TPC 10^7 CFU/g (ICMSF., 1986) was reached in 17 days for calamari mantle stored at 0°C. This occurred 5 days later than the 12 days reported by Paarup and Gou for squid (*Todaropsis eblanae*), (*Todarodes pacificus*) held at 0.1°C (Gou et al., 2010; Paarup, Sanchez, Moral, et al., 2002). This suggests that best practice methods applied throughout the supply chain confer a significant shelflife benefit.

The maximum quality shelflife for southern calamari mantle at 0°C was estimated to be 13-14 days post harvest at which point microbial counts were less than 10^6 CFU/g. This estimate represents a 100% increase in quality shelflife over calamari mantle stored at 5°C. This figure

correlates well with the equivalent days on ice multiplier of 2.09. During storage pH had gradually increased from 6.4 at day 0 to 6.8 on day 13 and driploss reached 4% confirming microbial numbers were rising to unacceptable levels (Figure: 7.1, 7.2, 7.3).

MAP at 5°C

The number of studies on the impact of packing cephalopods in modified atmospheres is limited. Based on the results of this study the maximum microbial shelflife of calamari packed in MAP at 5°C is 20 days to achieve a TPC of 10^7 CFU/g. The maximum quality shelflife should be set at 14 days. At this time microbial counts are less than 10^6 CFU/g, pH is similar to initial day 0 levels of pH 6.4, driploss is 6.7% and texture is slightly below day 0 levels. To further support this estimate, at day 13 the TPC for southern calamari packed in MAP at 5°C was only 2.1 x 10^4 CFU/g with pH of 6.4. This is similar to the published result for MA packed cuttlefish (95% CO_2, 5% O_2) at 4°C that on day 14 had a TPC of 10^4 and a pH of 6.61 (Speranza et al., 2009).

MAP 0°C

The maximum microbial shelflife of calamari packed in MAP at 0°C is estimated at 29-30 days. At day 27 the mean (n=2) TPC was 6.4 x 10^5 CFU/g indicating that the product was in the exponential growth phase and that maximum microbial shelflife of 10^7 CFU/g was near (Figure: 7.1). The maximum quality shelflife is estimated at 19-20 days. This estimate is primarily based on the day 17 preference test sensory finding of softening texture and increasing driploss as other measures did not indicate any issues with the product. At 19-20 days the microbial TPC was at 10^3 CFU/g, pH 6.5, similar to initial day 0 levels of pH 6.4, driploss 6.5% and texture 67% of the control day 5 maximum. The sensory analysis supports

this conclusion with the expert panel identifying the day 17 MAP as being slightly sweeter with a softer texture than the control, but overall, little difference. The Triangle test further supported this conclusion failing to identify a significant difference between control and treatment. The Preference test further confirmed the Triangle test result by failing to identify a significant difference between 17 day MAP, 14 day MAP and 7 day control.

pH

The initial pH of southern calamari was a mean (n=2) of 6.4. This compared to pH 6.49 for calamari meat 6-8 hours post harvest (Ozlap & Karakaya, 2009), 6.5 on day 2 (Lapa-Guimaraes et al., 2002), 6.61 after 48 hours (Gou et al., 2010) and initial pH of 6.6 (Olivas et al., 2004).

At day 4 the pH of MAP treatments at 0°C and 5°C had declined after packing in CO_2 to pH 6.1 and 6.2 respectively while the controls at 0°C and 5°C had increased to pH 6.7. The decrease in pH post MA packing in CO_2 is characteristic with the formation of mild carbonic acid and resultant pH decrease (Devlieghere et al., 1998; Dixon & Kell, 1989; Farber, 1991).

The pH of the control at 5°C commenced a rapid increase from days 10-13. This corresponded with bacterial TPC's that increased from 2.3 x 10^7 to 3.9 x 10^8 CFU/g. In calamari a pH of 7-7.1 and higher corresponded with elevated bacterial counts of 10^8 CFU/g and spoilage (Figure: 7.2). This outcome is similar to other studies on cephalopods at spoilage where pH of 7.3, soft texture, NH_3-N levels of 24 mg 100 g^{-1} and TMA-N levels of 12 mg 100 g^{-1} were recorded at day 13 (Paarup, Sanchez, Moral, et al., 2002) and at day 12, TPC of 7.2 x 10^7 and pH of 7.81 (Gou et al., 2010). In MA (95% CO_2) packed cuttlefish pH 6.9 at day 14 was indicative of the end of sensory shelflife (Speranza et al., 2009).

While pH is not a good predictor of microbial spoilage due to the changes occurring after the bacterial numbers have increased it is a useful confirmation of the presence of high microbial numbers in calamari.

Driploss

Calamari driploss was reported as 10.29% over 7 days at 0-4°C (Ozlap & Karakaya, 2009). This compares to 7.7% at 11 days for the calamari control at 5°C at which time the product was spoiled. The rate of drip for the control at 5°C was similar to the 0°C control and MAP treatments until day 5. After day 5 the rate of drip increased significantly over all other options which were similar (Figure: 7.3). The driploss profile of the control at 5°C is of a similar profile to the microbial growth curve indicating there may be some relationship between microbial spoilage and driploss in this treatment. The driploss from the 0°C options remained generally linear for 19 days while the MAP at 5°C deviated from linear after day 11.

Texture

Squid muscle is covered by four sheets of connective tissue on both the inner and outer surfaces (Sikorski & Kolodziejska, 1986). There are many differences between squid muscle and fish muscle. In squid there are no Z-lines, no protein bands characteristic of connectin and ATP degradation is different in invertebrates with squid not accumulating inosine-5'-phosphate (IMP) (Kagawa et al., 2002). The cause of textural deterioration of squid mantle is due to proteolysis caused by endodgenous and microbial enzymes (Ando et al., 1999; Olivas et al., 2004).

Squid texture degradation post mortem is reported to be rapid (Ando et al., 1999). The degradation of arrow squid shear strength has been reported to be within 9 hours of refrigeration (Ando et al., 1999). Kagawa reported changes in raw squid texture within the first 24 hours after death (Kagawa et al., 2002). These findings were not supported by this study where southern calamari mantle shear strength increased over the first 5 days 29% for the control and 100% for the MAP treatment. These increases are possibly attributable to rigor mortis. The mean shear strength at day 5 of 67 and 98 N for the control and MAP respectively exceeded the maximum mean shear strength of 32 N for Atlantic salmon confirming that calamari mantle muscle fibers and connective tissue are stronger than that of fish muscle (Olivas et al., 2004). The profile of the calamari shear strength degradation graph (Figure: 7.4) is similar to that of ice-stored jumbo squid (*Dosidicus gigas*) with shear strength at day 0 rising to day 3 before decreasing over the subsequent 15 days (Olivas et al., 2004).

This study did not find soft texture in southern calamari mantle after 10-13 days as reported by (Lapa-Guimaraes et al., 2002) and (Paarup, Sanchez, Moral, et al., 2002). At day 14 the southern calamari control was only 6% below initial measurements and the MAP treatment 30% higher than day 0 readings. Calamari shear strength demonstrated a progressive decline with 66% of the day 5 maximum strength retained at day 19 for the control and 46% retained for the MAP treatment (Figure 7.4). The sensory analysis supported the shear strength data. At day 10 the control shear strength was measured to be only 1.5% below the day 5 test maximum and the expert panel confirmed that the day 7 control was of excellent firm texture. The texture of the day 19 MAP at 0°C was 67% of the control maximum while the sensory assessments indicated the day 17 MAP was of good texture being indistinguishable from the day 7 control.

Sensory

The taste-active components of the mantle muscle of the oval squid (*Sepioteuthis lessoniana*) were determined to be glycine, alanine, proline, glutamate, arginine, adenosine 5 monophosphate, trimethylamine oxide, glycine betaine, as well as potassium, sodium, and chloride ions (Kani et al., 2008).

Sensory assessment has identified that whole gut in cephalopods have a relatively short shelflife with *Todarodes pacificus* held at 0°C classified as passable at day 6 (no smell and firm) and unacceptable after 10 days (putrid smell and very soft) (Lapa-Guimaraes et al., 2002) while *L. plei* squid held on ice had a shelflife of 7 days (Lapa-Guimaraes et al., 2002). A number of studies indicated 7-8 days for whole squid on ice as the maximum sensory shelflife (Sykes et al., 2009; P. Vaz-Pires, Seixas, P., Mota, M., Lapa-Guimaraes, J., Pickova, J., Lindo, A. & Silva, T., 2008). This reduced shelflife compared to skinless tubes is primarily due to initial autolytic spoilage with the gut as the primary source of enzymes and late spoilage bacteria (Paarup, Sanchez, Moral, et al., 2002; Paarup, Sanchez, Pelaez, et al., 2002; Sykes et al., 2009; P. Vaz-Pires et al., 2008).

In this study the control at 0°C was judged by the expert panel and preference test to be of excellent eating quality 7 days post harvest while the 17 day MAP at 0°C was not sufficiently different to the control, for a taste panel to discriminate. The quality of calamari skinless, eviscerated tubes was confirmed when retail purchased squid tubes were compared by Preference test to calamari treatments of MAP 17 days, MAP 14 days and control 7 days at 0°C. The retail tubes were judged to be inferior to all the experimental calamari options. The effect size was calculated to be large indicating that a very high proportion of tasters ranked the retail product least preferred.

7.5 Conclusion

This study found no evidence indicating that southern calamari had a shorter shelflife than fish with flathead and Atlantic salmon having similar quality shelflife estimates. There was also no evidence to support enzymatic degradation as the dominant spoilage mechanism in all treatments except MAP at 0°C. The maximum quality shelflife estimate of 20 days for MAP at 0°C is significantly longer than the 10-11 days found for cuttlefish mantles in the literature (Speranza et al., 2009). The case for temperature and hygiene control post harvest is supported by the results of this study. Maximum quality shelflives were established at 7 days in air at 5°C, 13-14 days in air at 0°C, 14 days in MAP at 5°C and 20 days in MAP at 0°C demonstrating the impact of temperature and MAP on quality shelflife.

8   Determination of Abalone Shelflife

8.1   Introduction

Molluscs

Blacklip abalone (*Haliotis rubra*) are a large marine gastropod mollusc which occur in rock reef habitats in southern Australia. They can live for up to 20 years and grow to 22 cm shell length and a weight of 3 kg. Blacklip abalone are caught commercially in Tasmania, Victoria, South Australia and New South Wales with a total catch of 3825 tonnes in 2010 (FRDC, 2012).

The principal market for this species is in canned, frozen or live forms in Asia. Recent market studies in China have indicated the need for alternative product forms (Seafood CRC). The chilled MA packed product form has not been well studied and represents an area where greater knowledge is required. Advantages of a packed chilled meat product include convenience, reduced weight resulting in air freight cost benefits, and the elimination of the need for infrastructure to support live animals, including circulating water facilities.

Experimental Objectives

- To determine the maximum quality shelf life of blacklip abalone (*Haliotis rubra*) by applying best practice handling, processing and preservation methods.

- To assess microbial shelflife at 0°C and 5°C temperatures under different atmospheres.

- To investigate the shelflife and sensory characteristics of abalone meat packed in MAP at 0°C as a potential alternative abalone product for the Asian market.

8.2 Methods

The methods are as listed in the General Materials and Methods section with the following specific details and/or modifications.

Microbiological

Two replicated trials were conducted in April-May 2010 and March-April 2012 to assess the microbial spoilage rate and associated microbial shelf-life of abalone meat packed as controls and in a modified atmosphere containing 100% CO_2 held at 0°C and 5°C or less. In trial 1, Total Plate Count (TPC) data was obtained on days 0, 7, 15 and 21. In trial 2, TPC, was measured on days 0, 3, 7, 11, 15, 21, 25.

pH

At day 0, 10 whole shucked meats with a mean weight of 103 ± 14 g (range 78-147 g) were randomly selected and the flesh pH measured. The individual meats were allocated to replicate treatments of MAP and control at 0°C for pH measurement on days 0, 3, 7, 11, 15, 21, 25 or until spoiled.

Drip Loss

The drip loss of abalone was determined for the shucked meat of 7 fish with a mean weight of 83.3 ± 2.6 g, (range of 52.5-129.5 g). Replicated treatments of MAP at 0°C was assessed for driploss on days 0, 3, 7, 11, 15, 21, 25.

Texture

The meat of 4 abalone (mean weight 157 ± 18 g, range 106-183 g) was assessed over time using an Agro Technologie (Forges Les Eaux, France) Agrosta 100 Statistic Durometer texture analyser. This is an example of a puncture analyser and had a modified tip for use with abalone. The texture was measured by inserting the analyser tip into the meat and the resistance recorded as a percentage of the total force required to fully compress the tip. The texture was measured at the point where the adductor muscle attached to the shell. 5 measurements were taken of each sample, 4 around the edges and 1 in the centre and the mean percentage calculated.
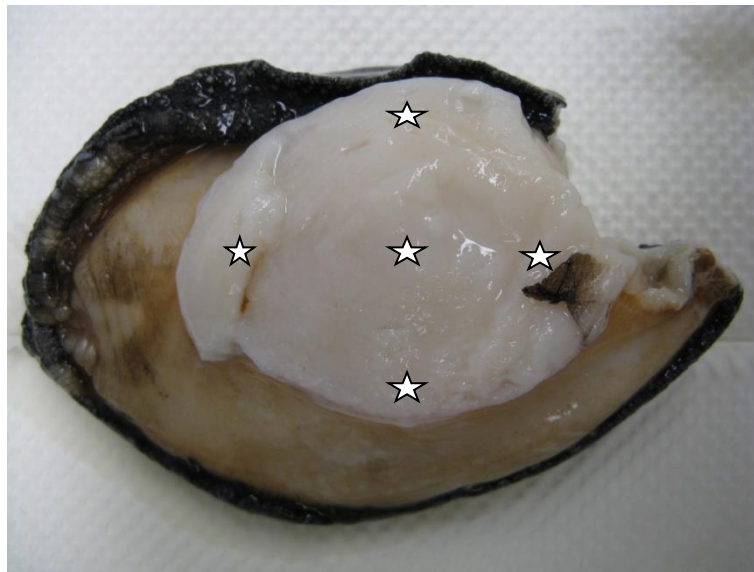
Plate 8.1: Shucked abalone meat showing the attachment point of the adductor muscle with the shell. Texture measurements were calculated as the mean (n=5) taken by inserting the puncture tip into the cut meat surface at the centre and at 4 locations around the edge as indicated by the ☆ symbol.

Sensory Analysis

Two taste panel assessments, a Preference ranking test and a Triangle difference test were conducted (Lawless & Heymann, 1999; Meilgaard et al., 2007). For these assessments 17 abalone were shucked, (mean meat weight 148 ± 24 g, range of 106 to 211 grams) and

randomly allocated to treatments (6,6,5). The controls (n=6) were stored at 5°C or less until

an equivalent time on ice of 11 days (11 days at 0°C) had been achieved before packing in

laminate pouches and freezing at -25°C for 3 weeks. Batches of MA packed samples (n=6)

were stored for 15 and 21 days (n=5) at 0°C before freezing and holding at -25°C.

Sample Preparation

All samples were water thawed (while remaining in the bag) at 17°C prior to testing. Thawed

meat was sliced to give each sample a replicate cross section of foot and adductor muscle

with a mean weight of 10 grams. Samples (n=5) of each treatment were allocated to

individual ceramic containers, covered by cling wrap and cooked for 40 sec on high in a

Sharp Carousel microwave oven (1100 watts) (150 grams abalone total per cook). This

method was judged to provide sufficient cooking to denature the protein while not

overcooking the samples and reducing typical characteristics.

After cooking, samples were assigned to numbered coded plates before transfer to individual

booths for assessment. The tasters were instructed prior to the activity on the objectives and

method of completing the Preference or Triangle tests.

Preference Ranking Test

The test was used to compare a control and 2 treatments for edibility attributes in accordance

with the method outlined in (Lawless & Heymann, 1999; Meilgaard et al., 2007). The

samples being assessed included the control of 11 equivalent days on ice and 15 and 21 day

MA packed in 100% CO_2 at 0°C abalone meat.

In this test, 16 panellists were instructed to rank the 3 samples from 1, most preferred, to 3, least preferred, based on personal preference. Samples were presented to panellists in a balanced random order. A total of 16 assessments were completed.

Triangle Discrimination Test

The triangle test was conducted to determine if a statistically significant difference existed between two treatments. The 11 day control abalone meat was considered to have good edibility attributes while the 21 day MAP was estimated to be nearing its suitability for eating shelflife maximum. Panelists (n=22) were required to identify the odd sample of 3, consisting of the 11 day control and the 100% CO_2 MAP treatment at 0°C for 21 days.

The assessment was conducted as per the method outlined in (Lawless & Heymann, 1999; Meilgaard et al., 2007) with 22 panellists recording a response. The panel was briefed prior to tasting on the objectives of the panel and the need to make a selection even if no difference could be detected.

The panellists were scheduled into groups of 5 to ensure randomisation within groups. Significance for a difference was determined at a level of 95% from tables (Meilgaard et al., 2007).

Statistical Analysis

A randomised complete block design was applied for the test. The data obtained were ordinal and therefore the rank values are not independently distributed indicating the need for a non parametric test. A Friedman's test was conducted to determine if there was any significant difference between treatments at 95% confidence limits. This was followed by a Wilcox

signed rank test to identify if significant, where differences might occur. All statistics were conducted using SPSS version 19.

Panel Demographics

The 22 panellists were recruited from a diverse group of seafood consumers and included mature age students in marine engineering, mature Pacific island students from a broad range of nations and academic and technical staff from the AMC's Beauty Point campus. The panellist ages ranged between 18 and 56 years of age. The sex ratio was split 60:40 male : female. All panellists were screened as being regular seafood consumers before selection to the panel.

8.3 Results

Storage Temperature

Samples stored at 5°C had a mean (n=503) temperature of 4.56 ± <0.1 °C over the 21 day trial. Samples stored at 0°C had a mean (n=503) temperature of 0.12 ± 0 °C over the 25 days logged.

Equivalent Days on Ice

Equivalent days on ice calculations were performed on the logged temperature data and are listed in the table below. A mean equivalent days on ice was calculated at 2.09 ± <0.1 for samples stored at < 5°C. It can be seen in Table 8.1 that storage at approximately 5°C more than doubled the equivalent days on ice, whereas storage at 0°C produced virtually no increase.

Table 8.1: Calculated equivalent days on ice for abalone stored at 0°C and 5°C in comparison to actual days.

Days	3	7	11	15	21	25
Calculated DOI 0°C	3.06	7.14	11.22	15.3	21.42	25.5
Calculated DOI 5°C	6.3	14.75	23.12	31.59	44.05	

Microbiology

The initial number of bacteria present on abalone portions pre-packing averaged 6.6×10^2 CFU/g (TPC) n= 2, ranging from $6.2 – 6.9 \times 10^2$ CFU/g.

Lag and Logarithmic Growth Phases

The mean counts of the controls indicated that the samples remained within the lag phase for 3 days at 5°C and less than 10 days at 0°C. The microbial TPC (4.4×10^7 CFU/g) of control at 5°C had exceeded the standard of 10^7 CFU/g ICMSF (1986) on day 11, while the control held at 0°C (7.5×10^6 CFU/g) exceeded it after 21 days post harvest. The microbial counts of the MAP samples at 5°C and 0°C indicated a lag phase lasting up to 21 days at 5°C and over 25 days for 0°C. The microbial count (2×10^2) of MAP 0°C at day 25 was similar to the initial day 0 count while the MAP samples held at 5°C (6.3×10^6 CFU/g) were nearing the standard at day 25 post harvest (Figure 8.1).
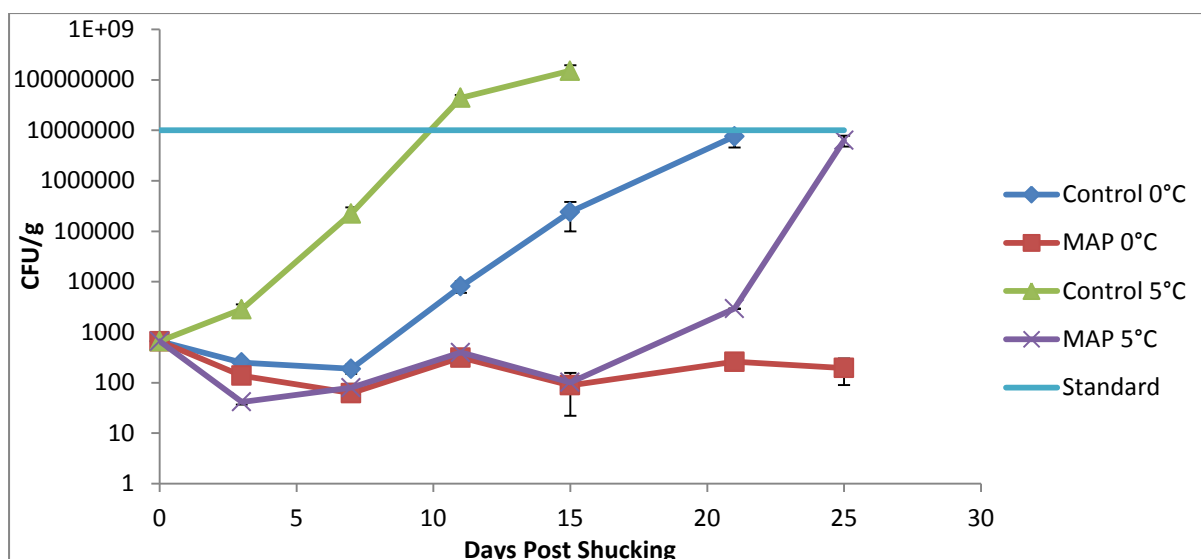
Figure 8.1: Mean (n=2) TPC (±SEM) for abalone portions, control and MAP (100% CO_2) at 0°C and 5°C sampled at 0, 3, 7, 11, 15, 21, 25 days. ICMSF (1986) standard of 10^7 CFU/g is indicated by the pale blue line.

The microbial spoilage trial was fully replicated in 2010 and 2012 (Figure: 8.2). Results showed almost identical spoilage rates. Initial microbial TPC for MAP at 0°C were trial 1 = 1.2 x 10^3 and trial 2 = 6.6 x 10^2 CFU/g. Counts decreased from day 0 until day 7 (93 and 63 CFU/g respectively). Counts remained stable until the trial was concluded at day 21 (1.7 x 10^2 CFU/g) and 25 (1.9 x 10^2 CFU/g) for trials 1 and 2 respectively.
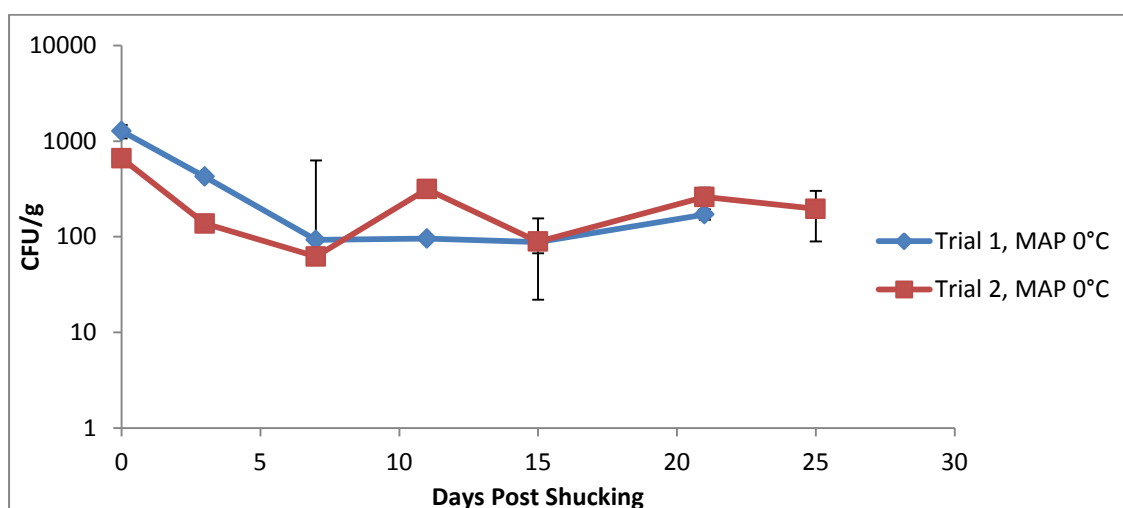
Figure 8.2: Replicated trials of TPC CFU/g (±SEM) of abalone portions packed in MAP (100% CO_2) at 0°C sampled at 0, 3, 7, 11, 15, 21, 25 days.

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pH

The mean (n=10) pH of abalone meat pre-rigor was 6.85 ± 0.03 range 6.73-6.99 (Figure: 8.3). Mean pH (n=2) declined for both control and MAP to 5.83 and 5.74 respectively on day 3. The pH remained close to day 3 levels for both control and MAP at day 15 values of 5.7 and 5.8 respectively. MAP pH was 5.7 at day 25 when the measurements concluded.
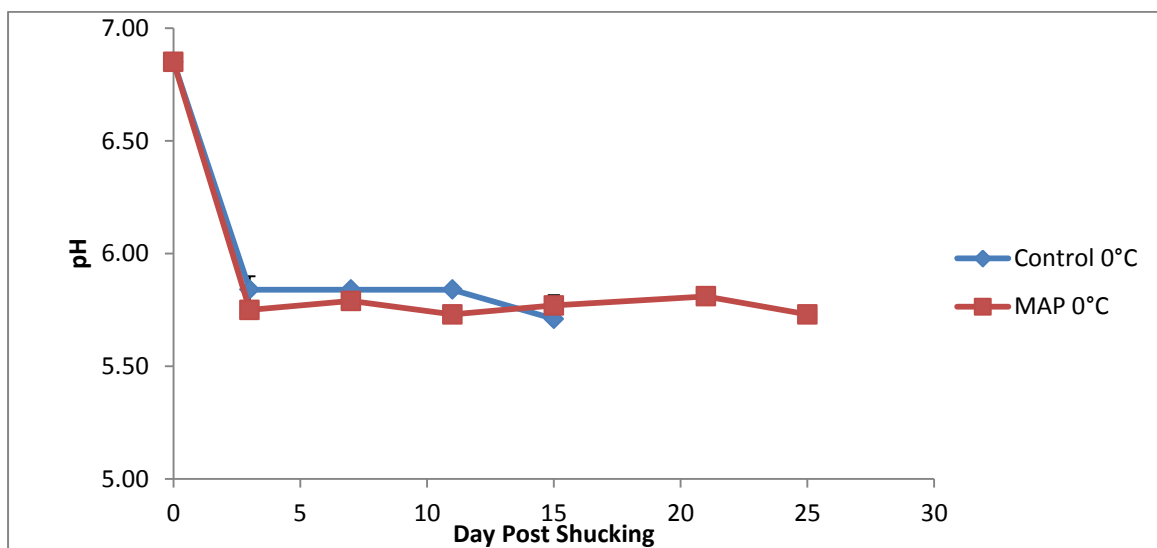
Figure 8.3: Mean (n=3) muscle pH ($\pm$ SEM) for portions stored as controls in gas permeable plastic bags and MA packs (100% CO_2) at temperatures of 0°C sampled at 0, 3, 7, 11, 15, 21, 25 or until spoiled.

Driploss

An initial mean (n=7) driploss of 3.7% for MA packed at 0°C samples occurred between days 0 to 3. By day 11 the mean drip percentage had only increased by a further 1.4% to a total of 4.3%. The rate of driploss again increased after day 11 to a total of 7.7% by weight at day 25 (Figure: 8.4).

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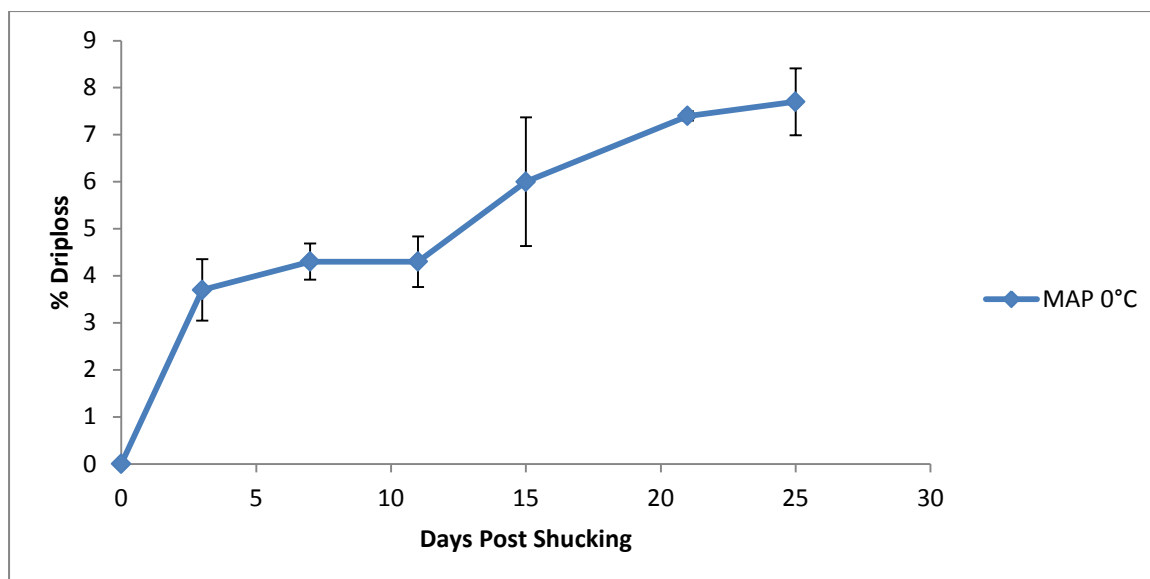
Figure 8.4: Mean (n=7) percent driploss (± SEM) for whole shucked abalone meat stored in MA pouches (gas ratio 100% CO_2) at 0°C sampled at 0, 3, 7, 11, 15, 21 and 25 days.

Texture

As determined by the puncture texture method, a 9% increase in puncture resistance for MAP at 0°C was observed from an initial pre-rigor mean (n=4) on day 0 of 54% ± 7% to 63% ± 4% at day 1. Following this initial rise, texture declined 27% from day 1 levels to a residual of 36.15 ± 3.7% at day 9. From day 9 to day 16 a slight drop of 2.55% was observed to a residual mean percentage of 33.6 ± 3.4% (Figure: 8.5).
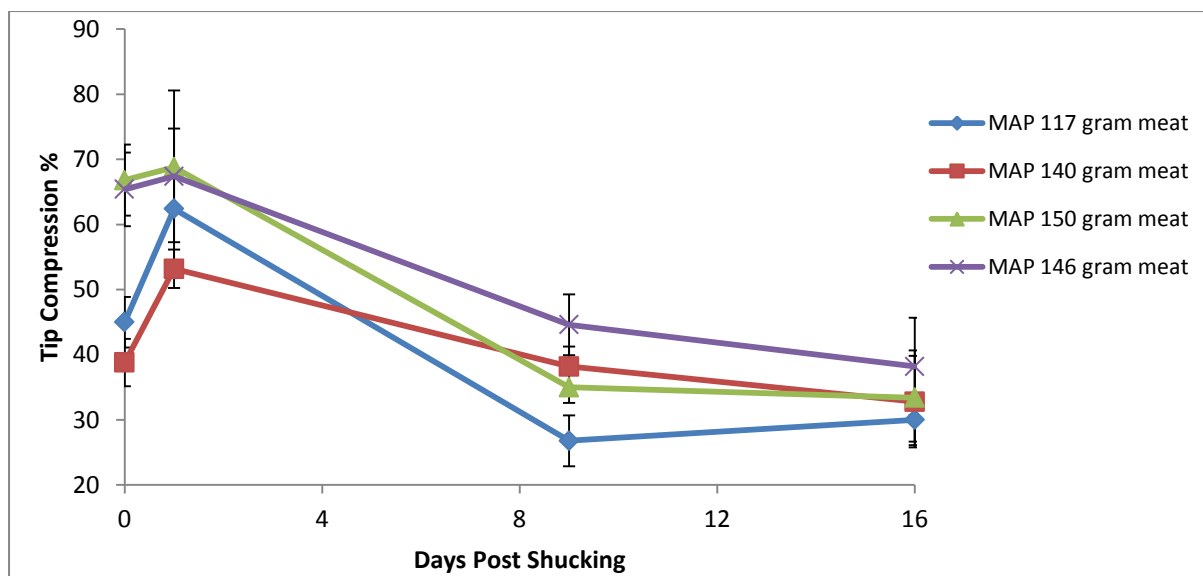
Figure 8.5: Mean (n=4) percent texture of abalone meat (±SEM) packed in a MA of 100% CO_2 at 0°C for 16 days. Measured on days 0, 1, 9 and 16.

Sensory

Preference

Sixteen tasters participated in the Preference test. A Friedman ANOVA indicated that rankings of taste preference for abalone at 95% confidence limits did not vary significantly across the three treatments, $\chi^2 F = 1.56$, $df = 2$, $N = 16$, $p = 0.459$.

Triangle Difference Test

The cooked abalone meat (control 11 days at 0°C and the treatment 21 days MAP at 0°C) was presented to 22 participants. 12 participants correctly identified the odd sample and 10 did not. The result was significant at $p = 0.05$ at a sensitivity of 50% indicating that a difference in the 2 products could be detected.

8.4   Discussion

The majority of abalone produced in Australia is exported to Asia. Key aspects of abalone quality include texture, driploss, spoilage and associated taste. The taste of abalone is different to fish and is associated with umami (Boudreau, 1980; Fletcher et al., 1990). The primary taste components of umami flavour are amino acids and 5'-ribonucleotides. Among the 5'-ribonucleotides, IMP, 5'-adenosine monophosphate (AMP) and 5'-guanosine monophosphate GMP contribute to the umami taste of natural foods (Fuke & Shimizu, 1993; Prescott, 2001). The umami taste of abalone is associated with AMP, glutamic acid and the sweet sensation produced by glycine and glycine betaine (Fuke & Konosu, 1991).

Sensory Assessment

One of the issues associated with sensory assessment of abalone in Australia is the lack of exposure tasters have to this product with little sold on the domestic market. The Triangle difference test is a method that requires the taster to determine if a difference exists between samples and does not require tasters to have extensive training. The Triangle test identified that the 21 days MAP was significantly different to the control at 11 days and was preferred to the control. The subsequent Preference test while not significant provided detail on the nature of the difference not available from the Triangle test. The 3 treatment options were ranked as 21 day MAP most preferred, 11 day control on ice and 15 day MAP least preferred. Anecdotal comments from tasters referred to texture being the primary difference with the 21 day MAP treatment being softer and judged preferable. Due to the lack of market specific knowledge relating to textural preference this finding could not be evaluated. It should be noted that softer texture may be a limiting factor in the Asian abalone export market.

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Microbial Shelflife

The initial mean (n=2) microbial TPC for shucked abalone meat was $6.6 \times 10^2 \pm 32$ CFU/g. This compares to initial counts of 10^1 CFU/g for 20 g farmed abalone (Briones et al., 2010) and 7×10^4 CFU/g (Sanguandeekul et al., 2008).

Air Stored at < 5°C

The effect of a temperature difference of 4.5°C on the growth rates of microbes on abalone meat stored in air was calculated as an equivalent days on ice multiplier of 2. The lag phase was estimated to be 3 days at 5°C and abalone at 5°C had a TPC of 4.4×10^7 CFU/g on day 11 post harvest (Figure: 8.1) at which point the product had exceeded the maximum microbial shelflife which was estimated to be less than 10 days. The less than 10-day maximum microbial shelflife outcome is comparable to the 9 days identified by organoleptic assessment as the initial decomposition point for abalone at 5°C (Watanabe et al., 1992). It is concluded that the maximum quality shelflife for abalone meat handled throughout the supply chain under best practice conditions is 7 days at 5°C. At this time the microbial TPC was less than 10^6 CFU/g. This estimate is supported by findings that during storage at 5°C abalone AMP increased until day 7 before sharply declining, at which time the spoilage indicator hypoxanthine was detected (Watanabe et al., 1992).

Air Stored at 0°C

The lag phase for the control at 0°C lasted for 10 days and the TPC reached 7.5×10^6 CFU/g on day 21. Twenty-one days should be regarded as the maximum microbial shelflife for abalone in air at 0°C. The maximum quality shelflife was estimated to be 15 days with pH

remaining at 5.71 and microbial counts reaching 2.4 x 10^5 at this time. This estimate is supported by the expert taste panel. The control at day 11 had a TPC of 8.1 x 10^3 CFU/g and exhibited taste and texture characteristics of good quality abalone. The estimate of 15 days quality shelflife is comparable to the findings of Watanabe on disk abalone (*H. discus*) held at 0°C where at day 11 no off odours attributable to polyamines (tyramine, putrescine, cadaverine, histamine, agmatine, tryptamine, spermidine) were detected (Watanabe et al., 1992). Initial detection of polyamines did not occur in the Watanabe study until day 13. In comparison, in this study the control on day 13 had a TPC of 10^5 CFU/g indicating it was still below the microbial standard. Watanabe found AMP, a key umami-related taste component of abalone, increased until day 11 before slowly declining (Watanabe et al., 1992). The inference that can be drawn from this is that the umami taste remained for some time after day 11 leading support to the 15 day maximum quality shelflife (Figure: 8.1).

MAP at 5°C

The MA packed samples at 5°C had a lag phase of 18 days before microbial counts rapidly increased and reached 6.3 x 10^6 CFU/g at day 25. Based on these counts 25 days should be considered the maximum microbial shelflife. Abalone in this study had 2 primary preservation methods, temperature control and MA packing in carbon dioxide. The effects of temperature can be seen on microbial growth rates between controls at 0°C and 5°C. The impact of CO_2 on growth can be seen between MAP at 0°C and control at 0°C. The cumulative effect of these hurdles can be seen in comparing the microbial TPC's between the control at 5°C (4.4 x 10^7 CFU/g) at day 11 and the MAP treatment at 0°C (1.9 x 10^2 CFU/g) at day 25.

MAP at 0°C

The decrease in microbial counts between day 0 and day 7 can be attributed to microbial selection and succession (McMeekin & Ross, 1996). In this process the initial flora found on the abalone react to the hurdles of temperature, CO_2 and pH. The outcome is an altered flora with some species dying, others suffering sub lethal injury or becoming unable to multiply due to the redirection of energy to maintain homeostasis (Leistner & Gould, 2002). This process continues over time and constitutes the lag phase, often resulting in the emergence of a specific spoilage organism (SSO) that ultimately dominates the environment (Powell & Tamplin, 2012). Even small enhancements to individual hurdles in a food has a definite effect on microbial stability when microbes are close to the growth - no growth interface (Leistner & Gould, 2002; McMeekin et al., 2000). The cumulative effect of hurdles and lowered energy reserves results in weakened organisms more susceptible to slight environmental changes. The combined effect of CO_2, temperature and lowered pH is the retardation of microbial growth and correspondingly increased lag phase as observed in the MAP at 0°C.

It was concluded that microbial spoilage was not the limiting factor impacting the quality shelflife of abalone packed in MAP at 0°C because the microbial count remained within the lag phase at day 25 while other indicators, particularly driploss reached levels of 7.5% and anecdotally texture declined to levels that would be commercially unacceptable. The number of studies investigating the packing of abalone in MAP environments is limited with only 1 reference being identified (Sanguandeekul et al., 2008). In this study abalone of 20 g whole weight were packed in MAP (40:30:30 CO_2, O_2, N_2) at 2°C and reported to be acceptable by sensory assessment at 15 days (Sanguandeekul et al., 2008). At day 16 microbial counts had exceeded 10^6 CFU/g for MAP treatments indicating a correlation between microbial and sensory estimates (Sanguandeekul et al., 2008). By contrast, in the current study at day 21,

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MAP 0°C samples were still in the lag phase of growth. Sensory assessment by the expert panel, Triangle and Preference tests determined that the product had good organoleptic and eating properties at day 21, but was significantly different to the control at 11 days and preferred to the control. The texture was identified as being softer than the control and was preferred by tasters. On this basis it is concluded that the quality shelflife for abalone meat packed in MAP at 0°C should be 21 days post shucking and that the mechanism responsible for shelflife limitation was enzymatic degradation of texture rather than microbial spoilage.

pH

In abalone, the main end product of the glycolytic pathway is D-lactic acid, the levels of which, at temperatures of 0°C post mortem, rise rapidly in the first 3 days and continue rising for 7 days before reaching a plateau thereafter (Watanabe et al., 1992). This result corresponds to the rapid decrease in pH post mortem observed in this study where pH values for the control fell from 6.85 at day 0 to 5.84 at day 3. This rapid decline in pH was also documented for *Haliotis midae* where initial readings of pH 6.58, 1.5 hours post shucking decreased to 5.73 after 2.7 days (Sales et al., 1999).

In MA packed abalone post mortem pH decline was also documented (Sanguandeekul et al., 2008). The magnitude of this decline was not as great (pH 6.43-6.28) as that found in this study (6.85-5.75) but these molluscs were only 20 g whole weight.

The energy requirements of a microorganism in a low pH environment are greater than the energy required at optimal pH values (Beales, 2004). The pH levels achieved in abalone post mortem may have contributed to the extended lag phase observed in the replicated study of MAP at 0°C. The terminal pH of abalone meat in MAP of 5.75 may be sufficiently low to

add another hurdle to microbial growth by causing the utilisation of energy to support

homeostasis. This may explain total plate counts of less than initial numbers at day 25.

Driploss

The initial fluid loss from abalone occurs almost immediately after shucking (James & Olley,

1970). This material is haemocyanin or abalone blood, the primary oxygen carrier. This loss

post shucking was not measured. The driploss of abalone packed in MAP at 0°C reached

7.7% on day 25. Observed driploss was not linear for this species (Figure: 8.4). The observed

results for MA packed abalone at 0°C did not differ significantly from results of air stored

abalone from other studies. By comparison in a study by Sales on *Haliotis midae* packed in

air at 7°C driploss reached 7.23% and 8.04% on day 7 (Sales et al., 1999) while a 9% driploss

was documented over 3 days at 0.5°C for blacklip abalone (*Haliotis rubra*) (James & Olley,

1970). At the levels observed in this study, driploss may constitute a significant economic

limitation to shelflife.

Texture

The meat of abalone is characterised by variable texture with 5 areas recognised as having

differing textures (Olaechea et al., 1993). Correlation was found between the collagen content

and meat toughness when measured instrumentally: the dorsal surface of the foot having the

highest collagen and correspondingly highest toughness levels (Olaechea et al., 1993). The

adductor muscle was instrumentally measured as being less tough than the foot (Olaechea et

al., 1993; Sanchez-Brambila et al., 2002) and this was confirmed in the present study when it

was found at day 21 that the texture meter tip could not puncture the abalone foot in any

location before exceeding the upper measuring threshold of the machine. By contrast, the

adductor muscle gave consistent measurements of texture as a compression percentage from day 0 to 16 (Figure: 8.5). A positive correlation was established by Sanchez-Brambila between instrumental hardness and chewiness measurements and sensory hardness and sensory chewiness in both adductor and foot muscle sections of *Haliotis cracherodii* (Sanchez-Brambila et al., 2002). This result supported the findings of this study in which instrumental and sensory texture assessment of MAP packed abalone meat at 0°C identified decreasing texture over time. Instrumental readings decreased from the mean (n=4) rigor high of 63% ± 3.5 compression to 36% ± 3.7 compression at day 9 and 33% ± 1.7 compression at day 16. This measured decline was supported by the sensory analysis where the Triangle test identified a significant difference between the day 11 control at 0°C and the day 21 MAP at 0°C. The Preference test provided insight into declining texture with the MAP at 0°C being reported verbally by tasters as most preferred due to softer texture. No literature on MA packed abalone could be identified to support the result of textural difference over storage time but textural decline over time is widely documented in other species (Hyldig & Nielsen, 2001; Kagawa et al., 2002; Love & Haq, 1970).

8.5   Conclusion

The results of this study estimate the maximum quality shelflife of abalone in MAP at 0°C to be 21 days subject to confirmation of texture and driploss suitability This result confirms the potential for the packing of abalone meat in modified atmospheres for chill distribution to export markets. At less than optimal temperatures of 5°C MA packed abalone can still be expected to have a microbial shelflife of 18-20 days while at 0°C air stored abalone can expect to have a quality shelflife of 15 days. This study clearly demonstrates the shelflife benefits of optimised handling and hygiene practices applied throughout the supply chain in combination with MA packing.

Acidified Sodium Chlorite Trials

Table 9.1: Summary Best Practice Supply Chain Experiments

Test area	Experimental aim	Storage characteristics	Test detail	Experimental design
Microbial	Test effect of acidified sodium chlorite (ASC) as a direct application sanitiser on flathead microbiology	5°C in unsealed gas permeable plastic bags	Total Plate Count (TPC) (AOAC 2000)	Control v treatment
Microbial	Test effect of acidified sodium chlorite (ASC) as a direct application sanitiser on Atlantic salmon packed in MA	0°C and 5°C in sealed gas impermeable bags 100% CO_2	TPC (AOAC 2000)	MAP ASC v MAP
Microbial	Assess the effectiveness of cleaning and sanitation method pre-processing and water rinsing during processing on bacterial numbers	Cutting boards	TPC (AOAC 2000)	Swabs at 5 locations x 25 cm^2 on cutting boards pre-processing and post processing

8.6 Introduction

Acidified Sodium Chlorite (ASC) Studies

The majority of preservative treatments applied to seafood do not cause complete inactivation of microorganisms but can induce sub-lethal injury. These injured cells have the potential to resuscitate and resume growth (Rajkovic et al., 2010). Damaged microorganisms become more exacting in growth requirements and more sensitive to other preservation factors (Lee & Kang, 2004). It was hypothesized that the direct application of Acidified Sodium Chlorite

(ASC) to fish could reduce microbial counts or weaken cells making them more susceptible to hurdles and further extend the lag phase.

Acidified Sodium Chlorite (ASC) was investigated for its potential as a sanitiser, to be applied as a spray to processed seafood. Acidified Sodium Chlorite is approved by the Australian Quarantine Inspection Service under Category 6 for use as a terminal sanitising rinse when used in accordance with manufacturers directions (Agranco Corp, 28/07/2003).

Experimental Objectives

- Assess the effect of direct spay application of activated sodium chlorite (ASC) on microbial numbers (TPC) at less than 5°C for air stored flathead (*Platycephalus bassensis*).
- Assess the effects on bacteria (TPC) of Modified Atmosphere Packaging (MAP) and MAP combined with Activated Sodium Chlorite (MAP/ASC) on Atlantic salmon (*Salmo salar*) at 0°C and less than 5°C.

8.7   Methods

The methods are as listed in the General Materials and Methods section with the following specific details and/or modifications.

The acidified sodium chlorite (ASC) and activator was obtained from Technica Pty. Ltd, Bayswater, Victoria with a concentration of 30 000 ppm. The required ASC (Vibrex Foodplus Sanitiser and Vibrex Activator C10^{TM}) mix strength of 500 ppm was attained by mixing Vibrex and activator in 1 litre of water according to the manufacturers instructions.

Samples were treated with ASC at a concentration of 500 ppm by series of 3, five-second sprays. After each spray, samples were manually mixed to ensure all surfaces were exposed. In total approximately 800 grams of fish were treated with 63 ml of ASC at a concentration of 500 ppm.

Equivalent Days in Ice

Equivalent days on ice was calculated for both the flathead at 5°C experiment and the Atlantic salmon MAP at 0°C and 5°C experiment.

Microbiology

Flathead samples held at 5°C or less were analysed for microbial counts (TPC CFU/g) on days 0, 6, 9.

Atlantic salmon samples held at 0°C and 5°C or less were analysed for microbial counts (TPC CFU/g) on days 0, 5, 8, 11, 14, 17, 20, 23, 26, 29, 32 or until microbial counts exceeded 10^7 per gram (ICMSF., 1986).

Statistical Analysis

Flathead treated with ASC and control, air packed at 5°C data were analysed with a Kruskal-Wallis ANOVA.

Atlantic salmon MAP, ASC/MAP at 0°C and 5°C was log transformed and analysed by ANOVA and Tukey's HSD (à of 0.05)

8.8 Results

Air Packed Flathead at 5°C

Equivalent Days on Ice

Equivalent days on ice calculations were performed on the logged temperature data and are listed in the table below. A mean equivalent days on ice was calculated for the flathead experiment at 2.12 ± 0.015 on logged (5°C or less) data and is presented daily in Table 9.1.

The mean storage temperature over the 9 days logged was 4.7°C (n=240) with a maximum of 5.2°C and a minimum of 4.3°C.

Table 9.2: Calculated equivalent days on ice for flathead stored at 5°C in comparison to actual days.

Days	1	2	3	4	5	6	7	8	9
Calculated DOI 5°C	2.11	4.7	6.3	8.4	10.5	12.7	14.8	16.9	19.1

Microbial counts

The mean (n=5) initial number of bacteria present on flathead control portions pre-packing (TPC) was 5.3×10^3 CFU/g while on ASC treated portions the counts was 2.1×10^3 CFU/g.
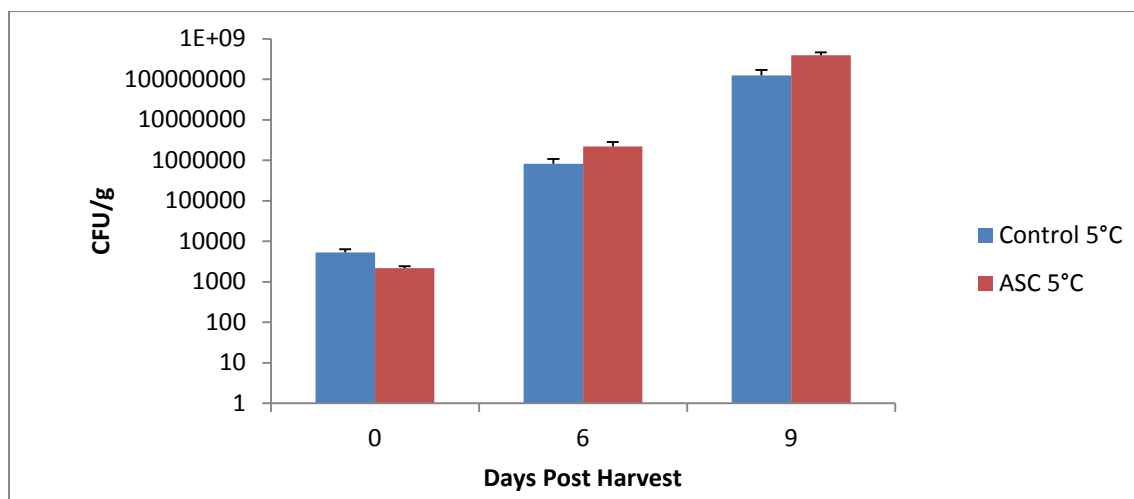
Figure 9.1: Mean (n=5) TPC (±SEM) for flathead portions, treated with acidified sodium chlorite (ASC) and untreated control at 5°C.

The mean counts of both control and ASC treated air stored samples at 5°C reached 8×10^5 and 2×10^6 CFU/g respectively after 6 days (Figure 9.1). This result is similar to that recorded for the flathead experiment where the control at 5°C reached 7×10^5 CFU/g at day 7 (Figure: 5.1). The mean microbial TPC of control and ASC treatment at 5°C had exceeded the standard (10^7 CFU/g) before day 9. This result compares to the flathead trial (chapter 5) where microbial counts had also exceeded the standard at day 9 (1.5×10^7 CFU/g) (Figure: 5.1).

A Kruskal-Wallis ANOVA was performed as log transformed data failed the Kolmogorov-Smirnov and Shapiro-Wilk tests for Normality and Levene's test for Homogeneity of Variances. No statistically significant difference was found in microbial Total Plate Counts (TPC) between the control (*Mean Rank*=14.6) and acidified sodium chlorite treated samples (*Mean Rank*=16.4) stored at 5°C over 9 days sampling ($H = 0.314$, $df = 1$, $N = 30$, $p = 0.576$).

ASC and MA Packed Atlantic Salmon at 0°C and 5°C

Equivalent Days on Ice

Equivalent days on ice calculations were performed on the logged temperature data and are listed in the table below. A mean equivalent days on ice was calculated for the Atlantic salmon experiment at $2.14 \pm <0.1$ on logged (5°C or less) data. It can be seen in Table 9.2 that storage at approximately 5°C more than doubled the equivalent days on ice, whereas storage at temperatures slightly above 0°C produced virtually no increase.

Table 9.3: Calculated equivalent days on ice for Atlantic salmon stored at 0°C and 5°C in comparison to actual days.

Days	5	8	11	14	17	20	23	26	29	32
Calculated DOI 0°C	5.1	8.16	11.22	14.28	17.34	20.4	23.46	26.52	29.58	32.64
Calculated DOI 5°C	9.16	17.1	22.8	28.42	36.38	42.8				

Microbial

The initial number of bacteria present on Atlantic salmon portions pre-packing and treatment (TPC) averaged 4×10^3 CFU/g. By day 5 counts had declined and ranged from 5×10^1 to 10^2 CFU/g for both ASC and MAP treatments at 0°C and 5°C (Figure 9.2).

Lag and Logarithmic Growth Phases

The mean counts of samples packed in MA and ASC, MAP at 5°C indicated that they remained within the lag phase for a maximum of 12 and 14 days respectively. Both MAP and ASC, MAP samples at 0°C remained within the lag phase for 20 days. Both 5°C treatments

were nearing a TPC of 10^7 CFU/g at day 20 indicating a maximum microbial shelflife of 21 days. ASC, MAP and MAP stored samples at 0°C had a TPC of 8.7 x 10^6 CFU/g and 1.8 x 10^6 CFU/g respectively at day 32 indicating a maximum microbial shelflife more than 32 days (Figure 9.2).
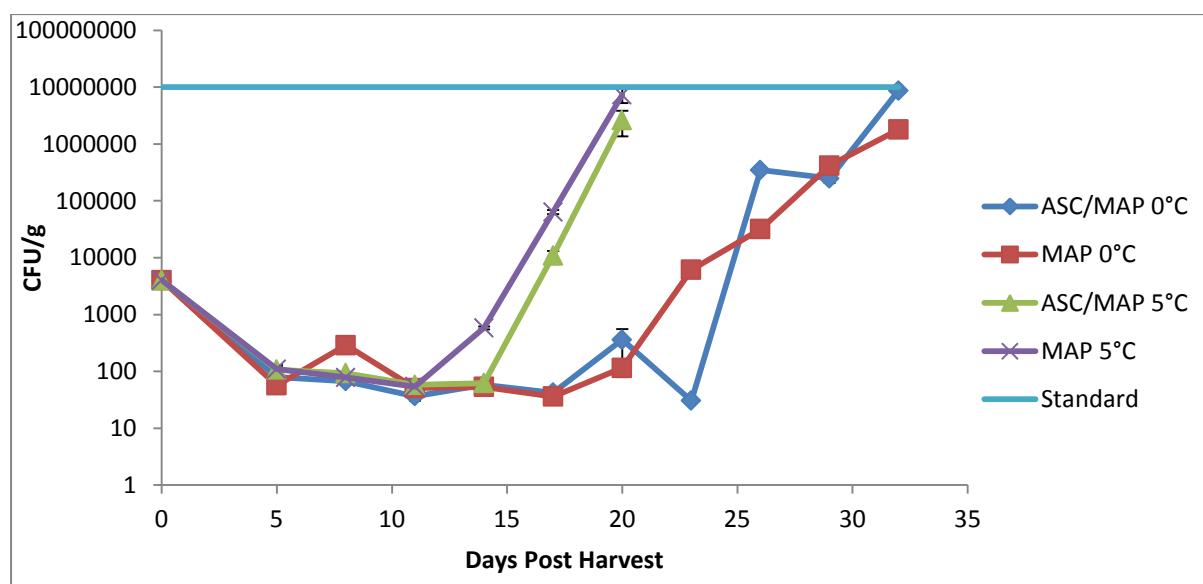
Figure 9.2: Mean (n=3) TPC (±SEM) for Atlantic salmon portions, ASC/MAP and MAP (100% CO_2) at 0°C and 5°C sampled on days 0, 5, 8, 11, 14, 17, 20, 23, 26, 29, 32 or until spoiled. ICMSF (1986) standard of 10^7 CFU/g is indicated by the pale blue line.

A one-way between-groups analysis of variance (ANOVA) was used to investigate whether Atlantic salmon portions treated with acidified sodium chlorite (ASC), packed in MA (100% CO_2) were significantly different (TPC CFU/g) from untreated MA packed (100% CO_2) portions at 0°C and 5°C.

Microbial count data are non parametric and all data were log 10 transformed before analysis. Levene's statistic was not significant, $F(3,116) = 2.2$, $p = 0.092$, and therefore the assumption of homogeneity of variance was not violated.

The ANOVA was statistically significant $F(3, 116) = 7.596$, $p = 0.000$, $\eta^2 = 0.164$.

Post hoc analysis with Tukey's HSD (using an à of 0.05) indicated that the significant difference was due to temperature and not the ASC treatment (Table: 9.3). Numbers of bacteria on portions treated with acidified sodium chlorite (ASC) and packed in MAP were not significantly different from MAP only stored portions at either temperature of 0°C or 5°C ($p = 0.979$), ($p = 0.831$) stored for 20 (5°C) and 32 days (0°C).

Table 9.4: Results of Tukey's HSD test indicate that means of 1 (ASC/MAP at 0°C) were not different from 2 (MAP at 0°C) while 3 (ASC/MAP at 5°C) was not different to 4 (MAP 5°C) but 1 and 2 differed from 3 and 4.

Tukey HSD[a]

| Treatment | N | Subset for alpha = 0.05 | |
		1	2
1.00 ASC MAP 0°C	30	2.6500	
2.00 MAP 0°C	30	2.8523	
3.00 ASC MAP 5°C	30		4.2400
4.00 MAP 5°C	30		4.6782
Sig.		.979	.831

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 30.000.

8.9   Discussion

Direct Application of Acidified Sodium Chlorite to Fish

Acidified sodium chlorite (ASC) is used commercially on a variety of food products to reduce bacterial numbers. The combined use of both ASC and MAP as a useful method of improving microbial safety was suggested by Lu (Lu & Xia, 2012). Its effectiveness on food pathogens and microbial populations has been validated in a number of studies on beef (Beverly et al., 2006; Bosilevac et al., 2004; Midgley & Small, 2006), chicken (EFSA, 2005;

Oyarzabal et al., 2004), and fish (Su & Morrissey, 2003; Tamplin, 2008). The premise underpinning the current study was that ASC would provide an additional hurdle and reduce initial microbial loads or cause sub lethal injury resulting in increased lag phase and product shelflife. In this study on flathead and Atlantic salmon, ASC did not provide a measurable benefit over controls packed in air (flathead) or carbon dioxide (Atlantic salmon) at 0°C and 5°C. This result is contradiction to the literature. A possible reason for this outcome is the surface area to weight ratio of the 20 gram portions to the ASC dosage rate. ASC application in reported trials has been as dips for fillets, whole chicken carcasses and beef that had a lower surface area to weight ratios. The result suggests the volume of ASC was insufficient for the surface area to be treated. The experiments on ASC as a direct spray-on product was discontinued when microbial factors were determined to not be the primary limiting factor of MAP at 0°C. The use of ASC directly on air packed seafood needs further investigation using whole fillets or adjusted ASC dose rates at typical processing temperatures.

Replicated Studies: Microbial Growth Variation in MA Packed Atlantic Salmon at 0°C

In trial 1, Atlantic salmon microbial counts of MAP samples at 5°C and 0°C indicated a lag phase lasting just under 10 days at 5°C and about 17 days at 0°C. The microbial count (2.6 x 10^7) of MAP 0°C had exceeded the standard at day 29, while MAP held at 5°C (1.2 x 10^7 CFU/g) exceeded it after 17 days post harvest (Figure 6.1). In the replicated study the MA packed samples remained within the lag phase for a maximum of 14 days at 5°C and 23 days at 0°C. The maximum microbial TPC (1.8 x 10^6 CFU/g) of MAP at 0°C exceeded the standard (10^7 CFU/g) after day 32, while the MAP held at 5°C (7.3 x 10^6 CFU/g) exceeded it after 20 days post harvest (Figure 9.2).

There a number of possible explanations for the microbial variation observed between the MAP 0°C treatments in the Atlantic salmon studies (Chapter 6 and 9). The initial low microbial counts imply a limited diversity of species on the product of mainly marine origin. Genetic methods (PCR) are identifying a much more diverse flora than had previously been thought (M.B. Hovda et al., 2007; Powell & Tamplin, 2012) on marine species. This variability in species may have implications for the energy requirements necessary to maintain homeostasis. A random variation in the flora or environment effect (possibly seasonal-temperature related) may make microbes more susceptible to hurdles leading to the observed variations.

An alternative or additional cause of the observed variation may be attributed to the susceptibility of microbes to small environmental changes close to the growth - no growth interface. Nearing this point even small enhancements to individual hurdles in a food can have a definite effect on microbial stability (Leistner & Gould, 2002; McMeekin et al., 2000). The combined effect of CO_2, temperature and pH will retard microbial growth differently based on species and correspondingly increase the lag phase as observed in the MAP at 0°C.

9   Cutting Board Cleaning, Sanitation and Rinse Confirmation Pre- and Post-Processing

9.1   Introduction

One of the key premises of this study is the lower the initial microbial number, the longer the lag phase at a given temperature. Poor cleaning procedures and poorly maintained equipment result in increasing microbial counts during processing and variable shelflife.

During processing, cutting boards and equipment (scales, tubs, trays) are contaminated with a variety of materials including protein, slime and offal. This material in combination with moisture at ambient temperatures provides an ideal medium for microbial growth. If this material is allowed to accumulate during processing, microbial numbers will increase and product will become increasingly more contaminated over time. To prevent this accumulation some form of regular cleaning process is required.

Experimental Aims

Three replicate trials were conducted using abalone and Atlantic salmon:

- To assess the effectiveness of the cleaning and sanitizing method pre processing.
- To assess the effectiveness of sprayed water to rinse contaminants from polyethylene cutting boards during and post processing.

9.2   Method

The methods are as listed in the General Materials and Methods section with the following specific details and/or modifications.

Three surface total plate count (TPC) assessments were conducted pre- and post-processing to assess the effectiveness of the cleaning, sanitation and rinsing method on the polyethylene cutting boards.

Swabs were taken along the centre of the cutting board pre- and post-processing at 3 locations during the first salmon trial and at 5 locations during subsequent trials with abalone and salmon.

Cutting boards were contaminated with fish based material during the processing of abalone and salmon. Abalone (n=19) were shucked, gutted, the radula removed and scrubbed on the cutting board over a 2-hour period. Whole Atlantic salmon (n=4, and n=11) were filleted. The fillets were skinned, trimmed and belly flaps removed before portioning. The processing bench was equipped with hoses and the polyethylene cutting boards were rinsed with town supply water after each individual animal was processed to remove visible offal and waste. Rinsing method involved 3-4 second spays with 300-400 ml of water and hand wiping if visible contaminants remained. The processing facilities and rinse method used in this study are shown in Plate 10.1.

Plate 10.1: Cutting board rinsing method applied during the study

9.3   Results

Pre-processing Cleaning and Sanitation

Cutting boards were found in all trials (n=3) to have low microbial colony numbers pre-processing indicating the cleaning methods and chemical sanitisers were effective.

Pre-processing cutting board colony numbers ranged from a minimum of 0 to a maximum of 7 CFU/cm^2. In total 62% of sites tested recorded no colonies and the mean number for all trials and all sites was 1.7 CFU/cm^2 which is below the limit of detection (25 CFU/ cm^2).

Post Processing Rinse Method

The cutting board rinsing method of spraying with water after processing each animal was found to be very effective at keeping bacterial numbers at low levels and preventing the build up of organisms during processing. The rinsing method removed gross contamination and material originating from the digestive tract, blood, slime and protein based materials from the cutting board surface.

Swabs conducted post-processing resulted in colonies being identified at 9 of the 13 sites (69%). Mean colony numbers per site ranged from a minimum of 0 to a maximum of 11 CFU/cm^2 with a mean for all trials and sites of 3.7 CFU/cm^2 (Table: 9.4). Count numbers in all trials were below the limit of detection (AOAC, 2000) and are reported as <25 CFU/cm^2.

Table 10.1: Pre and Post processing TVC counts confirming the effectiveness of the pre-processing cleaning and sanitation methods and cutting board washing methods and their effect on post processing microbial count numbers.

Species	Treatment	Number of sites	Mean CFU/cm^2	SE
Salmon	Preprocessing	3	0	0
Salmon	Preprocessing	5	3.50	1.5
Abalone	Preprocessing	5	0.90	0.6
Salmon	Postprocessing	3	0	0
Salmon	Postprocessing	5	4.80	1.5
Abalone	Postprocessing	5	2.40	1.0

9.4   Discussion

In Australian studies, Tamplin identified filleting benches that used trough sinks as being responsible for a 20-fold increase in pink ling TPC during processing (Tamplin, 2008). While Slattery identified cutting boards and packing tables as a source of cross contamination due to a build up over time of fish based material (S. Slattery et al., 1998). Gillespie observed an increase in the number of bacteria on Queensland fish during handling and processing (Gillespie & Macrae, 1975). Poor practices have been documented in the catching, processing and retail sectors of the fresh chilled seafood supply chain (Boulter, M. 2009, pers. comm. 5 October and Sedman, T. 2011, pers. comm. 7 June).

Cross contamination and resultant elevated levels of microbes can occur due to unclean surfaces, cutting boards, equipment, containers and ice. In addition damaged surfaces can be hard to clean and provide a reservoir of microbes (Tang et al., 2011). Elevated microbial numbers, poor temperature control in combination with a build up of fish-based material during processing can reduce product shelflife (Tebbutt et al., 2007).

The use of continuous running water or water rinsing during processing is widely practiced in Europe and New Zealand. SEAFISH UK, recommend this method in guidance to processors while in NZ Sealord group employ this method in the Nelson processing plant. This study confirmed the effectiveness of water rinsing in reducing the build-up of fish based material on cutting boards during processing.

10 General Discussion

"The long held tenet that industry could solve most of its problems of fish spoilage by more efficient chilling of fish is unquestionably reinforced by the findings in all 7 trials. Fish, whether stored in MAP or not, suffered marked reductions in shelflife when held at 5°C in comparison with fish held at 0°C. The beneficial effect of storage in CO_2 was also reduced at the higher temperature of storage" (Cann et al., 1983).

The effect of MAP, temperature and hygienic practices on product shelflife has been documented extensively over an extended period of time (Boknaes et al., 2000; Cann et al., 1983; Dalgaard et al., 2002; Devlieghere & Debevere, 2000; Dixon & Kell, 1989; Farber, 1991; Huss, 1995; Jakobsen & Bertelsen, 2004b; Mace et al., 2012; Ogrydziak & Brown, 1982; Powell & Tamplin, 2012; Sivertsvik et al., 2002; Valley & Rettger, 1927). The question then becomes why has the sector not addressed these issues identified by Cann? Some of the possible answers may include: a lack of best practice guidelines and clear benchmarks on which to assess shelflife performance; complexity and length of the supply chain (Anon, 2007); and a lack of knowledge and training (the seafood sector has low training participation rates).

There appears to be a general acceptance within the sector that poor practice particularly relating to temperature is acceptable. This is highlighted in an article in the FRDC magazine FISH, "Melbourne Fish Market Revival" (Norwood, 2013). On display in the full page picture associated with the story is a range of suboptimal icing and handling practices. This implies that these practices are accepted and endorsed by FRDC, a major funder of fisheries research. Time and temperature effects are cumulative and irreversible (Huss, 1995).

Optimised Model

The key aims of this project were to develop a best practice model and product shelflife benchmarks for the 4 species to act as a basis for comparative evaluation of supply chain performance. The best practice model applied practical methods throughout the supply chain to harvesting, chilling, handling, processing and packing that could be applied in order to establish the maximum microbial and quality shelflife. Aspects of the methods outlined in the optimised model are currently utilised by groups within industry and can be applied to any chain with little cost, as demonstrated at Southland Fish Supplies (Tamplin, 2008).

Sanitation, Hygiene and Temperature Control

Of initial concern, based on the literature, was microbial spoilage being the limiting factor in shelflife extension of chilled products (Huss, 1995; Reddy et al., 1992; Sivertsvik et al., 2002). To this end the initial focus of the project was to control microbial numbers. The initial hypothesis was that maximum lag phase and shelflife could be achieved by keeping initial microbial numbers as low as possible. This was achieved through the application of hygienic practices, time temperature control and optimal technologies throughout the supply chain.

To achieve this all equipment and surfaces were washed and sanitised prior to use, during processing, water rinsing of cutting boards and equipment occurred between animals that prevented the build up of offal, slime and fish based protein, preventing cross contamination. Temperature was controlled by the immersion of animals immediately post harvest in ice slurry where they remained until processing. During processing, product temperatures were kept to less than 7°C and product was not exposed to ambient temperatures for more than 1 hour before re-entering temperature control. The effectiveness of the hygiene, sanitation and water-rinsing method was confirmed by a series of pre and post processing tests at 16 cutting board sites. All sites were below the limit of detection (AOAC, 2000) and are reported as <25 CFU/cm^2 (Table 9.4).

The success of the method can be seen in 2 independent measures. The low TPC's on the cutting boards pre and post processing and the low initial TPC's of the processed product pre packing. The cutting board results (<25 CFU/cm^2) were 10-100 times less compared to other hygiene surveys of fish processing plants where TPC's for cutting boards and loading tables after 1 hour use ranged from 3.4 x 10^3 CFU/g to 4.4 x 10^4 CFU/g (S. Slattery et al., 1998).

The second measure of success can be seen in the low initial processed TPC's of the 4 species: all were < 4 x 10^3 CFU/g. In comparison, the initial TPC's sighted in the literature from Australian sources are listed in Table 11.1.

Table 11.1: Microbial TPC CFU/g for a selection Australian species prior to processing or during processing as reported by Slattery, Riley and Tamplin.

Source	Species	TPC CFU/g
Tamplin (2008)	Gould's squid	4.2×10^4
	banana prawns	5.8×10^5
	pink ling	5.1×10^5
	saucer scallops	4.2×10^4
Slattery (1998)	swordfish	5×10^5 and 5×10^4
	saucer scallops	4.3×10^4
	skin off fillets	1.1×10^6
Riley (2007)	herring	1.5×10^4
	tailor	4×10^4
	spangled emperor	5×10^3

All bar one (spangled emperor) of these data are at least 10 times higher than initial counts obtained here. It is recommended by Slattery that product with TPC's above 10^4 CFU/g should not be packed in MAP due to the lack of shelflife extension (Slattery, S. 2008. pers. comm. 28 August). This application of simple procedures of chilling and cleanliness enables a marked reduction in initial TPC of seafood.

Acidified Sodium Chlorite (ASC)

Acidified sodium chlorite was investigated as an anti- microbial compound for direct spray application to processed seafood products prior to packing to further limit microbial numbers and to extend the lag phase. This was found to be ineffective in reducing microbial numbers on skinless flathead fillets air packed at 5°C or Atlantic salmon fillets in combination with MAP at 0°C and 5°C (Figure 9.1 and 9.2). A possible cause for this was insufficient ASC spray to effectively cover the surface area of batches of 20-gram portions. This result is in

contradiction of the literature where ASC as a dip at 500 ppm was successful in reducing microbial numbers: in fish, by a log reduction of 0.5-1.5 CFU/g (Tamplin, 2008): in beef carcasses (Bosilevac et al., 2004; Midgley & Small, 2006) and in chicken broiler carcasses (EFSA, 2005; Oyarzabal et al., 2004). Further studies should be conducted using adjusted volume- to-surface area ratios to ascertain the potential for this product to contribute to shelflife extension.

Equivalent Days on Ice

Equivalent days on ice provides a simple calculation to convert variable temperature data to a base of 0°C. This allows the comparison of products held at different storage temperatures. By converting temperature data to a base of 0°C, studies become far more useful allowing direct comparison between studies at various temperatures. All 5°C microbial data was converted using logged temperature data to equivalent days on ice and plotted against 0°C microbial data (Appendix 1). In this study the equivalent days on ice multipliers were 2.2 for flathead, 2.13 Atlantic salmon and 2.09 for both calamari and abalone.

The work on equivalent days on ice provided information on the general accuracy of this method, its applicability to molluscs (abalone) and cephalopods (southern calamari) and its usefulness in bringing variable storage temperatures to a common standard of 0°C. The converted microbial data for flathead was analysed with a Mann-Whitney U test (0°C and converted from 5°C) and all air stored treatments were found not to be statistically different from each other, indicating the ability of the method to bring variable storage temperatures to a common standard (0°C). When applied to MAP microbial data the equivalent days on ice method was not able to accurately convert 5°C data for the logarithmic growth phase. Plots during this phase diverged significantly (Appendix 1). This result was to be expected with

MAP extending the microbial lag phase and decreasing growth in the logarithmic phase (TPC) when compared to equivalent air stored product (Cann et al., 1983; Church, 1994; Dixon & Kell, 1989; Farber, 1991; Ogrydziak & Brown, 1982; Venugopal, 2006; Zwietering et al., 1990).

MAP

One of the key areas identified as limiting experimental repeatability in MAP studies has been a lack of key information relating to pack gas volumes. Growth inhibition in MAP is determined by the concentration of dissolved CO_2 in the product (Devlieghere et al., 1998). This is in turn limited by the volume and pressure of gas in the head space (Devlieghere et al., 1998). Without knowledge of the volume of gas and product (gas/product ratio) the relative impact of CO_2 cannot be calculated nor studies compared. The full preservation effect of CO_2 will only be achieved if the CO_2 in the headspace exceeds the amount required to saturate the flesh. (Jakobsen & Bertelsen, 2004b). The lack of gas/product ratio information may be one reasons for the variability reported between MAP studies (Sivertsvik, 2007).

In all trials of the current study the gas/product ratio exceeded the critical 2:1 or 3:1 (Sivertsvik et al., 2002; Sivertsvik et al., 2004) and were typically 20 g seafood per 400 ml of gas determined by displacement. At these levels the water in the flesh should have been saturated with CO_2 resulting in maximum microbial inhibition (Jakobsen & Bertelsen, 2004a; Rotabakk et al., 2007). The packing of the samples while in pre-rigor should have maximised the absorbance of CO_2 due to the pH being at its highest level. At treatment temperatures of 0°C and 5°C, the volumes of gas absorbed into the flesh should not have been significantly different (Devlieghere et al., 1998).

Shelflife

Microbial Differences

A number of interesting observations were made in the study requiring further discussion. A pattern that was observed with MA packed calamari, taste panel flathead and Atlantic salmon at 0°C was a lag phase followed by logarithmic growth that typically reached TPC's of 10^7 CFU/g in less than 30 days. In a replicated flathead trial the lag phase was extended beyond 30 days and counts were still less than 10^6 CFU/g at 40 days. In abalone at day 25, TPC's were still at 10^2 indicating an extended lag phase, possibly well in excess of 30 days and similar to that observed for flathead.

Fletcher also observed this retarded microbial growth in King salmon where microbial numbers were less than 10^6 CFU/g at 45 days post harvest (Fletcher et al., 2002). While the mechanisms for this growth inhibition are not known it is suggested that small hurdle variations at the growth/no growth interface may result in an infinite or extended lag time (McMeekin et al., 2000). Microbes, when faced with a suboptimal environment, utilise energy to maintain homeostasis. Even small enhancements to individual hurdles in food has a definite effect on microbial stability when microbes are close to the growth, no growth interface (Leistner & Gould, 2002; McMeekin et al., 2000). The combined effect of low initial microbial numbers reducing microbial diversity, CO_2, temperature and lowered pH may be combining to retard microbial growth and correspondingly increase the lag phase as observed in the MAP treatment at 0°C.

Shelflife Extension Temperature and MAP

This study highlighted the marked reduction in shelflife of small temperature increases (<5°C) in the 4 temperate water species. At 5°C, the maximum microbial shelflives for flathead and abalone were 50% less than samples stored at 0°C, while the magnitude of the reduction was less for both Atlantic salmon and calamari, but still significant (Table 11.2). The impact of temperature on quality shelflife was more profound for all air-stored samples, which when stored at 0°C, had shelflife increases of 100% over samples stored at 5°C.

The benefit of MAP is that is can compensate for some of the loss in microbial shelflife that occurs as a result of storage at slightly elevated temperatures. This was demonstrated for all species where MA packed samples at less than 5°C had similar microbial growth curves with air packed samples at 0°C. This result is similar to that of Cann (Cann et al., 1983). The combined shelflife benefits of MAP and temperature (0°C) were in the order of 300% over for all 4 species air stored at 5°C (Table:11.2).

Table 11.2: Maximum microbial and quality shelflife in days for 4 species (flathead, Atlantic salmon, southern calamari, abalone), air packed or MAP at 0°C and 5°C.

Species	Maximum Microbial, Days to 10^7 CFU/g				Maximum Quality Shelflife, Days			
	Air packed <5°C	Air packed 0°C	MAP <5°C	MAP 0°C	Air packed <5°C	Air packed 0°C	MAP <5°C	MAP 0°C
Flathead	9	18	19-20	30+	6-7	13-14	15	21
Atlantic salmon	9	16	17	28	6-7	13-14	12	20
Calamari	10	17	20	29-30	7	13-14	14	19-20
Abalone	10	21	25	>25	nc	15	nc	21

nc: not calculated

Taste Panel

The taste panel method proved successful in solving some of the limitations and difficulties associated with taste panels (Lawless & Heymann, 1999; Meilgaard et al., 2007). Anticipated limitations were overcome by the combination of a small expert panel and full panel conducting discrimination and /or preference tests. The limitations cited in the literature include low participant numbers limiting statistical analysis, organisation and timing, and maintaining the panel over the length of the project, which in this case was 4 years. The expert panel was used to make ongoing quality assessments and the full panel was used to verify if in fact these perceived changes were occurring. The Triangle discrimination test does not require a fully trained panel and only requires the tasting of 3 samples. The simplicity of this test eliminates potential biases and errors that can occur with more complex tests. However Triangle tests are limited in that they do not provide any indication as to the direction, nature or magnitude of the difference (Lawless & Heymann, 1999; Meilgaard et al., 2007).

The Preference tests were used to reconfirm the findings of the Triangle tests and give indication of as to the direction of differences. For example, with abalone the Triangle test indicated a significant difference. The Preference test determined that the MAP treatment was preferred to the control thereby providing direction of the difference while the preference rankings provided indication as to the magnitude of the difference.

While the determination of shelflife is subjective, large numbers of regular seafood consumers as constituted these panels should be able to provide indication as to palatability, acceptability and difference potentially similar to the seafood buying public.

Maximum Quality Shelflife

The placement of these results in the context of the literature has proved difficult due to the lack of studies conducted on Australian species. In addition, international studies on whitefish, Atlantic salmon, squid and gastropods are often only indicative due to the lack of specific data relating to the methods used. For example studies by (Fernandez et al., 2009; Sanguandeekul et al., 2008; Speranza et al., 2009) failed to include specific information on gas-product ratios. The lack of information can include inadequate description of the origin and history of the raw material, gas product ratios or variable temperatures and measures of shelflife (Sivertsvik et al., 2002). In general, cod is considered to have a shelflife of 14-20 days at 0-3°C (Sivertsvik et al., 2002). This is in agreement with the maximum quality shelflife of flathead (whitefish) but is well short of the 40 days found for maximum microbial shelflife in the current study. Atlantic salmon is reported to have a sensory shelflife of 21 days when packed in MAP and superchilled at -2°C (Sivertsvik et al., 2003). This result is close to the 20 days found in the present study. Little directly comparable data can be found for abalone or southern calamari.

One clear outcome that can be drawn from this study is the influence of temperature. Temperature has a greater effect on both microbial and quality shelflife than MA packing (Table 11.2).

11 General Conclusion

This study has proved that by applying best practice handling, hygiene and packing technology throughout the seafood supply chain, extended quality shelflives can be achieved. These have been achieved using methods and equipment readily available to the commercial

sector. These shelflife estimates can provide a basis for the evaluation of commercial supply chain performance (Botta, 1995).

This study has highlighted the impacts of 5°C on shelflife and established that initial chilling and hygienic practices can significantly extend shelflife.

All participants in the seafood supply chain have a vested interest in the eating quality of the seafood consumers purchase. The community will continue to have difficulty in accessing quality seafood reliably until the suboptimal practices occurring throughout the chain are addressed. If these practices continue, revenue will be further reduced for all participants throughout the chain and result in a lack of consumer confidence in seafood as a regular reliable food product. Further studies should define where sub-optimisation is occurring throughout the chain. It is only when the causes of sub-optimisation have been rectified that the full potential of seafood as food product will be achieved.(Sykes et al., 2009)

12 Appendix 1

Data converted to Equivalent days on ice (0°C) and plotted for both air stored controls and MAP at 0°C and 5°C for each of the 4 species.
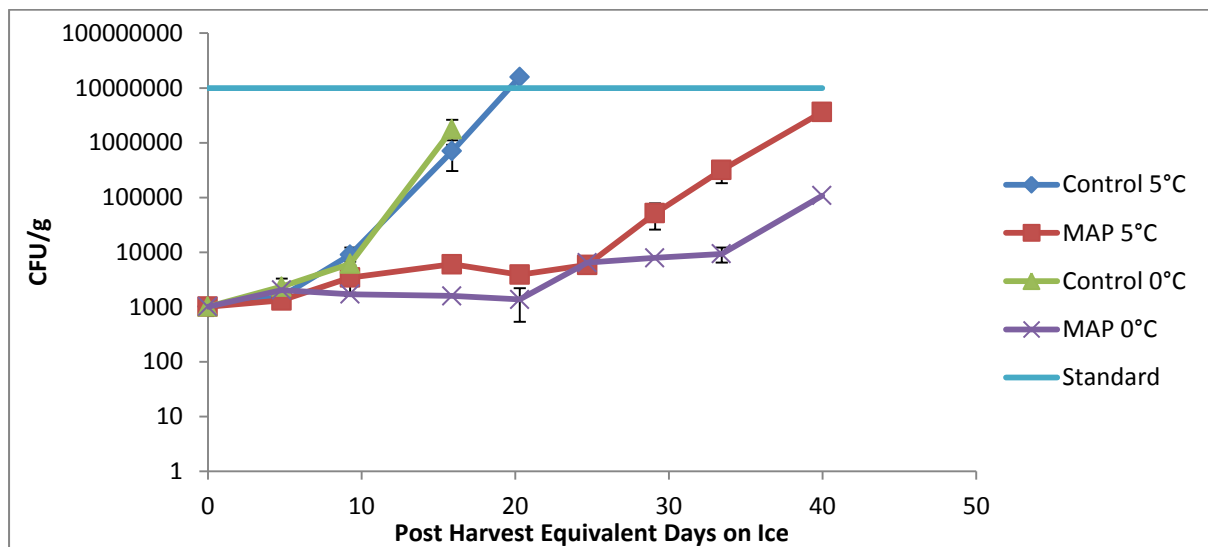
Figure 13.1: Mean (n=2) TPC for flathead portions, control and MAP (45:55 $CO_2 O_2$) at 0°C and 5°C when plotted as Equivalent days on ice. ICMSF (1986) standard of 10^7 CFU/g is indicated by the pale blue line.

Figure 13.1 shows that the equivalent days on ice microbial growth data for controls stored at 0°C and 5°C were not statistically different when analysed with a Mann-Whitney U test (U=7.5, z= -.145, p= .886, two-tailed).
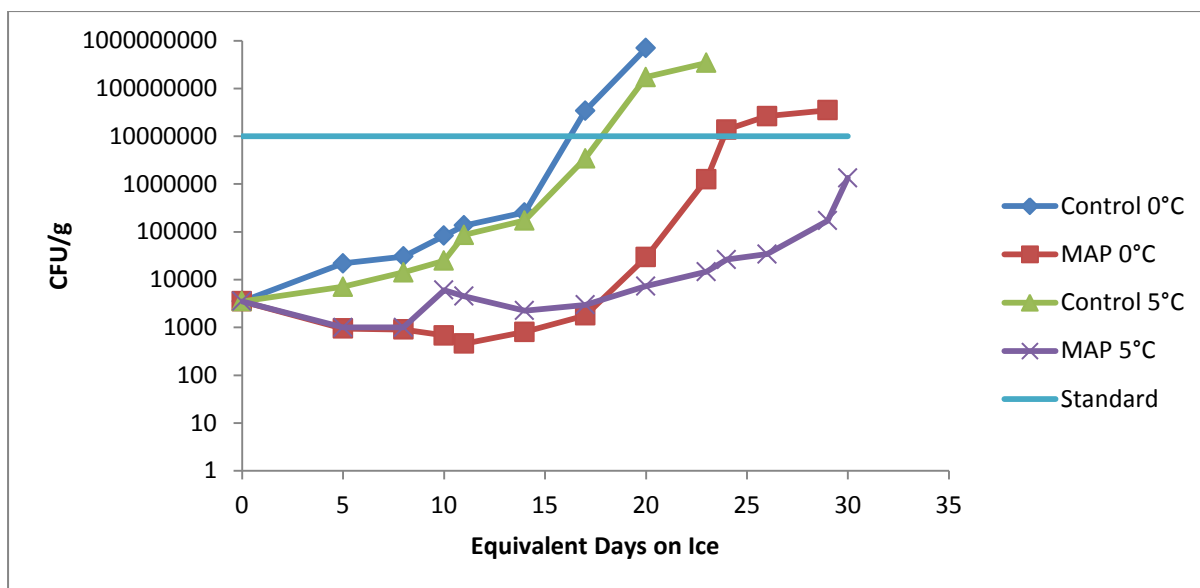
Figure 13.2: Mean (n=2) TPC for Atlantic salmon portions, control and MAP (100% CO_2) at 0°C and 5°C when plotted as Equivalent days on ice. ICMSF (1986) standard of 10^7 CFU/g is indicated by the pale blue line.
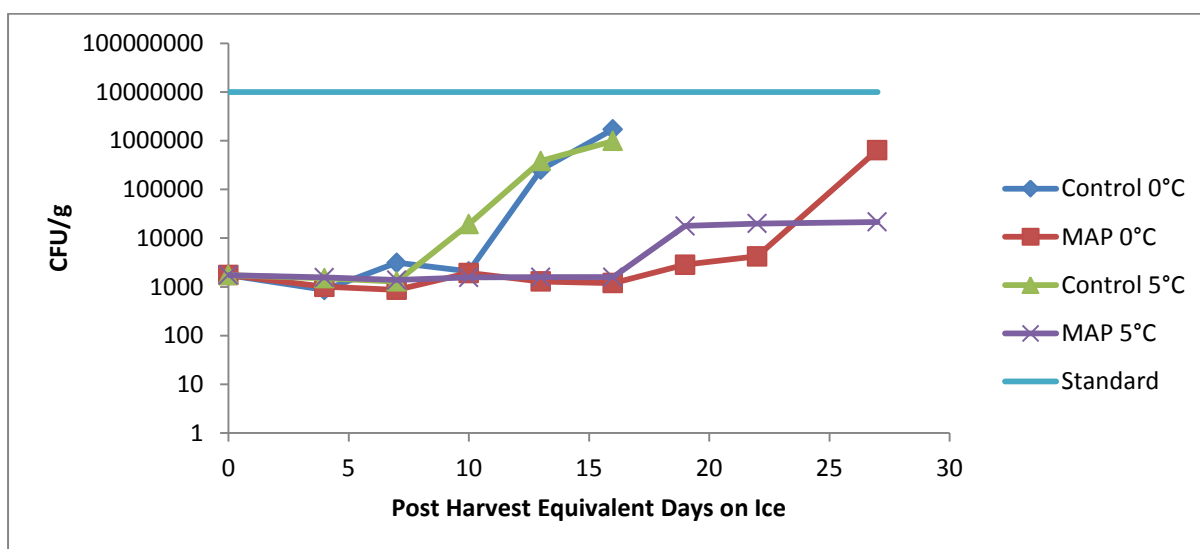
Figure13.3: Mean (n=2) TPC for calamari portions, control and MAP (100% CO_2) at 0°C and 5°C when plotted as Equivalent days on ice. ICMSF (1986) standard of 10^7 CFU/g is indicated by the pale blue line.
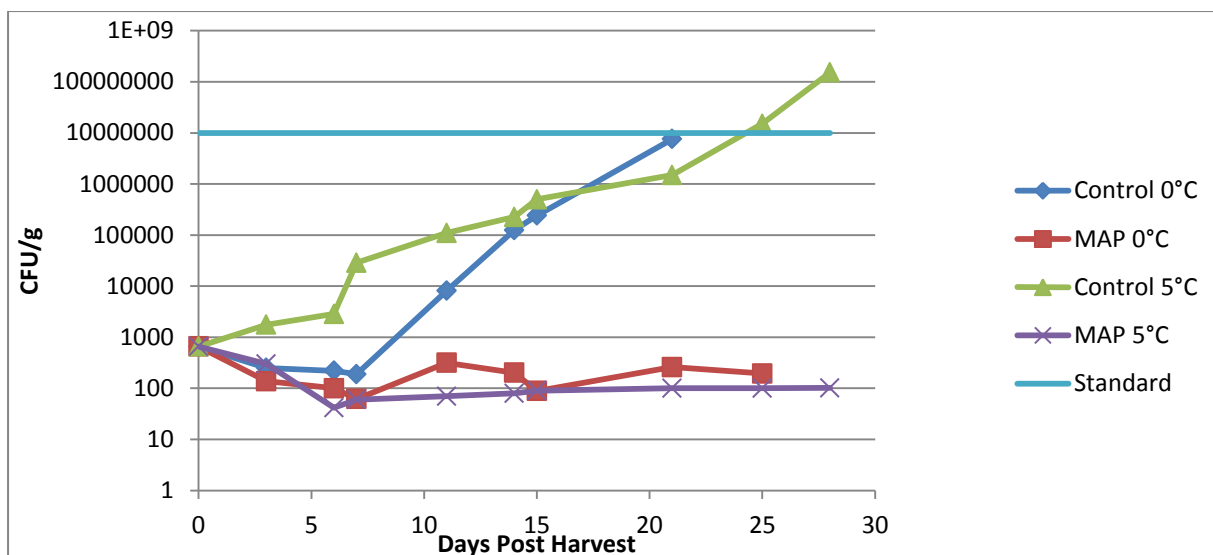
**Figure 13.4:** Mean (n=2) TPC for abalone, control and MAP (100% CO_2) at 0°C and 5°C when plotted as Equivalent days on ice. ICMSF (1986) standard of 10^7 CFU/g is indicated by the pale blue line.

13 Appendix 2

Taste Panel Methods

Flathead, 9 Point Hedonic Scale Acceptability Test

Name.. **Date**.......................................

Please select an answer below to indicate your recent level of consumption of this product:

In the last 3 months, about how often have you eaten fresh fish (tick one)

(   ) less than once per month

(   ) more than once per month but less than once per week

(   ) more than once per week

Sample number 245

Tick the box that best describes your overall opinion of each sample

☐　**Like extremely**

☐　**Like very much**

☐　**Like moderately**

☐　**Like slightly**

☐　**Neither like nor dislike**

☐　**Dislike slightly**

☐　**Dislike moderately**

☐　**Dislike very much**

☐　**Dislike extremely**

Example: Sample Coding Triangle Test

	Coding Triangle Test Salmon			
Number	A Control	A Control	B Treated	B Treated
1	862 A	245 A	458 B	614 B
2	862 A	458 B	245 A	
3	458 B	862 A	245 A	
4	614 B	458 B	862 A	
5	614 B	862 A	458 B	
6	862 A	458 B	614 B	
7	862 A	245 A	458 B	
8	862 A	458 B	245 A	
9	458 B	862 A	245 A	
10	614 B	458 B	862 A	
11	614 B	862 A	458 B	
12	862 A	458 B	614 B	
13	862 A	245 A	458 B	
14	862 A	458 B	245 A	
15	458 B	862 A	245 A	
16	614 B	458 B	862 A	
17	614 B	862 A	458 B	
18	862 A	458 B	614 B	
19	862 A	245 A	458 B	
20	862 A	458 B	245 A	
21	458 B	862 A	245 A	
22	614 B	458 B	862 A	
23	614 B	862 A	458 B	
24	862 A	458 B	614 B	
25	862 A	245 A	458 B	
26	862 A	458 B	245 A	
27	458 B	862 A	245 A	
28	614 B	458 B	862 A	
29	614 B	862 A	458 B	
30	862 A	458 B	614 B	

Triangle Test Assessment Sheet

Name	**Date**
Instructions Taste samples from left to right. Two are identical; Determine which is the **odd sample** and record the number in the centre column. If you are unsure guess. If no difference is apparent, you must guess.	

Sets of three samples	Which is the odd sample?	Comments
862 614 458		
223 183 393		
756 266 101		

Triangle Test Briefing Sheet

You have shown interest in participating in the taste panel assessment of a seafood product. The aim of this project is to establish shelflife benchmarks for 4 species of chilled Australian seafood. Today you will be tasting one of these species. The three samples will consist of control (good quality untreated raw material) and product treated by best practice handling, sanitation and packaging methods.

You can elect not to participate at any time and you are under no obligation to taste if you are concerned in any way with any product.

Due consideration has been taken to ensure the product is safe to eat including compliance with relevant food legislation.

An example of the taste panel form you will be required to complete is listed below. This form has no means of identifying individuals and the data collected will be subject to privacy considerations and will not be traded or on-sold. Completed forms will be stored in the NCMCRS archived for 5 years before disposal by shredding.
If you have any questions please ask.

Triangle Test		
Instructions Taste samples from left to right. Two are identical; Determine which is the **odd sample** and record the number in the centre column. If no difference is apparent, you must guess.		

Sets of three samples	Which is the odd sample?	Comments
862 245 458		
223 731 183		
756 432 266		

Preference Test Sheet: Calamari

Date:...............................

Taster Number:................................

      Please rinse your mouth with water before starting. You may rinse again at any time if required.

      Please taste the five samples in the order presented, from left to right. You may retaste the samples once you have tried all of them.

Rank the samples from most preferred to least preferred using the following numbers:
1=most preferred, 4= least preferred.
(If you have any questions, please ask the server now)

Sample	Rank (1 to 4)
387	....
765	....
254	....
196	....

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