

**INVESTIGATIONS INTO THE QUALITIES OF FARMED, FRESH
SOUTHERN BLUEFIN TUNA, AIR-FREIGHTED FROM PORT
LINCOLN, SOUTH AUSTRALIA TO TOKYO, JAPAN**



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March 25, 2007

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DECLARATION

I, Alistair Ewan Douglas, certify that this thesis does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

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Alistair Ewan Douglas

ACKNOWLEDGEMENTS

The challenges involved in multi-disciplinary and multi-industry research in two nations were significant. For example, directly involved in one experiment were a tuna farm and its staff (Blaslov Fishing), a processor (Australian Bight), a freight forwarder (Danzas), five Australian scientists, students and staff (Flinders University and Allan Bremner & Associates), six Japanese scientists, students and staff (Tokyo University of Fisheries), five staff of an auctioneering company (Toichi), four staff of a wholesaler (Sugahei), and finally, eight research staff and forty five general staff of a major Japanese trading company (Nippon Suisan). Organization of this particular experiment was not only challenging – its execution was complicated by a storm and the tuna involved delayed in Sydney for twenty four hours due to the dangers of the originally booked aircraft breaking apart during landing (as lighter flowers had been previously loaded onto the plane unbalancing the trimmings of the aircraft). The times of just being allowed on a local plane covered in tuna blood, crossing the equator, and not sleeping on a bed (or futon) for over sixty hours to do an experiment really did make the beer and sake taste that much better at the end of the day(s)!

This work therefore resulted from the collaborative efforts, both directly and indirectly, of so many people representing research, government and commercial groups in Australia and Japan, that it would require too much paper here to personally thank everyone. However I must name the following:

In the Australian Industry: Justin Nelligan & Ric Lehmann of Blaslov Fishing; Rick Kolega, Semi Skoljarev & David Warland of Sekol; Stephen Moriarty of Southern Waters; Terry Richardson of Australian Bight; Brian Jeffriess of TBOAA., and David Ellis of Fishing Industry Services. In Australian R&D organizations: Peter Montague & Emily Downes of Aquafin CRC; Stephen Clarke of SARDI; Stephen Nottingham of CFT; Mark Thomas, Kelie Stead, staff and resident researchers of the LMSC.

In the Japanese industry: Tatsumi Taguchi, Ryozo Moriwaka, Masuo Murakami & Kenichi Kurihara of Toichi; Tsunenori Iida, Toichiro Iida & staff of Hicho; Takeshi Ueda of Naito; H. Tanaka & staff of Sugahei; Joji Kojima & staff of Yamajo. In the Japanese R&D organizations: Takafumi Arimoto, Toshiaki Ohshima, Etsuo Watanabe, Chiaki Imada, Naoko Hamada, Takeshi Kobayashi, Sohn Jeong Ho, staff and students of Tokyo University of Fisheries; Testu Mori, Tomoko Kohata, Itaru Shioya, Kazuyuki Yuguchi, & Minako Obama of the Central Research Laboratories of Nippon Suisan

In closing I would like to thank my supervisors Dr. John F. Carragher and Dr. Philip M. Thomas for all your efforts and advice along the way. I am greatly indebted to Prof. H. Allan Bremner - this research would not have been completed without your involvement, your support, and timely and detailed correspondence – thank you. Finally, my family and friends for your unconditional support throughout – Cheers!

David this is a little bit better mate...

ABSTRACT

The establishment of the Aquafin CRC enabled Japan-based research into the qualities of farmed, fresh Southern bluefin tuna to be conducted. This occurred via industry collaboration in both Australia and Japan, and through the establishment of a memorandum of understanding between Flinders University of South Australia and the Tokyo University of Fisheries (now the Tokyo University of Marine Science and Technology), and an agreement between the Aquafin CRC and Nippon Suisan, the product was profiled, and instrumental and sensory investigations into the qualities of this valuable product were able to be developed and undertaken.

Although the three major cuts of tuna white muscle, known as Akami, Chutoro, and Otoro, are compositionally different, they were shown to have similar patterns of change post-mortem for a selection of bio-chemical parameters commonly associated with 'quality', potentially allowing for the indirect assessment of the more valuable cut (Otoro) from the destructive sampling of the less valuable cut (Akami).

Further, the establishment of a correlation between expert subjective assigned ranks of 'quality' and a ratio of derived red, green, and blue (RGB) values from digital images of the flesh, offers a new objective quality assessment technique that is both rapid and non-destructive. In addition, a balanced and statistically robust analytical protocol was developed for the sensory assessment of the whole carcass qualities of tuna flesh. The protocol allows for the affect of any

on-farm or in-chain manipulations on the sensory properties of the flesh that are directly perceptible to consumers to be assessed.

As the product has a reputation for short colour shelf-life on the market, the effects of using vitamin supplements as a counter measure (as per the industry practice) on the concentrations of vitamins in the flesh and on the colour shelf-life of the end product were investigated. Vitamin supplementation was categorically proven to aid in the colour retention of the flesh of farmed Southern bluefin tuna with low to medium levels of fat both in Australia and in Japan.

Harvest stress is known to affect the qualities of fish flesh, and in this study the effects of a prevalent industry harvesting practice on a selection of sensory and biochemical quality related characteristics of tuna flesh were investigated. Although there were no significant differences in the majority of the sensory and biochemical indicators of quality between fish harvested at the beginning or at the end of a commercial tuna harvest, expert-calibrated RGB ratios and the sensory descriptors of transparency and brightness resulted in significant deleterious effects of harvest stress on the Akami and the valuable Otoro sections respectively.

Finally, the time-temperature management of chilled tuna carcasses when air-freighted to Japan, as well as the effects of shipping on the day of harvest or the day after harvest on flesh quality were investigated. Within the cold chain, the most likely periods when temperature control could be violated were

shown to be during the loading and off-loading of the tuna coffins at the airports. And, although there were no statistical significant differences between the sensory and biochemical parameters measured from fish shipped on the same day as harvest when compared to those shipped a day after harvest, averages favoured the latter where recorded carcass temperatures were lower and more stable.

Finally this body of work demonstrated that collaborative market-based research can be undertaken in Japan, and that product quality needs to be measured in a way that is sympathetic to customer culture and expectations.

1. INTRODUCTION

1.1 Background

Southern bluefin tuna (SBT) – *Thunnus maccoyii* – is a member of the Thunnus family of tunas which include Northern bluefin tuna, Yellowfin tuna and Bigeye tuna. They are large, fast swimming pelagic fish found throughout the Southern Hemisphere mainly in the waters between 30 and 50 degrees south. Their only known breeding ground is in the Indian Ocean south east of Java, from where the juveniles migrate down the coast of Western Australia, around Cape Leeuwin to school in the waters of the Great Australian Bight (Caton, 1991; Fig. 1). It is in these waters that the Southern bluefin tuna fishing and canning industry of Port Lincoln in South Australia was established in the 1950s by a group of pioneering immigrants (Fig. 1.1).

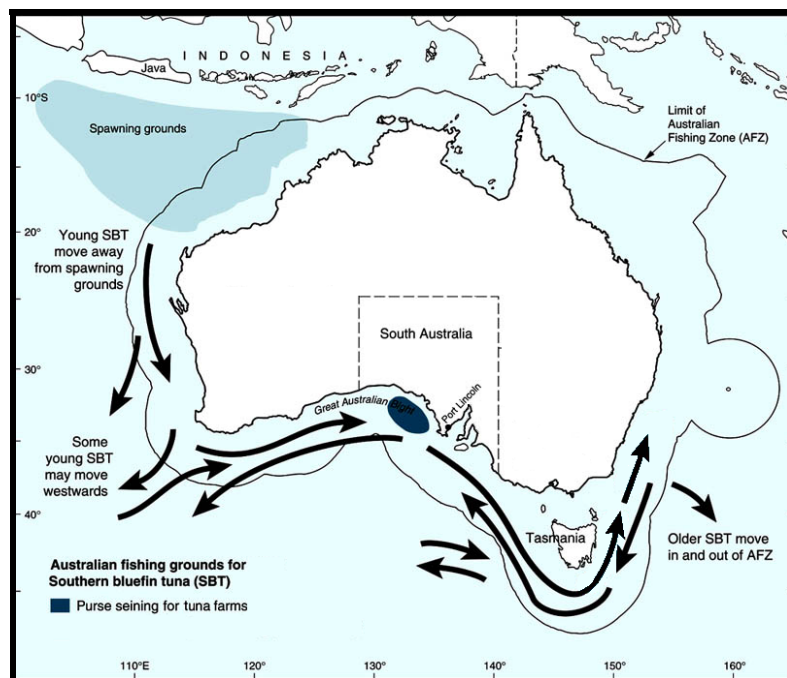


Figure 1.1: Spawning, fishing grounds, and migratory routes around Australia for Southern bluefin tuna (*Thunnus maccoyii*). Source image: Caton (1991).

The species however, was over fished in the 1970s and 80s, and in response to poor catches and poor economic returns from the canned tuna market, a research and development venture involving the Tuna Boat Owners Association of Australia, the Japanese Overseas Fisheries Cooperation Foundation and the South Australian government was established in the early 1990s to examine the feasibility of transporting live, wild captured fish from the Great Australian Bight to the waters off Port Lincoln for shipment to the lucrative Japanese sashimi market. The relative success of this first trial led to a two year program funded by the Fisheries Research and Development Corporation (FRDC) to investigate the holding, maintaining and marketing of the product (Clarke & Bushell, 2001). It is from these beginnings that the SBT farming industry of Port Lincoln evolved.

Instead of being landed at sea in December to March each year, the industry now corrals the tuna in purse seine nets and transfers them underwater into tow cages. These cages are then towed from the fishing grounds to the waters of the Spencer Gulf off Port Lincoln where they are once again transferred into larger grow out cages. The tuna are kept between two to six months in the Spencer Gulf being fed a variety of baitfish species. When they are ready for market they are hand-harvested, placed immediately into iced sea water, processed on land, either placed into freezer containers or loaded into refrigerated trucks, and then shipped or air-freighted to Japan respectively.

A Commonwealth Government agreement in 2001 saw the establishment of an aquaculture focussed Collaborative Research Centre (Aquafin CRC) with

the aim of providing critical technologies for the rapid and sustainable growth of finfish (particularly Atlantic salmon (*Salmo salar*) and SBT) aquaculture in Australia. Set up for a seven year period (2001-2008) with a planned investment into Australian aquaculture totalling \$34 million, the Aquafin CRC provided the Farmed Southern Bluefin Tuna Aquaculture Sub-Program, initiated by the FRDC in 1997, with a substantial funding boost.

Within the R&D sub-program there are five major areas of research including farm husbandry and management, feeds and nutrition, environmental monitoring and mitigation, fish health, and product quality. In the early years, the industry members and researchers of the product quality team had a limited understanding of the raw product they were producing and shipping to Japan and what quality characteristics constituted a sashimi grade product. Therefore, it was first necessary to characterise the product qualitatively into its parts, and quantitatively via physico-chemical analyses, before determining if there were effects of any pre- and post-harvest processes on the qualities of the product. Along with a variety of instrumental techniques, a subjective flesh colour ranking scheme was developed to investigate and monitor the effects of various treatments such as harvest stress and vitamin supplementation on the colour stability of the product. This sensory method soon became a cornerstone of the research program and a well trained and experienced panel now exists at the Lincoln Marine Science Centre in Port Lincoln.

The funding boost provided by the Aquafin CRC brought with it opportunities to investigate the qualities of the product in its market in Japan. To facilitate this,

a Memorandum of Understanding was signed between CRC participant Flinders University and the Tokyo University of Fisheries (now the Tokyo University of Marine Science and Technology). This allowed for research to be undertaken not only in Australia but also Japan, and for analytical techniques to be shared, developed, and applied in an atmosphere of collaboration. Another major step was the signing of a collaborative agreement between the Central Research Laboratories of the large Japanese seafood importer and processor Nippon Suisan Pty. Ltd. and the Aquafin CRC. These agreements made possible physico-chemical and sensory research into the qualities of Australian farmed Southern bluefin tuna at the point where it is sold and consumed. The following research chapters detail the methods employed, developed, and investigated in both countries to define, sample, characterise and measure the instrumental and sensory qualities of this unique product.

1.2 Defining Quality

“Quality is an unusually slippery concept - easy to visualise and yet exasperatingly difficult to define” Garvin D.A. (1988) *Managing Quality: The Strategic & Competitive Edge*, The Free Press, MacMillan Inc. New York.

According to Payson (1994), prior to any discussion on quality, a specific perspective must be established with regard to its meaning. The term quality is positioned third within the fourteen Aristotelian categories, following substance and quantity, and its various definitions occupy more than two pages of the Oxford English Dictionary (OED 2nd Edition, 1989). The word has been used to refer to the character, disposition, nature, capacity, skill, accomplishments, title, social position, profession, fraternity, and the mental and moral attributes of both humans and animals. It is used to define objects by their attribute, property, manner, style, habit, power, substance, nature, and kind. Its synonyms are many. Furthermore, quality is contextual and relative. Thus, the quality of identical items can be judged differently at either the same time in a different context or in the same context at a different time (Meiselman, 2001).

In developed economic societies the importance of product quality to both producers and consumers is rarely questioned, however, the determinants of quality and its meanings are often poorly, if at all, defined (Bremner, 2000; Meiselman, 2001). Garvin (1988) asks the questions: Is quality objective or subjective? Is it relative or absolute? Is it timeless or socially determined? Can it be divided into narrower and more meaningful categories? According to Payson (1994), as modern humans are economic beings, quality legitimizes us as providers of goods and services, and, in some sense is our *raison d'être*.

Economists study quality and ways to measure it in order to figure out quantity. This is opposed to businesses, political organizations, and research organizations whose struggle is to foster its improvement (Payson, 1994). The same author proposes that a "good's quality is an inherent aspect of the good itself, whether or not one can actually measure it". However, such a definition only leads to the frustrated retort "*I know quality when I see it!*" - a statement often used by middle management to their subordinates when struggling to define quality despite it being the goal of all firms (Taormina, 2001).

A global institutional approach to defining and standardizing the qualities of both products and services, the International Organization for Standardization (ISO), claims that a lack of standardization can affect the quality of life itself. That the standardization of screw threads helps to keep chairs, children's bicycles and aircraft together, and that for the disabled, for example, they are able to access and use consumer products, public transport, and buildings because the dimensions of wheel-chairs and entrances are standardized.

In his attempt to define quality, Garvin (1988) proposed the following five categories; Transcendent Quality, Product-Based Quality, User-Based Quality, Manufacturing-Based Quality, and Value-Based Quality. Transcendent Quality is synonymous with innate excellence and somewhat beyond definition but attainable via experience. Product-Based Quality is viewed as a precise and measurable attribute of a product. User-Based Quality centres on the premise that beauty lies in the eyes of the beholder. Manufacturing-Based Quality is based on the conformance to requirements in engineering and manufacturing

practices. And finally, Value-Based Quality provides conformance or performance at an acceptable cost or price. However, a much more simple approach to quality was proposed by Crosby (1979) where he states that *“Quality means conformance to requirements, and that’s all it means. If you start confusing quality with elegance, brightness, dignity, love, or something else, you will find that it has different ideas. Don’t talk about poor quality or high quality. Talk about conformance and non-conformance.”* Although suitable for screws, such a definition alone would hardly be welcomed by the food and beverage industries other than the technologists.

The Total Food Quality Model (TFQM), originally proposed by Grunert et al. (1996), is an attempt to integrate a number of approaches to analyse consumer quality perception and decision-making. The model distinguishes between before and after purchase evaluations with the dimensions of quality categorized into search, experience and credence characteristics (Darby & Karni, 1973). Which category a product is placed in depends on when the consumer can ascertain quality attributes. A search quality (like the appearance of a piece of meat) can be evaluated before the purchase, an experience quality (like the taste of the meat) can first be evaluated after the purchase, and a credence quality (like the healthiness of the meat) can, under normal circumstances, not be evaluated by the average consumer at all, but is a question of faith and trust in the information provided. Many characteristics of a food product, like taste, cannot be ascertained before purchase. Therefore most food products have search characteristics to a limited degree. In order to make a choice, the consumer will develop expectations about quality — but it

is only during and after consumption that experience quality can be determined, and even this is limited in the case of credence characteristics like the healthiness of a product. The consumer and technologist alike must therefore rely on attributes that, in their experience, imaginary or not, link with the experience quality

The term quality, according to Bremner (2000), is properly used in advertising, sales, and marketing to create an impression in the minds of subjects without having to be specific about the meaning. However, in the scientific field a more specific meaning of the word quality is required, and along with the words fresh or freshness, the author claims it is probably the most misused word in food science. Further, according to Meiselman (2001), despite food quality being multidimensional, most attempts to define and measure it in the food sciences are one dimensional such as the sensory characteristics or the microbiological status of food. Studies in food quality have centred mostly in product development and have utilized technical approaches to food science and technology, microbiology, consumer research, and market research (Meiselman, 2001). However, definitions of quality may vary according to these differing viewpoints. A technologist may list only safety, nutrition, availability, convenience, integrity, and freshness as the quality defining attributes of foods. Other qualities such as value for money, legal value, technological value, socio-ecological value, psychological value, and political value could be included in a definition when the viewpoints of other stakeholders and professionals are considered (Bremner, 2000).

With quality a national obsession in Japan, and the colour, shape and arrangement of a meal as carefully thought out as a painting (Garvin, 1988; Yoshida & Sesoko, 1989), how does the Japanese market define the quality of tuna used in sashimi or sushi – the origins of which dates back over 1200 years (Tayama, 1981)? Do Northern bluefin tuna, caught in the waters off Japan since the Manyo Period (8th century) require a separate definition to that of a Southern bluefin tuna, which Japanese fisherman only commenced fishing for in 1952-53 in the southern hemisphere when on-board freezer technologies were developed? Are definitions universal and static or do they change according to the time of season, fishing ground, region, or fish size? Can the quality of an individual tuna be assessed or is the quality of each of the differing cuts of a tuna assessed separately? Do the quality definitions of these differing cuts change depending on form or intended use – raw cuts used in sashimi or sushi, minced in sushi, or baked?

Finally, is the assessment of quality standardised or do the definitions change between the differing stakeholders – the wholesalers, retailers, restaurateurs, chefs, consumers, regulators, etc.? Amongst consumers, do notions of quality change depending on whether sea birds or dolphins were caught along with the tuna, whether the fishery is sustainable, whether the species is rare and expensive, or will it vary whether the consumer is a man or a woman, or between women who are or who are not pregnant etc.?

Tunas, according to Ebisawa (1996), were not considered a high quality sushi ingredient until the beginning of the Second World War. Indeed, one of the first

specialist sushi restaurants established in 1684 stated on the entrance curtain that '*As tuna goes off quickly it is not served here*'. At the beginning of the Showa Period (1926-1989) the high fat *toro* regions of the tuna became regarded as higher quality than the low fat *akami* regions. The reason behind the fascination with *toro*, Ebisawa (1996) claims, is deeply tied to the 'violent surge' of American culture following the Second World War and the westernization and 'fattening' of the food culture of the Japanese.

As a result of this, the high fat *Hon Maguro* (Northern bluefin) and *Minami* or *Indo Maguro* (Southern bluefin) are considered the best tuna species with a single piece of sushi (approximately 10g) costing as much as 2-3000 yen (Tayama, 1981; Ebisawa, 1996). Southern bluefin however, according to Tayama (1981), is a relative newcomer and Japanese consumers have not been acquainted with this species for very long when compared to Northern bluefin. According to Ueda (2003), a 'good' Southern bluefin will be comparable to a Northern bluefin in flavour, and thus, they are considered number two (for raw consumption in Japan).

The wholesale market for tuna in Japan is artisanal with some wholesaling operations being family concerns spanning ten or more generations. There is neither a definitive nor market-wide systematic grading scheme used to describe and assess tuna quality. Instead, it is referred to and communicated colloquially amongst wholesalers who are trained over many years to distinguish 'good' from 'bad' quality tuna. This is done prior to auction, where wholesalers assess tuna quality visually and tactilely, and descriptors used are

based mostly on the colour, fat, and moisture levels of the flesh, and the shape of the tuna. Individual wholesalers will occasionally use symbols to represent the quality of the tuna they are interested in bidding on in the auction and will write them alongside an individual fish's auction number. Such symbols can be developed by individual wholesalers for their own use or handed down and shared amongst other employees of the same company.

There are many general terms used to describe the quality and shape of tunas on the market. Terms such as the onomatopoeic '*gari*' refers to the sound a knife makes when inserted into the carcass of a low fat specimen, and '*rakkyo*', which refers to a fish that's shape resembles that of a shallot where the head appears large and the tail is thin. These are often fish that may have recently spawned and have minimal fat in their flesh and are therefore considered 'low quality' in the current market. Shallots (*rakkyo*) and ginger (also *gari* in Japanese) are often used as condiments when eating raw tuna – possibly along with these low quality tuna as they may be considered necessary to add flavour (Tayama, 1981). Other examples include *mizumaguro*, or 'water tuna', which are tuna whose flesh lacks consistency and is too moist when eaten, and *akabero*, which refers to flesh that has a red jelly like appearance and texture (Tayama, 1981). In addition, a whole range of terms and expressions exist to describe the flesh of diseased, damaged, or poorly processed tuna (for a full list see pages 15-17 of *The Australian Tuna Handling Manual – A Practical Guide to Industry* (Erica Starling & Geoff Diver). Seafood Services Australia, Queensland, Australia.

As a large quantity of tuna is sold on auction floors throughout Japan, it may be argued that the average price paid per kilogram by Japanese wholesalers is a useful determiner of 'poor' and 'good' quality tuna. However, for similar reasons economists have problems pricing oil, gold or water, tuna catch and supply can vary greatly on any given day or within any given season. Furthermore, and unlike those other commodities, demand for tuna is elastic as there are substitutes on the market – both as a source of sashimi tuna and as a source of protein. Moreover, buyer and consumer behaviour can be erratic with auction battles leading to extreme prices such as the 20.2 million yen (AUD\$310,000) paid for a single 202kg Northern bluefin tuna by a wholesaler in Tokyo who told reporters after the auction, "I just wanted to buy the highest quality tuna" (Anon, The Japan Times, 2001). Although such outliers can be removed from analyses, the issue of arbitration and the need to collect a whole range of market-related data (e.g. indicators of demand, supply, price and availability of substitutes etc.) would complicate the use of auction price as a meaningful quality indicator to assess the outcome of flesh quality experiments over time.

With no definition and standardised grading system on the markets in Japan, tuna quality as it is assessed and communicated appears to fit into the first of Garvin's (1988) five definitions of quality (transcendent quality) where the author claims that quality cannot be defined precisely - that it is an un-analysable property one learns to recognise only through experience. Such a definition and system of assessment, however, is not particularly suitable for scientific investigation. Therefore, it is necessary to remove the

psycho-socio-economic aspects of quality, and examine tuna solely as a muscle food that is comprised of water, proteins, fats, carbohydrates, vitamins and minerals, and a variety of other organic and inorganic constituents; with the relative mix of these constituents determining the conformation, appearance, odour, texture, flavour, nutrition, and safety of the product at any one time prior, during, and after consumption. These are the physico-chemical and sensory qualities of a single cut of tuna muscle - its 'qualitas'. It is only when these qualities are combined with hedonic properties, such as the 'peace of mind' a consumer may feel if the tuna came from a sustainable fishery or the feeling of 'exclusivity' a consumer may feel if consuming a rare tuna etc., that the 'total quality event' peculiar to the individual consumer and the cut of tuna in question is produced.

In the case of fisheries and aquaculture, as with other industries, it is the role of marketers, industry organizations, technologists, and company management to identify, define and improve the psycho-socio-economic qualities of a product and service where it is possible to do so. Alternatively, it is the role of fishers or farm managers, processors, and distributors to ensure the product meets defined safety standards and possesses the physico-chemical and sensory qualities that best satisfy its end users. It is this concept that best reflects the ISO 8402 Standard for Quality which defines quality as "the totality of features and characteristics of a product or service that bear on its ability to satisfy stated or implied needs". These 'needs' can be identified by establishing links between the physico-chemical and sensory qualities of the product to the results of blind consumer preference testing. Prior to

accomplishing this, however, it is first necessary to identify and/or develop methods to directly measure and profile the inherent variations in both the physico-chemical and sensorial qualities of the product.

1.3 Measuring the Qualities of Fish

The quality attributes of fish can be analysed instrumentally or sensorially. However, as fish is a food product the ultimate judge of quality is the consumer and instrumental methods need calibrating to sensory techniques (Gill, 1995). The appeal of instrumental techniques, according to that author, is that they allow for the setting of quantitative standards such as the tolerance levels of chemical spoilage indicators, and eliminate the need to base decisions on personal opinions and time-consuming microbiological methods.

1.3.1 Instrumental Techniques

There are many instrumental techniques available for making an assessment of the qualities of fish – be they measures of body composition or freshness. Body composition assessment methods can be divided into five levels: the atomic level, the molecular level, the cell level, the tissue level, and the whole-body level (Durnin, 1995; Duerenberg & Schutz, 1995). All levels are related and one can calculate total body composition from each level, assuming constant and equal relationships in all individuals. Methods can be either direct (chemical analysis), indirect (making use of data based on chemical analysis), or doubly indirect (based on a statistical relationship between easily measurable body parameters and data obtained by direct or indirect methods) (Duerenberg & Schutz, 1995). Nearly all of the techniques

used for estimating body composition are indirect measurements. That is, they measure some physical property of the body which is related to body composition, and then make use of the assumed constancy of the relationship to calculate composition (Nord & Payne, 1995). In Neutron Activation Analysis (NAA), for example, the body is infiltrated with fast neutrons of a known energy level. The neutrons are captured in the body by specific chemical elements, depending on their energy, resulting in the formation of specific isotopes with initially higher energy levels. The energy is then emitted in the form of gamma rays with a well-defined energy level dependent on the isotope formed. It is from these energy emissions that the amounts of nitrogen, calcium, chlorine, sodium, phosphorus, and oxygen can be calculated, and then, via stoichiometric relationships, it is then possible to determine the amount of body proteins, bone mass, extracellular water, and fat levels (Duerenberg, & Schutz, 1995).

The focus of body composition analysis in the aquaculture industry is often on the lipid and protein fractions as they relate to and affect fish growth and reproduction, as well as the storage, appearance, and organoleptic properties of the end product. This is especially the case for tuna where the fatty part of a carcass is the most valuable cut and its levels can have a significant effect on its colour, texture, and taste (Rye, 1991; Sigurgisladottir *et al.*, 1997). Water, as a key component in food systems including fish flesh, is also a fraction of interest as it influences most process variables, product characteristics, and stability attributes. Moisture levels influence all diffusion-controlled reactions (e.g.: enzymatic activity, crystallisation processes, and browning) and usually

the most important factor determining the thermodynamic properties of flesh, and therefore, its quantification, along with fat and protein levels, allow for a better understanding of flesh qualities (Jepsen *et al.*, 1999).

With the freshness of fish being a major quality, health and safety concern, a number of instrumental techniques to gauge fish freshness have been developed. The techniques can be physical or chemical and can be linked to the sensory qualities or microbiology of the flesh under certain storage conditions. The most common methods measure the accumulation and/or degradation of volatile compounds, oxidised lipids, or adenosine tri-phosphate, or changes in the texture, microstructure, or electrical properties of the flesh (Gill, 1995). Instrumental measures of freshness are often used in the resolving of issues regarding the marginal qualities of fish. However, unlike sensory techniques, they can be time consuming, lack sensitivity, and unable to determine notions of 'good' and 'bad' quality (Gill, 1995).

1.3.2 Sensory Techniques

The assessment of fish 'quality' was for centuries exclusively a sensory appraisal of the aesthetic appearance and freshness of fish (Nielsen, 1995). Although still a satisfactory method for fishmongers purchasing from artisanal fishers whom after fishing for a few hours return to sell their catch while the fish is still alive or very fresh (Huss, 1995), it is not satisfactory for the now global fish market where fishing grounds and consumers are often thousands of miles apart. In order for regulators, wholesalers and retailers to be sure that fish distribution channels are supplying consumers with safe and healthy fish and

fish products it is necessary that sensory methods be performed scientifically under carefully controlled conditions so that the effects of the test environment, personal bias, etc., can be reduced (Nielsen, 1995).

More objective approaches to the sensory assessment of fish freshness included an indexing system developed by the Torry Research Station in the 1950s, and the EU scheme, introduced in 1976, which grades fish into one of three quality levels - E (Extra), A, and B where E is the highest quality and anything below B is the level where fish should be discarded for human consumption (Nielsen, 1995).

The Quality Index Method (QIM), originally developed by the CSIRO Tasmanian Food Research unit is now widely used throughout the EU (Hyldig & Green-Petersen, 2004). Based on characteristic, well-defined changes in several significant sensory parameters, QIM is a practical rating system of the freshness of raw fish species. Inspecting fish for changes in their outer appearance, a score from 0 to 3 demerit index points is assigned to each characteristic (eyes, skin, gills, odor etc.). A score of zero is given for very fresh fish while increasingly larger scores result as a characteristic deteriorates (Bremner *et al.*, 1987; Nielsen, 1995). The scores for all characteristics are then summed to give an overall sensory score of an individual fish. Further, as the QIM scale has been devised to produce a linear correlation between the demerit score and storage life on ice, it is possible to predict remaining storage life on ice.

With limited control over the compositional qualities of fish caught in the nets or on the hooks of fishers, instrumental and sensory techniques used to assess fish quality have focussed on measuring the so-called freshness of fish in order to identify ways to best preserve quality through post-harvest approaches. Aquaculturalists though, have greater control over the compositional qualities of the fish they harvest and therefore their concerns have expanded from not only measuring and checking the marginal qualities of fish, such as its fitness for consumption, to measuring the hedonic qualities of the product – those attributes and characteristics that make a fish look and taste ‘good’. Sensory evaluation, therefore, is now of great interest to the aquaculture industry as a tool to scientifically measure the effects of on-farm or in-chain practices on the characteristics and attributes directly related to the 'eating experience' of their customers when consuming the end product. However, as sensory evaluation techniques are mostly used in the assessment of processed foods, it is necessary to review the processes and considerations of sensory evaluation and assess the unique challenges of applying a particular technique to an unprocessed fish product such as farmed Southern bluefin tuna.

1.3.3 Process of Sensory Evaluation

The concept of sensory evaluation began to appear in the literature post World War II, and although a relatively new discipline, along with microbiological safety and nutrition, the sensory properties of foods are one of the primary determinants of our food preferences (Frijters, 1984; White, 1996). The modern discipline of sensory science draws upon the theories and practices of food science, physiology, psychology, and statistics to arrive at analysable

responses from stimuli perceived by the five senses (Piggott et al., 1998). According to Frijters (1984), there exist three main elements (the stimulus object, the sensory perception, and the sensory response), and two relationships (psychophysical and psychometrical) that are of keen interest to the sensory scientist. Psychophysics deals with the relationships between the physical properties of the stimulus object and the sensory characteristics of perception. Psychometrics confronts the relationships between the sensory stimuli perceived and the responses of subjects (Frijters, 1984).

The methods that examine the psychometric and the psychophysical relationships between foods and humans can be considered either affective (subjective), and involve the examination of consumer preferences and/or their acceptance of products, or analytical (objective) using trained panellists and which centre on the measurement of the qualities of foods and their differences or similarities (Larmond, 1987). Complicating the study of these relationships is the fact that sensory perceptions are private events and therefore, as they are not directly observable, sensory measurements are classified as derived measures (Frijters, 1984). Consumer trials using untrained subjects can reveal a particular demographics' acceptance, preference, or bias toward a particular product or product's characteristic, but they cannot quantitatively describe or discern the sensory characteristics of, or between, two products with acceptable degrees of accuracy or precision. To achieve the latter we use sensory evaluation techniques with panellists whose sensitivities have been tested and who have been trained to differentiate, describe and evaluate the characteristics of a particular subject matter. Although sometimes criticised for

its lack of reliability due to subject error, sensory evaluation of the properties of foods is considered to be the most direct and the most sensitive measurement technique when compared to quantitative methods (Frijters, 1984).

The first step in the development of a sensory evaluation strategy of a food or material is the identification of the test objective (Stone & Sidel, 1987). Once identified the selection of an appropriate testing method will depend upon the product and its requirements, the logistical and cost constraints of the chosen test, and the qualifications and availability of test subjects. Following test selection it is necessary to consider the measurement technique, the type of response scale, the experimental design, and how the response data is to be analysed (Fig. 1.2).

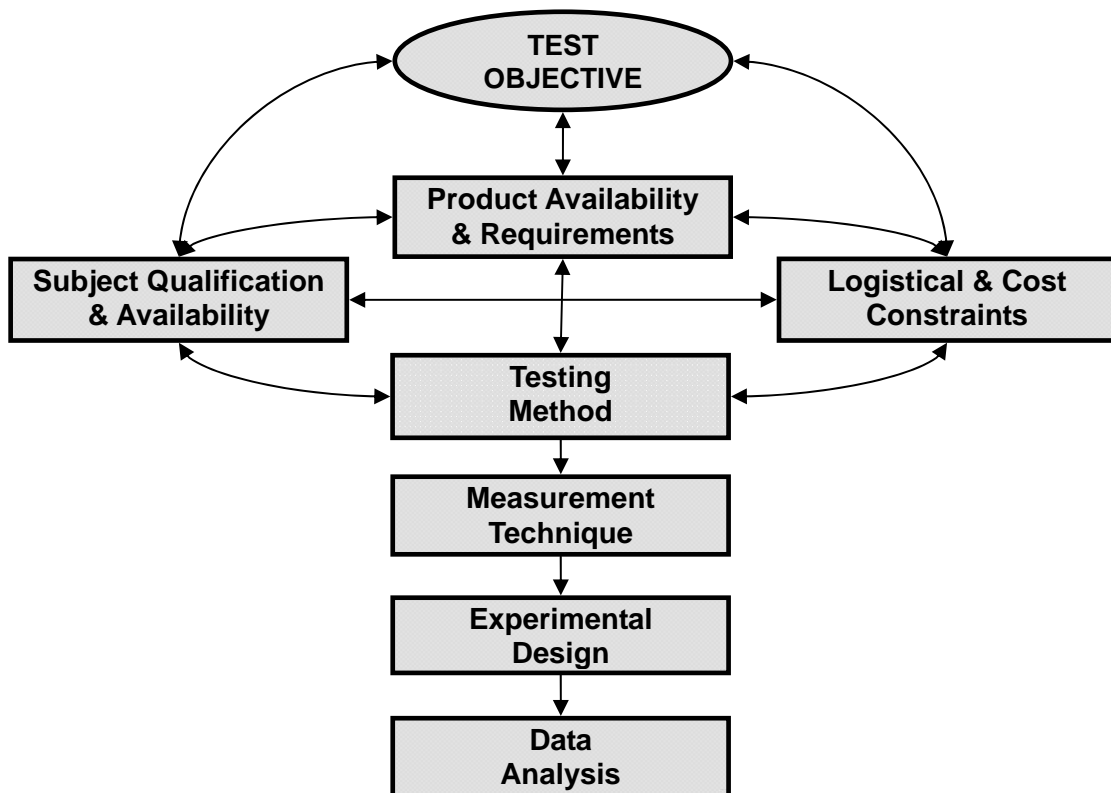


Figure 1.2: Connectivity and order of the considerations in the formulation of a sensory testing strategy.

Identifying the Test Objective

Central to the success of any sensory test is a clear understanding and statement of the test objective (Larmond, 1987). The stated test objective, the testing method, the experimental design and analysis, and measurement technique all need to tackle the identified problem and relate well to one another in order to yield reliable and valid product information (Stone & Sidel, 1993).

Testing Methods

Test objectives in sensory science fall into one of two major forms of sensory testing method – difference or descriptive tests (Piggott, 1998). Difference techniques aim to identify whether or not products are perceivably different, and descriptive methods attempt to identify and measure the sensory composition of foods, or determine the presence and/or intensity of particular attributes of a food (Piggott, 1998).

Difference tests are procedures used in many disciplines including biology, psychology, economics and market research to measure comparative judgements and choices (Frijters, 1984). In sensory science they are used to discriminate between two different types of stimuli or products. The simplest difference test is called a *duo test* where two products are presented to one or more subjects with the instruction to select the stronger of the two with regards to a pre-specified attribute. A *duo-trio test* removes the need of attribute specification prior to the test, as a sample of one of the two products to be examined is first used as a reference. The subject is then asked to select which of the two products differs most from the reference (Frijters, 1984).

Another type of difference test, known as a *triangle test*, presents subjects with multiple sets of three coded samples with half the sets containing two samples of product A and one sample of product B, and the other half containing two samples of product B and one of product A. The subjects are then informed that two of the samples are the same and one is different, and for the test, instructed to identify the odd sample of the three (Frijters, 1984; Larmond, 1987). In the instance where the assessor cannot detect a difference but is forced to choose the odd sample out, *triangle tests* are more efficient than *duo-trio tests* in that the probability of selecting the correct sample by chance is 33% rather than 50%. However, with less tasting required, *duo-trio tests* can be preferred to triangle tests when strongly favoured products are being investigated (Larmond, 1987). Variations of the same theme include the *tetrad test* and the *hexagonal test* where multiple reference and treatment samples are provided.

Ranking tests are difference tests which present subjects with three or more samples and subjects are instructed to order the samples according to the levels of intensity of a particular characteristic. Although rapid, the test provides no indication of the size of the difference between samples and as samples are evaluated in relation to each other results from one set of ranks are not comparable to the results from a differing set of ranks (Larmond, 1987). According to Stone & Sidel, (1993) it is probably this latter limitation that has resulted in the infrequent use of ranking in sensory evaluation.

Difference tests provide no indication of the dimension of difference and, as the sensory nature of 'oddity' is often not specified, the subject is left to him/herself to decide upon the sensory attributes which are relevant for discrimination (Frijters, 1984; Larmond, 1987). Mostly used in quality control or ingredient-substitution investigations, where the products are relatively homogenous, all sensory difference tests are forced-choice procedures in which the subject is required to select a sample that is different even though the subject may not be able to detect any discernible differences (Frijters, 1984; Larmond, 1987). When not discernible, the choices, considered random guesses, are either correct or incorrect and the probability of a correct response is easily determined with the use of binomial or chi-square testing where it is possible to calculate whether the differences in the responses were due to chance (sampling variability) (Frijters, 1984). As sensory difference tests are based on a statistical comparison of the distribution of correct and incorrect responses and the expected theoretical distribution of random responses, they are not founded on the principles of sensory perception according to Frijters (1984), but on the combined principles of guessing behaviour and probability theory (Frijters, 1984).

Descriptive sensory analyses utilize trained and experienced subjects that examine and evaluate food products to provide detailed descriptions of appearance, flavour, and texture. Of available descriptive methods flavour profile analysis (FPA), texture profile analysis (TPA) and quantitative descriptive analysis (QDA) are the most widely known and used (Larmond, 1987). These descriptive scaling methods attempt to describe the perceptible

factors, the intensity of each, their order of perception, aftertaste, the overall impression, and qualitatively and quantitatively describe the mechanical and geometric characteristics of foods (Larmond, 1987). QDA combines descriptive analysis, unstructured scales, and repeated measures to characterise the sensory attributes of products in order of appearance, the intensities of each attribute, and then statistical techniques to determine whether or not significant differences exist between the intensities of sensory characteristics (Larmond, 1987). Although highly valuable tools these descriptive sensory techniques require highly trained and motivated subjects (Larmond, 1987).

Magnitude estimation is an experimental technique that attempts to quantitatively scale how much of a particular sensation subjects are experiencing. Subjects are presented with a series of samples that vary in a particular characteristic and are instructed to assign a number to the first sample (or a number is assigned by the experimenter), and then rate each following sample in relation to the first. If, for example, a subject rated the sweetness of a liquid first in a series as being '10' then any of the following samples considered 'half as sweet' by that subject would score a 5, and a sample considered 'twice as sweet' would score a 20 (Snodgrass *et al.*, 1985; Larmond, 1987).

In summary, *triangle*, *duo*, and *duo-trio tests* indicate a difference only between two samples, *ranking tests* indicate if the samples differ in a particular characteristic and the direction of the difference, descriptive scaling methods provide information on the size and direction, and magnitude estimation

provides information on the proportions of differences (Table 1.1; Larmond, 1987).

Test Name	Type of Test	Information Obtained			
		Difference	Direction	Size	Proportion
<i>Duo, Duo-Trio, Triangle</i>	Difference	✓	×	×	×
<i>Ranking</i>	Difference	✓	✓	×	×
<i>FPA, TPA, QDA</i>	Descriptive Scaling	✓	✓	✓	×
<i>Magnitude Estimation</i>	Descriptive Ratio Scale	✓	✓	✓	✓

Table 1.1: Type of sensory testing method and the information obtained.

When selecting a sensory testing method it is necessary to consider the product's requirements and availability, subject qualifications and availability, and the logistical and cost constraints associated with the test objective.

Product Availability & Requirements

Although the amount of sample presented to subjects is often limited by the quantity of raw material available, the amount should be as constant as possible, adequate for the task, and allow for re-tasting if deemed necessary by the subject (Larmond, 1987). The Sensory Evaluation Committee of the American Society of Testing and Materials (ASTM, 1968) recommends that each panellist receive at minimum 1 oz (28gm) of a solid for use in a discrimination test, and double that amount for preference testing. Further, in order to obtain meaningful results, the samples that each subject receives must be representative of the product and be physically uniform for each treatment (Larmond, 1987).

The homogeneity and the stability of the samples or product being tested also require consideration. Unlike the visual and auditory modes, where exactly comparable stimuli can be produced, it is impossible to produce two physically identical food products such as apples or fish for all subjects to evaluate, and therefore some of the random variability attributable to subjects may be associated to stimulus variability (Land & Shepherd, 1984). The flavour, texture, and appearance of red meat, for example, with varying degrees of fat marbling and connective tissue, as well as the changes in colour that occur with the oxidation of the pigment myoglobin from deoxymyoglobin (purple) through to oxy-myoglobin (cherry red) and finally to metmyoglobin (brown), need accounting for when processing, preparing and presenting samples for evaluation.

Logistical & Cost Constraints

With all test objectives in sensory science there are logistical and cost constraints particular to each experimental method that have to be determined and met prior to execution. These may include:

1. Facilities and tools: the testing facility, its size and appropriateness; the need and availability of storage (constant high/low temperature-humidity); preparation tools and processing equipment.
2. Transportation: the transportation of products/samples from the place of production to the place of testing may be needed.

3. Human resources: staff to conduct the experiment and subjects to participate in the experiment; the need for staff and/or subject training; consultants, statisticians, butchers, chefs, survey staff, and translators may also be required.
4. The product: its size, and number. Also a product may require harvesting, capture, slaughter (possible ethical and religious considerations), manufacture, processing, baking etc.
5. Analysis: the time and cost of any physico-chemical testing; statistical analysis.

The available budget will also restrict the test objective, the testing method, the number of subjects and their level of training, the quantity of sample, and all experimental design factors such as how data is to be collected (on paper or electronically) the staff available (cutters, presenters), whether the analysis can be outsourced, and how many times the experiment can be replicated.

Subject Qualifications & Availability

People who perform sensory evaluations (also referred to as assessors or judges) can be categorised as subjects, selected subjects, panellists, or experts. Subjects are any unqualified persons involved in a test; selected subjects have been tested, trained and chosen for their proven ability; a panellist is a member of a select group with no particular expertise or abilities; and an expert is someone with considerable experience and proven ability in the assessment of a given product (Land & Shepherd, 1984). The availability of subjects, either un-qualified or qualified, will determine the type of sensory

testing that can be conducted.

Threshold concentrations that elicit a taste sensation can vary by up to two orders of magnitude between subjects, and thus inter-individual variation in subject responses can result from the presence or absence of specific receptors (genetic makeup); the sensitivity to, and discrimination between, stimuli (sensory experience); redundancy, duplication and nuances associated with the terms used to describe the sensory experience (semantics); and the cognitive transformation of sensory inputs into a quasi-numeric form (reporting) (Frijters, 1984; Plattig, 1984; Brown et al., 1996). Intra-individually, responses can vary according to the time of day, hunger and satiety, hormonal influences, and age (Plattig, 1984). Limits to the magnitude of a physical difference between two stimuli that can be perceived can result in a Type II error where two physically different stimuli from two different products can elicit two identical responses, or, alternatively a Type I error where subjects respond differently to the same stimulus (Frijters, 1984).

In sensory evaluation, subjects and panels are the analytical instrument, and, as with all instruments, they require calibration to ensure they are as objective, accurate, and precise as possible. Where feasible subjects should be screened, trained, and the reproducibility of their evaluations examined (Larmond, 1987). Pre-screening, using the Munsell Colour Vision or the Ishihara tests for sensitivity and blindness for example, or threshold testing, enables those panellists with colour vision defects, or insensitivity to a characteristic or stimulus of interest to be identified and disqualified from a

particular test where they would bias, skew or corrupt results. Training panellists assists to develop familiarity with the product and its characteristics, to develop a common language to describe these characteristics, to identify differences, and improve and standardise the consistency of results. Untrained subjects often struggle to disregard personal preferences and, despite understanding the terms used, do not use them in a consistent manner leading to scattered responses and statistical non-significance (Larmond, 1987). Apart from the pre-screening and training of subjects, efforts to minimize this variation and standardize the judgements of subjects include repetition, and statistical modelling (Brown, et al., 1996).

Measurement Techniques

Scales are the tools subjects use to express their perceptions, and knowing the properties and limitations of the measuring instrument is of vital importance (Land & Shepherd, 1984). The sensory scientist needs to consider the test objective, the product, and the subjects when deciding upon a response scale as inappropriate scales that are not optimised for subject use in a particular task can result in poor response data and lower motivation levels (Land & Shepherd, 1984; Stone & Sidel, 1993).

Scales used for the rating or scoring of samples are a continuum divided into equally spaced, successive values that can be graphic, descriptive or numerical. They can be uni-polar with a zero at one end or bipolar with antonyms at either end (Land & Shepherd, 1984). Too broad a scale loses discrimination but too fine a scale can introduce error.

According to Stone & Sidel (1993), in order to derive the most value from a response scale it should be:

- a. *Meaningful to Subjects*: the words used must be familiar, easily understood, readily related to the product and the task, and unambiguous to the subjects. Jargon and technical terms familiar to a researcher may be meaningless to an untrained panellist.
- b. *Uncomplicated to Use*: the task and scale must be easy to use. If complicated, measurement error can increase and product differences not detected.
- c. *Unbiased*: it is important that the scale be balanced and that all number and word categories are equally represented so as to not influence the test outcome.
- d. *Relevant*: chosen scales should only measure the intended attribute, characteristic or attitude, and not combine an element of quality and preference for example.
- e. *Sensitive to Differences*: the length of the scale and the number of categories can influence the sensitivity for measuring differences.

f. *Provide for a Variety of Statistical Analyses:* to determine whether effects are a result of chance or an applied treatment the response scales need to be amenable to statistical analyses. Less flexible and less sensitive scales limit inferential power.

Stevens (1951) proposed four categories in which all response scales fall. Nominal scales for naming and classification, ordinal scales for ranking, interval scales measuring magnitudes that are equidistant between categories, and ratio scales.

Nominal scales detail only class association or recognition with no quantitative relationships existent between classes. The main feature of nominal scales is the total independence of the order between categories, and although considered a low-order scale, the assignment of ranks or percentages based on frequencies to nominal categories allows the use of statistical methods available to ordinal data. Valid analytical techniques include frequencies of occurrence, modes, chi-square, and contingency correlation (Land & Shepherd, 1984; Stone & Sidel, 1993).

Ordinal scales denote the increasing or decreasing nature of an attribute or class without providing any magnitude or distance between non-interchangeable categories (Land & Shepherd, 1984). When used in multi-product tests, all products must be examined before judgements are made and therefore sensory fatigue, which is confounded by the interactions between products which have lingering flavours or odour, can be minimised. If

subjects are not trained or qualified to perform evaluations of a certain product's attributes, such as the ranking of flavour intensity, there can be no assurance that the subjects actually perceived and were able to rank flavour (Stone & Sidel, 1993). Valid analytical techniques of ordinals ranked data include all those available to the analysis of nominal data as well as the non-parametric Wilcoxon signed rank test, Mann-Whitney, Kruskal-Wallis, Friedman Two-Way ANOVA, and Kendall's coefficient of concordance (Land & Shepherd, 1984; Stone & Sidel, 1993).

An interval scale is defined by equi-distances between categories on a scale which has an arbitrary zero point, and, therefore, is unable to measure the absolute magnitude of an attribute (Stone & Sidel, 1993). Mathematical comparison is possible using the arithmetic mean, standard deviation, and a variety of non-parametric and parametric analyses such as Pearson correlation factor analysis, or discriminant analysis (Land & Shepherd, 1984; McDowell, 2006).

Ratio scales differ from interval scales in that a constant ratio exists between points on a scale and zeros are absolute therefore possessing geometric properties rather than just the arithmetic properties (Land & Shepherd, 1984). Similar to natural ratio scales, there are no arbitrarily limited endpoints, and, as the choice of numbers will vary amongst subjects, the scores are transformed so that the geometric mean of all subject data equals 1.00 and the logarithms are analysed by analysis of variance (Larmond, 1987). In moving from nominal to ratio scales the demands on subjects increase both in their comprehension

of instructions and their ability to respond.

In order to minimize potential variations in the interpretation of scale values by subjects, sensory scientists sometimes develop and use standards which are then used to train subjects (Land & Shepherd, 1984). As described by Cardello and Maller (1987) the question of the validity of the scales of sensation is a 'thorny' one, where no one scale can be considered better than another when differing methods of judgement are used.

Experimental Design

Experimental design is an organized approach to the collection of data and requires population definition, randomization, administration of treatments, consideration of the sample size requirement, and sound statistical analysis (Gacula, 1988). The chosen design will affect the accuracy of results and the feedback into the test objective. A well designed experiment reduces costs, simplifies the interpretations of the results, and yields useful and meaningful outcomes (Larmond, 1987; Gacula, 1988).

The number of subjects used in a sensory test will influence the statistical significance of the results with too few a number used requiring subject responses to be considerably different between treatments in order to produce a significant result (Land & Shepherd, 1984; Larmond, 1987). Although there is no set number, consumer preference/acceptance testing requires the number of subjects often to be in the hundreds in order to cover demographic, regional, and cultural differences. When using trained or expert panellists Larmond

(1987) states a panel numbering 10 subjects is common and that a 5 subject panel is the recommended minimum number.

According to Gacula, (1988) when accounting for Type I and Type II errors and desired test sensitivity, it is possible to determine appropriate sample sizes using the following formula:

$$n = [(Z_{\alpha} + Z_{\beta})^2 \sigma^2] / (\mu_1 - \mu_2)$$

where Z is the area under the curve of the standard normal distribution; σ^2 is the variance and $\mu_1 - \mu_2$ is the desired difference to be detected. Gacula (1988) noted that in order to detect a difference of 0.5 on a nine point scale (assuming $\sigma = 1.0$) with $\alpha = 0.05$ and a power of 0.90, 52 panellists would be required per treatment. By changing the detectable difference value from 0.5 to 0.4 and 0.6 increases and decreases the required number of panellists to 81 and 36 respectively.

It is considered 'best practice' to run two sessions in one day with each subject participating in both sessions to obtain replication in the judgements, provide a measure of consistency of the panellists, and to minimise exposure to systematic errors (Larmond, 1987).

One important aspect of sensory testing is the number of samples that are presented to subjects for evaluation. Physiological fatigue of the sensory organs and/or tiredness, boredom and confusion of the subjects can result

from presenting too many samples for evaluation (Land & Shepherd, 1984). Determining the appropriate number of samples relates to the type of stimulus being evaluated and the complexity of the task. For example, the visual evaluation of samples is less taxing on subjects when compared to the evaluation of a sample's flavour or texture. The swallowing of samples rather than being able to spit them out and the need to taste unpleasant samples can affect both the required evaluation time and subject enthusiasm (Land & Shepherd, 1984). The expectations of subjects regarding the number of evaluations, the number of samples, the reason for doing the test, the importance of the test and their relationship to the experimenter can also affect subject performance (Land & Shepherd, 1984).

Environment, Sample Allocation, & Presentation

To assist the subject to concentrate at the task at hand and provide the optimum setting for unbiased judgement, subjects should be alone in an environment with negligible distractions and interruptions (Land & Shepherd, 1984; Larmond, 1987). The testing area should be quiet, comfortable, free from foreign odour (maintaining positive pressure in the testing room), and preferably be maintained at a constant temperature and humidity. Individual booth's with neutral colours and uniform lighting that does not distort the colour of samples is considered the optimum environment (Larmond, 1987).

Samples need to be prepared in an area with sufficient counter space to allow for their efficient allocation and serving. Preliminary testing is usually necessary to determine efficient methods of preparation and allocation

(Larmond, 1987). Samples should also be served at the temperature in which they are normally consumed and held constantly at that temperature prior to and during testing. If held for a long period of time precautions should be taken to prevent samples from drying out and any quality changes from occurring (Larmond, 1987).

All samples should be individually coded as subjects may learn, or believe they know the identity of a particular sample and start to rate it consistently according to its label and not its attributes (Land & Shepherd, 1984). The code assigned to samples should not introduce any bias or provide any indication of the identity of the treatments, and, according to Larmond (1987) three digit random codes from random number tables are the most appropriate and are widely used.

Serving containers that are identical and that do not impart any taste or odour to the product should be chosen for sample presentation. Colourless or white containers are best in order to not mask any colour differences between samples (Larmond, 1987).

Psychological Error and Allocation (Randomization)

As the subjects of a sensory test are human they are prone to a number of well documented psychological errors when conducting sensory evaluations. Even a well trained panel can respond poorly if the experiment is designed poorly and psychological errors are not taken into account. These errors include: time-order errors; errors of central tendency; errors of expectation, habituation

and anticipation; stimulus errors: logical and leniency errors; halo effects; proximity errors; contrast and convergence errors; and range-frequency effects.

The first-sample effect is a time order error where the first sample or product presented in a multi-sample or multi-product test is often evaluated higher on measurement scales than when the same sample or product is evaluated later in the series of presentation (Stone & Sidel, 1993). To control for this phenomenon each sample of a multi-sample test should come first an equal number of times in the sampling series.

Errors of central tendency occur when subjects avoid both poles of a bi-polar, and the upper end of uni-polar response scales, and tend to score around the central position. This can result in products or samples being less differentiated than otherwise would occur with trained subjects or subjects familiar with the range of stimuli being evaluated (Land & Shepherd, 1984; Stone & Sidel, 1993).

Errors of expectation, habituation and anticipation occur when prior knowledge or experience generate expectations within a subject for specific attributes or differences between samples and products. Habituation occurs when multiple stimuli nullify the subject's perception of an actual change in sample or product. Anticipation errors occur if there is a perceived change when in fact no actual change in sample has been presented to the subject (Stone & Sidel, 1993).

Stimulus errors occur when subjects respond in an atypical manner owing to the fact that they have or believe that they have some prior knowledge on the products or samples being used in the test, and, according to Stone & Sidel (1993), reinforces the notion that participants involved in the setting up of the test should not then become subjects.

Logical errors arise when subjects follow their own logic in determining the requirements of the task at hand and results from unacquaintance with the protocols of the employed technique. Leniency errors are the result of the subject allowing their feelings toward the experimenter influence their scoring of attributes or when subjects try to comply with what they feel the experimenter desires (Land & Shepherd, 1984; Stone & Sidel, 1993). 'Double blind procedures' attempt to minimise these effects by using an experimenter unfamiliar with the trial objective, the classification of subjects, or the treatment conditions (Land & Shepherd, 1984).

The halo effect results when a response to one particular question by a subject influences successive responses by that subject and are common with untrained panellists or consumers who are attempting to justify a preference rating or are suffering from physiological fatigue due to numerous re-tastings (Stone & Sidel, 1993).

As the name suggests proximity errors are said to occur when attributes measured in close proximity to one another are scored more similarly than those attributes that are measured farther apart during evaluation. Possible

solutions include randomizing the order in which the attributes are assessed by subjects.

Contrast errors can occur, for example, when a product of high intensity in a particular attribute is immediately followed by a product of low intensity. This can result in the scored difference being far greater than the actual difference, with the low intensity product score being more exaggerated than if the preceding product was closer in intensity. Convergence is the opposite effect where like products are presented in close proximity (Stone & Sidel, 1993).

Both the range and frequency of presented stimuli can influence the evaluations made by subjects. Similar to the error of central tendency subjects adjust the centre of the rating scale in the direction of the centre of the stimulus range (Land & Shepherd, 1984).

Proper experimental design is used to decrease the risk of introducing bias, and randomization is one of the fundamental principles of good experimentation (Piggott *et al.* 1998). To account for psychological errors the order of presentation of samples to subjects should be randomized or balanced with the ideal situation resulting when every possible order occurs an equal number of times (Larmond, 1987). It is also necessary to randomly allocate experimental materials to treatments so that each has an equal chance of being assigned to a particular treatment in order to guarantee that a statistical test will have a valid significance level (Gacula, 1988).

Data Analysis & Statistical Considerations

The physical and sensory complexity of some foods can result in as many as forty sensory attributes being measured, as well as numerous instrumental measures (Cardello & Maller, 1987). The power of multivariate statistics to deal with such large numbers of variables is often employed to expose the underlying physical and perceptual dimensions (Cardello & Maller, 1987).

Non-parametric tests, according to Stone & Sidel (1993) provide the sensory professional with additional tools for data analysis when there are reasons to justify their use, otherwise, in agreement with O'Mahony (1986), these authors note that parametric methods are preferable owing to the fact they use scale data obtained from the subjects.

As with all data sets the reliability, validity, and the amount of replication used to gather data underpins analytical results. In sensory science, reliability refers to the ability of subjects to respond repeatedly in the same manner from the same stimulus. Low subject numbers make it important that the experimenter has confidence in the ability of those subjects to reliably respond. Validity refers to the accuracy of those responses. It is of no value to the sensory professional if subjects are repeatedly responding to stimuli in an invalid manner. Although difficult to assess, two types of validity are commonly referred to in sensory science – face and external validity. Face validity is said to prevail where responses are in line with expectations, and external validity occurs when a different and/or larger set of subjects respond in accordance with the original set of subjects (Stone & Sidel, 1993).

Independent re-evaluation in identical experimental conditions that allows for the consistency of individual subjects and panels to be determined is the goal of replication (Piggott *et al.* 1998). Practical considerations such as product availability, preparation requirements, product stability, subject availability (both in numbers and frequency), and the information required all affect the ability to replicate (Stone & Sidel, 1993). Replication in foods such as meat is not a simple task as the individual animals themselves, the place and method of slaughter, and the storage time and temperature variables introduce variability (Piggott *et al.* 1998). Products, according to Stone & Sidel (1993), are often more a source of variability than subjects, and can be out of the control of the sensory professional.

Concordance Analysis, an application of Principal Components Analysis, is a powerful tool for analysing the performance of sensory panellists, and can reveal whether panellists agree or not, panellists that cannot reproduce their evaluations, and distinguish panellists who have problems with a particular attribute.

Sensory-instrumental analysis compares and contrasts a data set containing a collection of sensory assessments on a number of products with a data set containing a number of instrumental measures on the same products (Dijksterhuis, 1997). According to Dijksterhuis (1997), multivariate methods to study the relationships between sensory and instrumental data sets can differ in three respects – symmetry, measurement level, and criterion.

The symmetry refers to the way in which the data is treated by the analytical method. Asymmetric methods, that include Partial Least Squares Regression, Principal Components Regression, Redundancy Analysis and Multiple Regression, attempt to predict one data set from another and treat both data sets differently (Dijksterhuis, 1997). Symmetrical methods, including Canonical Correlation Analysis and Procrustes Analysis, investigate only the relationships between the data sets with neither set used as the object of prediction (Dijksterhuis, 1997).

Non-linearities at the measurement level in sensory-instrumental analysis presents the experimenter with the difficult task of finding the right balance between imposing linear restrictions with the risk of missing interesting relations, and imposing hardly any restrictions with the risk of fitting noise (Dijksterhuis, 1997). The criteria in which the relationship between the sensory and instrumental data sets are defined differ according to the multivariate procedure, and can be based upon maximal covariances, maximal correlation or minimal variances (Dijksterhuis, 1997).

Principal Components Analysis (PCA) is an often used statistical technique in sensory science and functions to reduce a set of individual items (variables and data) into components. The first component has maximum correlation with all variables in the data set and accounts for the greatest amount of variance, the second component accounts for the second-largest amount of variance etc. This trend continues until all variation as practicable has been accounted for

(Powers, 1984). Finally, asymmetric Partial Least Squares Regression analysis (PSLR) is often used to examine the relationships between data sets, such as those between instrumental (x) and sensory (y), by predicting one from the other, as well as attempting to find the 'best' solution of X that will explain variations of the Y variable set (Chung et al., 2003).

An understanding of the principles of sensory evaluation and the techniques available for the analysis of food makes it possible to consider and develop a sensory testing protocol for a raw fish product such as Southern bluefin tuna. Once such a tool is at hand it would be possible to measure the affects of variations in, or manipulations of, any on-farm or in-chain practices on the sensory profile of the end product. Furthermore, if linked to consumer preference or acceptance analyses, the results could help to identify practices that maximise the product characteristics associated with a 'good quality eating event' – the reason d'être of all farm managers.

1.4 Aims & Objectives

The first objective of the Japan-based research effort was to identify, investigate, and compare the instrumental techniques used by researchers at the Tokyo University of Fisheries to measure the qualities of fish flesh with those used by the tuna flesh quality research team in Australia. Secondly, in collaboration with Japanese researchers and industry representatives, to develop new, and appropriate, instrumental and sensory techniques for the measurement of the qualities of Southern bluefin tuna – reviewed in the methods section.

The flesh qualities of the product in Japan, the effects of on-farm and in-chain manipulations on selected quality characteristics of the end product could all be examined using sensory and instrumental techniques. Chapter three examines the effects of using vitamin supplements as per the industry practice on the flesh concentrations of vitamins and the colour shelf-life of the end product. Chapter four examines the effects of an industry harvesting practice on the sensory and biochemical characteristics of quality. Finally, chapter five investigates the time-temperature management of air-freighted SBT to Japan, as well as the subjective and objective quality associated outcomes of two industry shipping procedures.

2 . SAMPLING AND MEASURING FLESH QUALITY AND QUALITIES OF FARMED SOUTHERN BLUEFIN TUNA

2.1 Flesh Sampling Techniques

Core Sampling: This was undertaken in order to obtain flesh samples from the carcasses of farmed Southern bluefin tuna for physico-biochemical analyses and colour panel assessment. Coring tools of various dimensions were developed in 1999, and a medium-sized coring tool which removes a cylindrical flesh sample of approximately 15-20g in weight, 1.2cm in diameter, and 8cm in length was selected for use in the following experiments. The samples were removed immediately after the fish was killed from the bleed cut incision which is in the region 5cm posterior to the pectoral fins for each tuna. The core sample provided a cross-sectional sample of the carcass that included portions of the heavily vascularised red meat and the low-level (Akami) and medium-level (Chutoro) lipid sections of skeletal muscle from a tuna carcass (Fig. 2.1). Core samples for biochemical analysis were immediately stored in liquid nitrogen. Samples used for colour shelf-life examination were sealed in small plastic bags and placed on ice for the journey (approximately 4 hours) from the sampling location to the constant temperature refrigerators at the Lincoln Marine Science Centre in Port Lincoln.

Tail Cut Sampling: The grading of the flesh quality of farmed fresh Southern bluefin tuna in Port Lincoln by Japanese technicians involved the removal of a small section of tissue from the redundant section of the tail. This cut is generally taken from the left side of the carcass and between the 3rd and 4th finlet of the tail (Fig. 2.2).

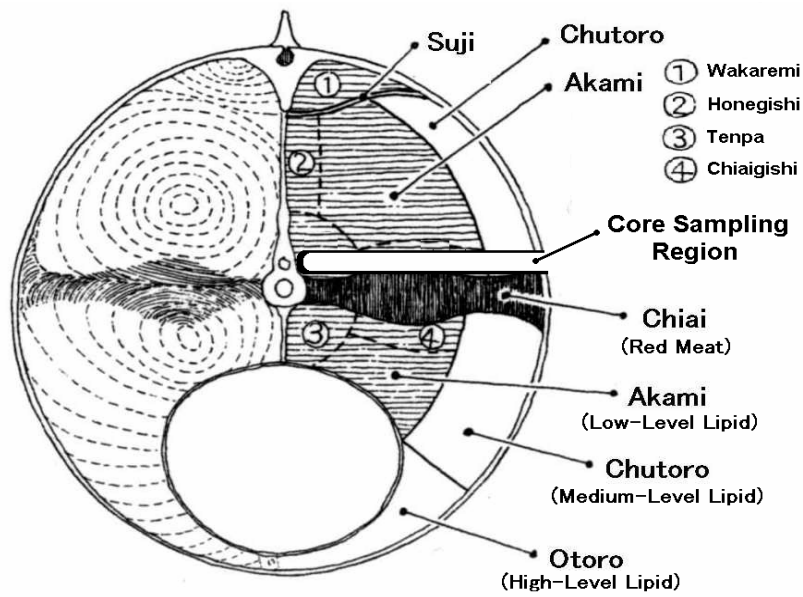


Figure 2.1: Cross-section of a tuna carcass showing the core sampling region used to gather flesh samples for both physicochemical analyses, and for use in colour shelf-life trials in Port Lincoln (Diagram translated from Ueda, 2003).



Figure 2.2: Removal of the tail section during processing in for flesh quality grading.



Figure 2.3: Tail-lopped tuna prior to auction and removing flesh from the redundant tail-section for flesh quality assessment.

In Japan, prior to auction, the whole tail is cut off from between the 4th and 5th finlet allowing the wholesalers to visually grade the whole tail region and remove flesh from the redundant tail sections (Fig. 2.3). When conducted at the Tokyo University of Fisheries panel assessment of colour shelf-life used sections of the tail (as removed by the Japanese technicians in Port Lincoln) but with the samples taken at the Tsukiji wholesale market.

2.2 Physico-Chemical Quality Measures

2.2.1 *pH* - The muscle pH level is widely used as an objective measure of flesh quality in the red meat industry (Warriss & Brown, 1987; Gariepy et al, 1994; Sante,1996). Accelerated levels of aerobic and anaerobic metabolism occurring immediately pre and post-mortem deplete muscle glycogen stores resulting in a build up of lactic acid and a drop in muscle pH. In the beef industry, meat with a low ultimate pH is often red in colour and is juicy, and therefore considered for the valuable 'table cut' market. In contrast, muscle which has low glycogen reserves at slaughter will produce little lactic acid and the fate of this high pH 'dark and dry' meat is the low-value manufactured meat market (mince, breakfast or luncheon sausage) (Chrystall & Daley, 1996). The pH of the tuna flesh cores was measured along the cores and averaged using a surface probe and a hand-held pH meter (*Eutech Instruments Model Cyber Scan pH 310*).

2.2.2 *Tri-Stimulus Colour*: Tri-stimulus instrumental colour measurement involves the quantification of the three primaries of reflected light: pure red, green, and blue. Imaginary primaries x, y, and z, are then used to plot any

colour as a point in a sphere (Hunt, 1977). Several such spheres, known as colour spaces, exist, and include the well known CIE $L^*a^*b^*$ and CIE LUV colour spaces. The CIE $L^*a^*b^*$ colour space has the advantage over CIE LUV in that it is closer to the visual perception of humans. According to Hunt (1977) differences between colours as judged by humans approximate the same differences as perceived by the $L^*a^*b^*$ system. Within the $L^*a^*b^*$ system the L^* value describes the 'lightness', the a^* value the 'redness', and the b^* value the 'yellowness' (Fig. 2.4).

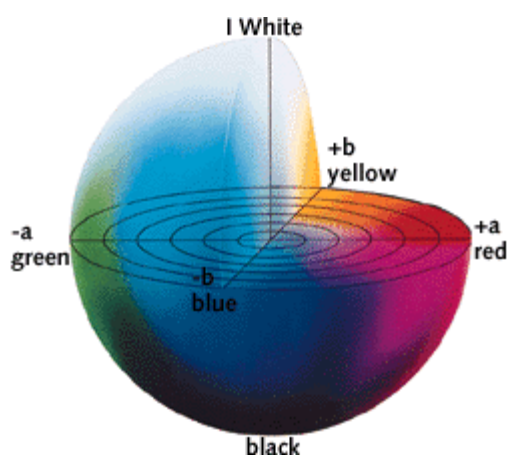


Figure 2.4: CIE $L^*a^*b^*$ (1976) Colour space. L^* = lightness; a^* = redness, and b^* = yellowness.

CIE $L^*a^*b^*$ colour space measurements have been used extensively in the examination of various food stuffs (Baardseth *et al.* 1988), the flesh colour and pigmentation of cultured salmon and trout (Little *et al.*, 1979; Blanc & Choubert, 1985; Anderson *et al.*, 1990), and the colour of flesh in the pork and red meat industries (Hegarty, 1969; Frankie & Solberg, 1971; Birth *et al.* 1978). In the present study colour CIE $L^*a^*b^*$ measurements were recorded directly from the flesh samples with a calibrated *Minolta CR-310* colorimeter (aperture 8mm). The samples, of equal thickness (~10mm), were placed on standard white tiles during colour measurements, which were the average of three readings. It

must be noted that CIE a^* and b^* values are polar coordinates and are used together to plot the colour (hue) and colour intensity (chroma) of the sample. The *Minolta Colorimeter* however is designed for hard surfaces and the translucency of tuna flesh results in the absorption and scattering of much the incidence light leading to a hue and chroma in grey colour space. Indeed, according to McLaren (2007), hue angles calculated using the CIELAB recommended method can result in errors of up to 35° for translucent samples. Therefore only the CIE L value and the polar coordinates are examined as indicators of changes or differences in the reflective and physical properties of the flesh rather than as indicators of 'colour'.

2.2.3 Hydroperoxides: The pentadiene structure, found in most of the polyunsaturated fatty acid acyl chains common in fish, is especially vulnerable to oxidation, and begins with the autocatalytic abstraction of a hydrogen atom from the central carbon of the alpha-methylene group in the chain. The remaining lipid radical (L^{\cdot}) reacts quickly with atmospheric oxygen to produce a peroxy-radical (LOO^{\cdot}) which, in-turn, can abstract a hydrogen molecule from a neighbouring acyl chain and result in formation of a lipid hydroperoxide ($LOOH^{\cdot}$) and new radical (L^{\cdot}), with the former often used as measure of the degradation of fish flesh quality (Jorgensen, 1995).

Total lipid hydroperoxides were analysed by flow-injection system with a detection system using diphenyl-1-pyrenylphosphine (DPPP). The mobile phase, with a flow rate of 0.5 ml/min, was passed through a Shimadzu HPLC pump (Model LC-9A, Kyoto, Japan) and transferred to a stainless steel reaction coil (0.25mm i.d. \times 40m). At the inlet of the reaction coil a DPPP

solution (3mg/200ml of 1-butanol and 100ml of methanol) was pumped (Shimadzu LC-10AS) and mixed with the eluent in a T-connector at a flow rate of 0.3ml/min. The fluorescence intensity of the DPP oxide was monitored at an excitation wavelength of 352nm and an emission wavelength of 380nm with a Shimadzu fluorescence detector (Model RF 535). The NBD-labelled PC used as an internal standard was detected at an excitation wavelength of 460nm and an emission wavelength of 534nm with a Shimadzu fluorescence detector (Model RF-10A) set in the flow line behind the first fluorescence detector. The intensities of the fluorescence signals were integrated with Shimadzu chromatographic integrators (Model Chromatopac C-R6A).

2.2.4 Myoglobin-Metmyoglobin: The relative amounts of the three derivatives of a globular haem protein known as myoglobin primarily determines the surface colour of most muscle foods including tuna. Reduced myoglobin (deoxymyoglobin) is purplish in colour; myoglobin, which forms oxymyoglobin in the presence of air, is responsible for the characteristic red colour; and metmyoglobin, which occurs when oxygen binds to the ferric derivative of myoglobin, is brown in colour (Hood & Riordan, 1973).

In the present study, the measurement of metmyoglobin was conducted according to Bito's method (1976). Two grams of sample were added to 10ml of cooled distilled water. The sample was then homogenized on a small white plate and left for 20 minutes. Following filtration using filter paper 5A (*Advantech Co.*) the pH of the filtered liquid was adjusted to 7.0 using 1N NaOH. The liquid was then spun in a chilled centrifuge at a rate of 8,500 RPM

per gram (of sample) for 5 minutes. Then, according to the method described by Kakuta & Uchiyama (1986), the upper fraction was filtered using a filter membrane of pore size $0.3\mu\text{m}$ (*Saltrius Co.*). Immediately following this step $100\mu\text{l}$ of the filtered fraction was analysed for haemoglobin and myoglobin content by HPLC using two PROTEIN-PAK 300 gel filter columns in series (*Waters Co.*), with a flushing speed of 1.0ml/min with a 0.01M phosphoric acid buffer containing 0.2M sodium sulphate ($\text{pH } 6.58$). The absorbance spectra of the flushed fluid was measured using a multi-channel detector MCPD-3600 (*Otsuka Denki Co.*) at wavelengths covering $280\text{-}704\text{nm}$. Using Bito's simplified method, a beta peak for oxymyoglobin at 540nm , a beta peak for metmyoglobin at 503nm , and the beta peak for myoglobin at 700nm were used to establish absorption areas (absorption degree x time), that is, the ratio of absorption areas of E_{503} , E_{540} and E_{700} .

$$\text{Absorption Area} = \frac{E_{540} - E_{700}}{E_{503} - E_{700}}$$

From the calculated absorption areas, and interpolation using Kakuta's myoglobin and metmyoglobin ratio calculation curve (Fig.2.5), ratios of metmyoglobin were determined. It took approximately one hour to obtain the reading for myoglobin from the sample. The extraction was conducted in a temperature controlled room set at 4° Celsius and the HPLC room was operated in a constant temperature room set at 20° Celsius.

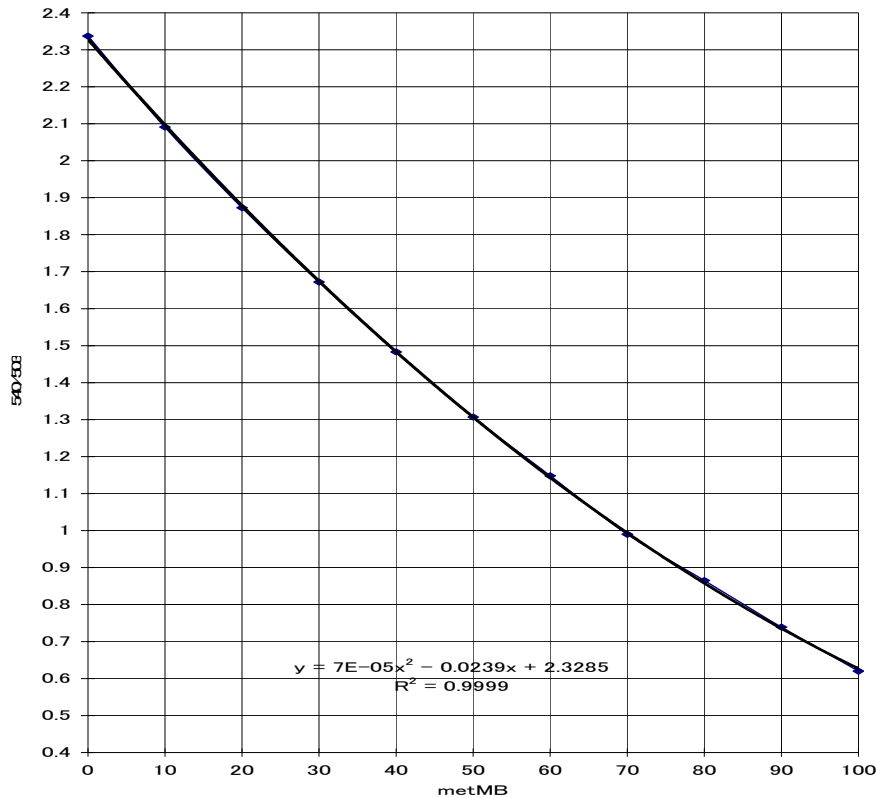


Figure 2.5: Myoglobin -metmyoglobin ratio calculation curve (Kakuta & Uchiyama, 1986)

2.2.5 Nucleotide Catabolites: Post-mortem, the nucleotide adenosine triphosphate (ATP) breaks down into adenosine diphosphate (ADP), adenosine monophosphate (AMP), inosine monophosphate (IMP), inosine (Ino) and hypoxanthine (Hx), with the latter associated with bitter off-flavours in fish (Saito *et al.*, 1959). The degradation of ATP from one catabolite to another proceeds in the same way with most fish and the relative ratio of the concentrations of these breakdown products at any one time, known as a K value, is often used as an indicator of fish freshness (Saito *et al.*, 1959; Gill, 1995).

In the present study, nucleotides were extracted from 5-6g of tuna muscle homogenized with 15 ml of 6% Perchloric acid at 0⁰ Celsius. The homogenate was then centrifuged at 3000 g for 10 min, and the supernatant adjusted up to

20 ml. An aliquot of 1 ml of supernatant was neutralized with 1.0M potassium hydroxide. After standing at 0⁰ Celsius for 10 minutes, potassium perchlorate was removed by centrifuging at 4⁰ Celsius. The supernatant was then filtered (0.22µm) for subsequent HPLC analysis. Separations were carried out in an Asahipak GS-320 HQ column (7.6 X 300 mm; Showa Denko) with a guard column (GS-2G) equilibrated at 25⁰ Celsius. A mobile phase of 0.02 M Na₂H₂PO₄ (disodium dihydrogen orthophosphate, pH 2.9) dissolved in MilliQ purified distilled water (Millipore, Bedford, MA) was used at a flow rate of 1 ml/min. The eluant was monitored at 260 nm (Saito et al., 1959).

2.2.6 Tissue Lactic Acid: Excessive energy demands pre-mortem results in most teleosts, including tuna, producing energy via anaerobic glycolysis. This more inefficient process has principally lactic and pyruvic acids as its end-products and measures of their concentrations are often used as indicators of pre-mortem stress which can affect the qualities of the flesh (Driedzic & Hochachka, 1978).

Lactic acid concentrations were analysed from 5-6g of tuna muscle homogenized with 15 ml of 6% Perchloric acid at 0⁰ Celsius. The homogenate was then centrifuged at 3000 g for 10 min, and the supernatant diluted up to 20 ml. An aliquot of 1 ml of supernatant was neutralized with 1.0M potassium hydroxide. After standing at 0⁰ Celsius for 10 minutes, potassium perchlorate was removed by centrifuging at 4⁰ Celsius. The supernatant was then filtered (0.22µm) and concentrations were determined by high-performance liquid chromatography (Shimadzu Organic Acid Analysis System, Shimadzu, Kyoto,

Japan) using a Shim-pack SPR-H column (250mm × 7.8mm internal diameter; Shimadzu), and an electro-conductivity detector. Prior to injection, all samples were diluted 1/40 with distilled water and then filtered through 0.22µm cellulose acetate membranes.

2.2.7 Lipid: In order to determine the fat concentrations of tuna muscle a method was adapted from the Norwegian Standard NSF, (NS 9402 E 1st edition December 1994 UDC 639.2). Wet tissue of $12 \pm 0.5\text{g}$ was homogenised and then two portions of $5 \pm 0.5\text{g}$ of the homogenate were transferred into a tared weighing beaker and the accurate weights recorded. The tissue was then ground in a mortar with $20 \pm 0.5\text{g}$ of anhydrous sodium sulphate until dry and uniform in appearance and then transferred to a 250ml flask containing $40 \pm 0.5\text{ml}$ of ethyl acetate. The flask was agitated for 1 hour at a medium rate (using a suitable shaker or agitator). The contents were then centrifuged for 5 minutes at 4000 rpm. The supernatant was then transferred into a weighed drying beaker which was then placed in a water bath of approximately 80°C under a fume hood until all the solvent had evaporated. The beaker was then placed in a drying chamber / oven set at 60°C for 15 minutes and the extracted fat and the beaker were then weighed. Percentage fat = (fat weight / dilution factor / tissue wet weight) x 100.

2.2.8 Moisture: In order to equate moisture levels in tuna muscle two portions of approximately 5 grams of finely chopped flesh were placed into pre-weighed and tared foil pans. The weights were accurately recorded and the foil pans and flesh samples dried in an oven for 24 hours at 100 degrees Celsius.

Following complete desiccation, the dry weights were recorded and flesh moisture was equated as the averaged ratio of the dry weights to the wet weights of both samples.

2.2.9 Vitamin E, Vitamin C, and Selenium: The vitamin E (alpha-tocopherol) and dl-alpha-tocopherol acetate concentration were determined by a HPLC method based on the method of (Huo *et al.*, 1999) modified for SBT muscle by D'Antignana *et al.* (unpublished). Vitamin C was determined by a technique based on the HPLC fluorescence detection method of Brown and Miller (1992), which was adapted to SBT muscle in the laboratory at the Lincoln Marine Science Centre with the cooperation of Malcolm Brown of CSIRO Hobart, Tasmania. Muscle samples analysed for selenium were sent, stored in dry ice, to Regional Laboratory Services, Benalla, Victoria, Australia. Selenium concentration was determined using a method based on the fluorimetric technique of Watkinson (1966) modified as per Paynter *et al.* (1993).

2.3 Method Development

2.3.1 A Rapid, Objective and Non-Destructive Technique to Measure the Quality of Sashimi Tuna

Originating in the 1960s and based on camera-computer technology the use of computer vision has expanded into areas such as medical diagnostics, forensics, the biological and ecological sciences, automatic manufacturing and surveillance, remote sensing, and autonomous vehicle and robot guidance systems (Glasbey & Horgan, 1995; Villafuerte & Negro, 1998; Sun, 2004). More recently, due to the cost effectiveness, consistency, superior speed and accuracy of the technology it has been applied to the sensory analysis of food and agricultural products, and traditional visual quality inspection performed by human inspectors has the potential to be replaced by computer vision systems. (Brosnan & Sun, 2004).

Human beings, both at a conscious and subconscious level, classify objects based upon their visual appearances. For food, following an assessment of colour, consumers ascribe values for quality, freshness, and palatability based upon certain criterion and standards (Zhang *et al.*, 1998). According to Zhang *et al* (1998) this process requires reproduction in any automated system where colour is a quality criterion. Most commercial digital cameras mimic the human colour vision system. Digital cameras scan images with a matrix of hundreds of thousands of microscopic photocells, creating pixels where colour is recorded as brightness values of between 0 to 255 for the primary colours red, green, and blue (RGB) (Villafuerte & Negro,1998).

With expert human assessment as the standard, computer vision technology and image analysis has been used to predict and measure the lean colour and marbling of beef (Gerrard *et al.*, 1996; Kuchida *et al.*, 2000a; Kuchida *et al.*, 2000b), the colour of pork chops (Lu *et al.*, 2000; O'Sullivan *et al.*, 2003), the colour of chocolate (Kumara *et al.*, 2003), the colour and shelf life of lettuce (Zhou *et al.*, 2004), and the colour and classification of muffins (Abdullah *et al.*, 2000), potatoes (Tao *et al.*, 1995), apples (Heinemann *et al.*, 1995), and cheese (Wang & Sun, 2002). Indeed, Kuchida *et al.* (2001a) manufactured a fixed dome section that enabled digital image capture of the cross section of beef at a constant angle, distance, and illumination. Using image analysis the predicted beef marbling scores were 97% +/- 1 correlated to expert assigned marbling scores. Kuchida *et al.* (2001b) also found high correlation coefficients between the camera captured R,G, B and luminance values and expertly graded beef colour scores of -0.98, -0.91, -0.82, and -0.99 respectively. Further, in a demonstration of its broader application, computer vision proved to be more accurate than the untrained human eye and by the colour of their plumage able to differentiate two sub-species of partridge in Spain (Villafuerte & Negro, 1998).

The qualities of farmed Southern bluefin tuna are assessed via a visual inspection of the tail section of individual tuna by Japanese technicians and wholesalers in Port Lincoln and Japan respectively. These technicians, trained to discriminate the quality attributes of colour, fat content (visual and tactile), and carcass conformation use varied and subjective ranking systems in order

to express and communicate quality within their respective companies. The attributes of fat and colour are either assessed independently of each other, or a 'hybrid' grade including both fat and colour is ascribed to the flesh. The red colour and the levels of intramuscular fat in tuna are inversely related with increasing levels of colourless fat diluting the amount of muscle pigmentation, and therefore, reducing the hue and its intensity of colour in the muscle (Gjerde, 1987; Sigurgisladottir *et.al.*, 1997). On average, farmed tuna have higher and more consistent levels of fat in their flesh when compared to wild tuna, and therefore of less significance when grading. However, farmed tuna have a reputation on the wholesale market in Japan as having a short colour shelf-life. As a result, it is the colour of the flesh of farmed tuna, and indicators of colour longevity (e.g. surface moisture levels) that have become the most important quality criteria used to assess the quality to the farmed product in Japanese markets.

On a day to day basis, the various grading systems of the Japanese technicians employed in Port Lincoln by Japanese importers appear to serve their needs adequately, however, they do not provide an objective, repeatable and transferable measure of quality over time for the farmers or researchers. The ability to more objectively evaluate the flesh quality of sashimi tuna will enable researchers and the industry to quantify the flesh quality outcomes of any pre-harvest or post-harvest manipulation from one farming season to the next, and between differing companies and farming locations.

Tri-stimulus colorimeters are expensive and due to the translucent nature of tuna flesh and the consequent scattering of the equipment's incident light, the recorded colour data occupies the grey instead of the red colour space, therefore rendering the machines suitable only for examining changes in the reflective properties of the flesh in time series such as occurs in shelf-life examinations (Douglas, 1999 – unpublished data). Digital camera technology and associated softwares have in recent years become increasingly affordable and therefore commonplace as a research tool with more applications for digital image analysis being discovered each year. As vision is the primary sense involved in the quality grading of sashimi tuna, digital images of the flesh of Southern bluefin may contain valuable information that could relate to the current 'standard' - that is the trained eye of Japanese technicians and buyers. The rapid, non-destructive, and low-cost nature of this technology offers advantages to the tuna farming industry in Port Lincoln considering both the high value of the product and the short period of time between the landing and packaging of the product.

Aims and Objectives

The aim of the current investigation was to examine whether RGB values extracted from the digital images of tuna flesh could be used as an objective tool in the analysis of the quality of sashimi grade tuna.

Materials & Methods

Tuna, Quality Grades and Price Information: Throughout the course of the 2002 Southern bluefin tuna farming season the tail section of a total of 235

SBT were taken pre-auction at the Tsukiji Wholesale Markets. An individual auctioneer with more than 25 years experience grading and selling tuna at the the Tsukiji Wholesale Markets in Tokyo was used to provide overall tuna quality ranks of either A, B or C for each of the 235 tuna. Grade A being of the highest 'quality' and C the lowest. Following auction the price paid per kilogram for each of the 235 fish was recorded.

Digital Camera and Lighting: A Minolta DiMAGE 5 digital camera (white balancing performed each session) was used to collect images. As colour quantification is effected by the degree and type of illumination, as well as the angle and distance of the lighting system from the target object (Zhang *et al.*, 1998; Kumara *et al.*, 2003), a constant luminescent light source was set at a constant angle and distance from the surface of the flesh (Fig 2.6). The light source was designed for the sensory assessment of foods by the *Centre for Food Technology*, Queensland Dept of Primary Industries, Australia.

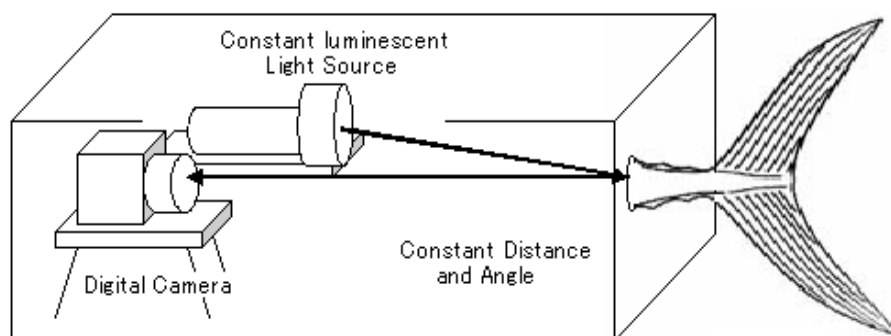


Figure 2.6: Method of capturing a digital image of flesh from the tail of individual tuna.

Data Extraction: Red, green and blue (RGB) values were obtained from the images at the central muscle block of the upper-right portion of the tail (Fig. 2.7). This muscle block was chosen due to its proximity to the two areas of

interest to the wholesalers grading the tuna – the inner section for red colour and the outer section for marbling with fat. To obtain the values the images were opened in *Adobe Photoshop 6.0* software and using the 'Zoom Tool' the area of interest was enlarged to its maximum possible level of magnification avoiding areas containing any reflections, connective tissue, or dark spots. Using the 'Blur' function in the 'Filter' options the colour data of each pixel in the enlarged area were blended. Then, using the 'Eyedropper Tool' set to the '5 by 5 Average' setting in the 'Sample Size' option, the R, G, and B values of the images were recorded from the centre of the image. Total quality ranks ascribed to the tuna by an experienced Japanese tuna auctioneer were then compared to a ratio of the red value to the sum of the green and blue values ($R/(G+B)$) and the data was analysed using two-tailed t-tests. The ratio was chosen to emphasize the key role of redness in the quality assessment of sashimi grade tuna.

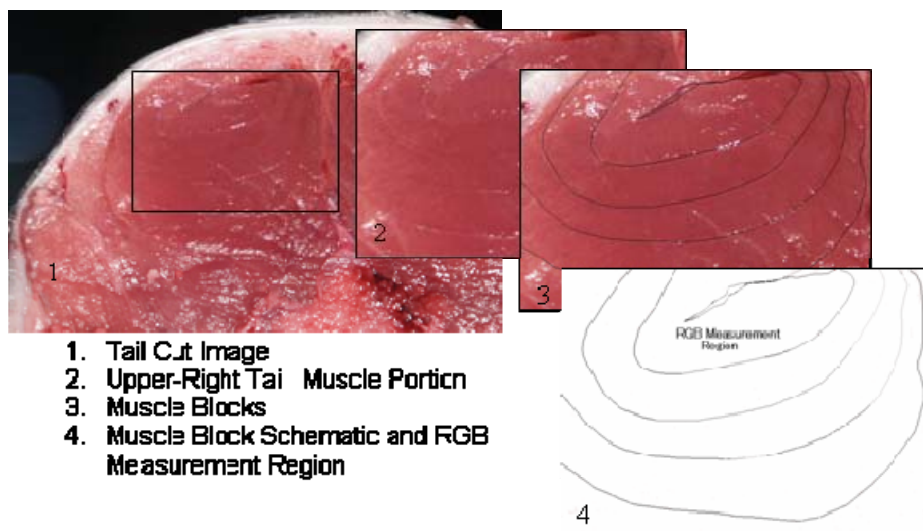


Figure 2.7: Area of the tail cut used to gather the RGB data of the flesh.

Data Analysis: The quality ranks ascribed to the tuna by the experienced Japanese tuna auctioneer were compared to a ratio of the red value to the sum of the green and blue values ($R/(G+B)$) and the data was analysed using two-tailed t-tests (*SPSS*).

Results & Discussion

There were significant differences between the RGB ratios of quality ranks A and B ($P < 0.001$) and B and C ($P < 0.05$). Significant differences were also measured between the average prices paid per kilogram at auction for the quality ranks A and B ($P < 0.001$) and B and C ($P < 0.05$) (Fig. 2.8).

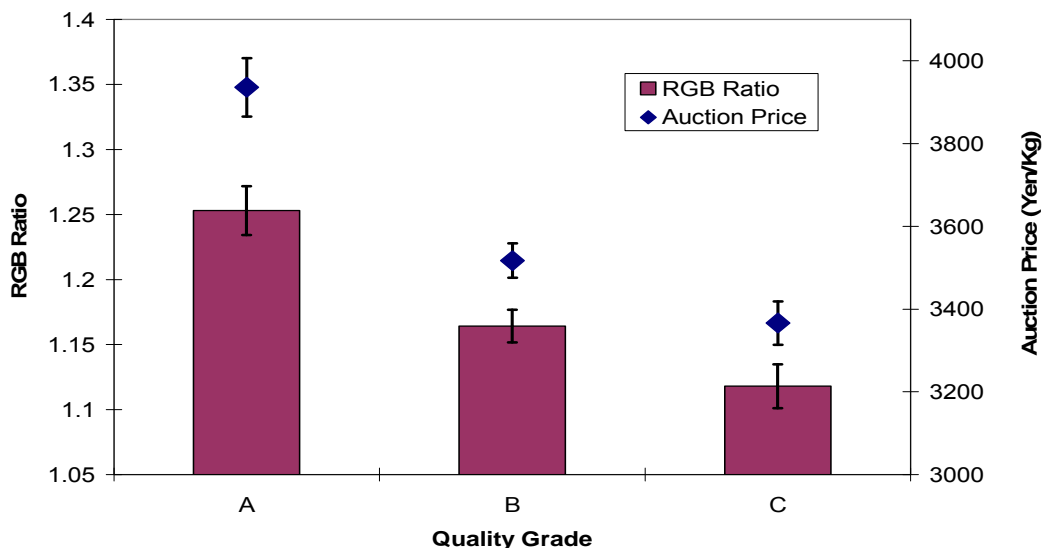


Figure 2.8: RGB ratio ($R/(G+B)$; \pm standard error) and auction floor price as a function of quality grade (A = High Quality, $n=64$; B = Medium Quality, $n=146$; C = Low Quality, $n=51$) as ascribed to air-freighted, farmed Southern bluefin tuna from Port Lincoln, Australia at the Tsukiji Wholesale Markets, Tokyo by an expert grader in the 2002 season.

A relationship between ranks of sashimi tuna quality and colour information extracted from digital images has never been demonstrated previously, and this result indicates that digital colour analysis can be used as an objective and repeatable measure of overall tuna quality in the marketplace. As the colour and fat levels of sashimi grade tuna are said to be the main determiners of

quality, and there is a known relationship between the concentration of intramuscular fat (and therefore moisture) on the colour of tuna flesh, the visual assessment of quality by wholesalers on the Tsukiji Wholesale Market are measures of the overall appearance of the flesh and a synthesis of the colour and fat characteristics. Subsequently, digital colour analysis of the flesh is not only a measure of the colour characteristics, but also accounts for the other major market determiners of sashimi quality – the fat and moisture levels of the flesh.

In addition to RGB data, other information may prove beneficial. Villafuerte & Negro (1998) found that further standardization was obtained by dividing each colour component by its luminosity (L), which is a function of the three primary colours. This way, according to the authors, indices for the amount of red (R/L), green (G/L), or blue (B/L) could be used to compare these colours among individuals. However, Zhang *et al.* (1998) found colour classes to be consistently orientated with the intensity axis in RGB space for most biomaterials except in the cases of monochromatic objects such as red meat. In the present study, where a constant luminescent light source was used, a greater level of significance was found by expressing the red values as a ratio of the green and blue values combined.

The quality rankings ascribed to tuna by an individual expert auctioneer also correlated well to the auction floor price. Assuming that wholesalers pay for quality, an auctioneer's assessment of quality therefore appears to be in agreement with the purchasing wholesalers' assessments of quality. However,

price alone is not a reliable indicator of flesh quality at any one time as the relationship between flesh quality and price can vary along with changes in macro- and micro-economic conditions.

A premium is paid for fatter fish on the market and for the higher quality ranks ascribed to larger and rounder individuals (Fig. 2.9), meaning that digital colour analysis could also be combined with rapid analytical techniques of body composition and carcass conformation. However, as opposed to wild tuna, farmed tuna have relatively consistent and high levels of fat, and they are commonly criticised for weak colour and a comparatively short colour shelf life. Therefore objective measurement of the colour characteristics of the flesh of farmed tuna would be of greater significance in its quality ranking when compared to that of wild tuna.

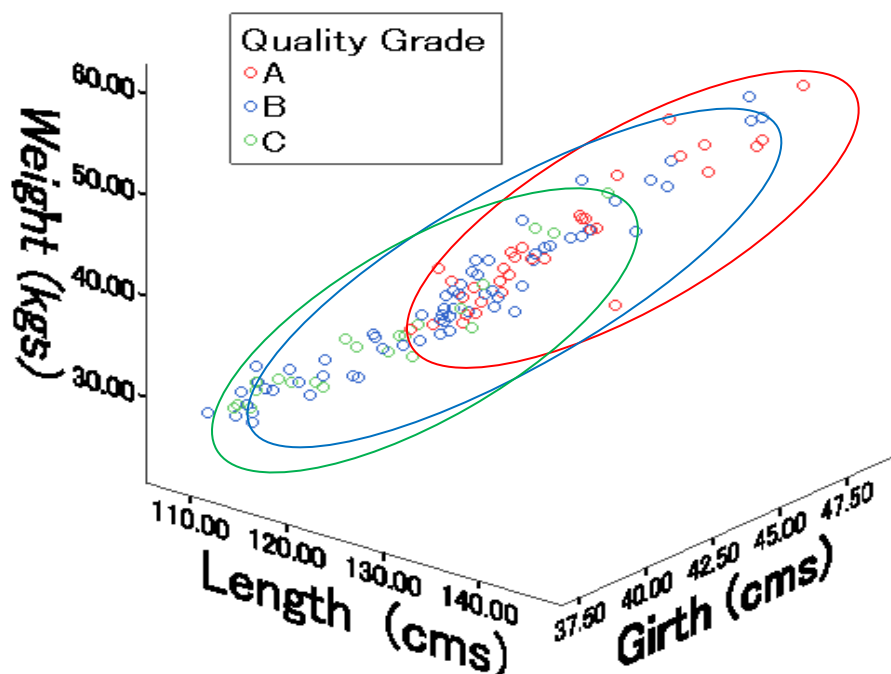


Figure 2.9: Weight, length and girth (measured from the front of the secondary dorsal fin to the front of the anal fin) and quality grade (A = High Quality; B = Medium Quality; C = Low Quality) as ascribed to air-freighted, farmed Southern bluefin tuna from Port Lincoln, Australia at the Tsukji Wholesale Markets, Tokyo by an expert grader in the 2002 season (n=225).

Additionally, to account for changes in quality characteristics of the flesh over time, digital colour analysis and an indicator of time-temperature history ('freshness') such as K Value could, with regular calibration to an expert grader, form the basis of a quality ranking system (Fig. 2.10). In such a scheme, for example, the flesh of two tuna each recording an RGB ratio of 1.25, but with K Values of 5 and 15, would result in B and D quality grades respectively.

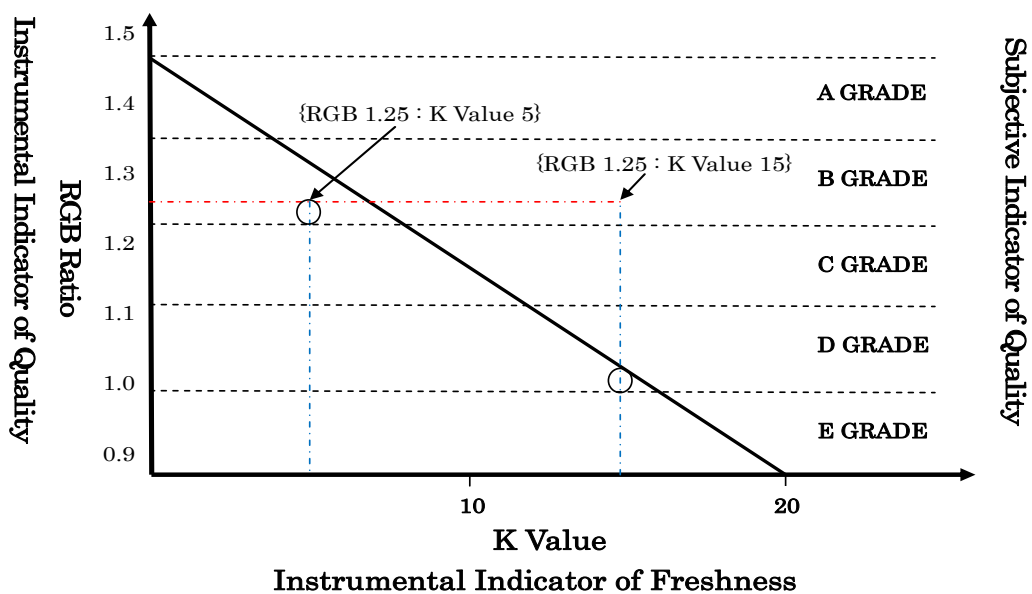


Figure 2.10: Expert calibrated (Subjective Indicator) flesh quality grading scheme incorporating instrumental measures of quality (RGB Ratio) and freshness (K Value). The area below the diagonal at which the blue line first intersects determines the quality grade. The examples above show a B grade and D grade fish.

Digital colour analysis is a promising technique for the objective quality assessment of sashimi tuna. Software already developed by Villafuerte & Negro (1998) that creates a new file for the RGB values of each pixel from an image on a clipboard and saves the data with two identifiers - one for photo

identification and the other an area code, could help expedite the use of this technology into the industry. Any automated quality assessment system though, would require periodic calibration to the eyes of expert tuna graders. In the classification of pork into five classes that reflected pre-existing grading standards, Zhang *et al* (1998) found that the expert grading varied somewhat and suggested the variances may have been due to each individual meat packers standards in the US and dependent upon customer specifications.

The current study only gathered expert ascribed tuna quality ranks from the Tsukiji Fish Markets in Tokyo, central Japan. The calibration of an automated system for the assessment of sashimi of tuna for the Japanese market may need to take into account possible regional variations in the quality ranking of tuna. Furthermore, with fresh tuna carcasses leaving Port Lincoln not fully chilled and in a biochemically labile condition, the technique would require validation, and possibly calibration for use on tail cut samples removed at processing either just prior to shipment or following a time in storage. However, the digital analysis of sashimi grade tuna flesh, according to the results of the present study, shows much potential in enabling the rapid and relatively non-destructive assessment of the qualities of the flesh as it relates to market value.

2.3.2 Development of a Sensory Analytical Method for Southern Bluefin Tuna and the Relationships between the Sensory and Biochemical Properties of the Carcass

Southern bluefin tuna are highly valued by humans as a food. Health and safety considerations aside, a 'good' or a 'poor' quality specimen is ultimately defined by the amount of pleasure that is derived from its consumption. The level of derivable pleasure can vary between individuals and across cultures, as is evidenced by the fact almost 100% of Australia's catch of Southern bluefin tuna is consumed in Japan, it is eaten raw, and prices paid for a single slice (~15g) can vary between AUD\$1.00 and \$15.00 at restaurants. This contrasts significantly with prices obtained in the 1950's through to the 1980's when Southern bluefin tuna caught in Australian waters were cooked and canned at a price of AUD\$2.50/kg and mostly consumed domestically.

A great deal of research has been conducted into the biochemical indicators of 'quality' for fish without any prior definition of what 'quality' is or means, and often authors have merely stated that the parameters they measured, such as pH, K value, or bacterial loading, are related to 'quality'. These measures are certainly related to the quality characteristics of the flesh, its physico-chemical makeup, and to the health and safety of the product, but how are they related to the concept of quality as used by consumers? Fish on a supermarket shelf or on a restaurant plate is a food item competing with other foods not only as a source of protein and other nutrients but also as a source of gastronomic pleasure with the sensory attributes of appearance, odour, flavour, and texture. So what does a pH of 5.9, a K value of 15, and a bacterial loading of 10^3

cfu/cm² on the skin surface of fish mean to a consumer or customer? Indeed, what does it mean to a fisher, an aquaculturist, or a freight manager looking to improve upon the 'quality' of the product he or she produces and supplies to his or her customers?

To find out what makes one product 'better' than another requires some form of hedonic testing – a consumer preference trial. These are often both expensive and difficult to perform as they require a large number of test subjects to be representative and take into account cultural, regional, and demographic differences within and across the consumer population. However, prior to any large scale consumer preference trials that help define the 'product quality' target it is first necessary to profile the product instrumentally and sensorially in order to be able to objectively assess whether any changes in production and post-production procedures move the end product closer to or further away from this target. It is therefore necessary to develop a sensory testing protocol that is statistically robust with sufficient subject and sample numbers, that is representative of the whole product, and that is manageable and cost effective. The following section documents the development of a sensory testing protocol for sashimi tuna of the average size of farmed Southern bluefin tuna, combines it with some instrumental profiling of the carcass, and relates both the sensory and instrumental profiles of eight carcasses taken on one occasion in the farming season.

Aims and Objectives

The objectives of this investigation were to formulate a descriptive sensory testing strategy for the sashimi of Southern bluefin tuna that could be used as a blueprint for future treatment testing of the product, to investigate a range of physico-chemical and sensory characteristics of the farmed product, and examine the relationships between these physico-chemical and sensory indicators of quality.

Method

Product Availability and Requirements

The carcass of a sashimi-grade tuna, regardless of the species, is partitioned, and the cuts distinguished by their locality on the carcass and their fat concentration. The carcass is initially cut into four major loins by wholesalers and is called *yotsuwari* - meaning 'divided into four' (Fig. 2.11). These four loins are separated by their respective positions on the horizontal and lateral planes. The right side of the fish, which is normally the side of the fish which is laid down for presentation, is referred to as *shitami* - meaning 'downside meat', and the left side as *uwami* - meaning 'upside meat'. These sections are then further divided into either dorsal (*se* - meaning back) and ventral (*hara* - meaning belly) portions. Moving posteriorly from the head to tail, the carcass is split into three major sections – the *kami*, the *naka*, and the *shimo* section. The *naka* section is further split into two smaller sections and these are distinguished by their locality to either the *kami* or *shimo* regions.

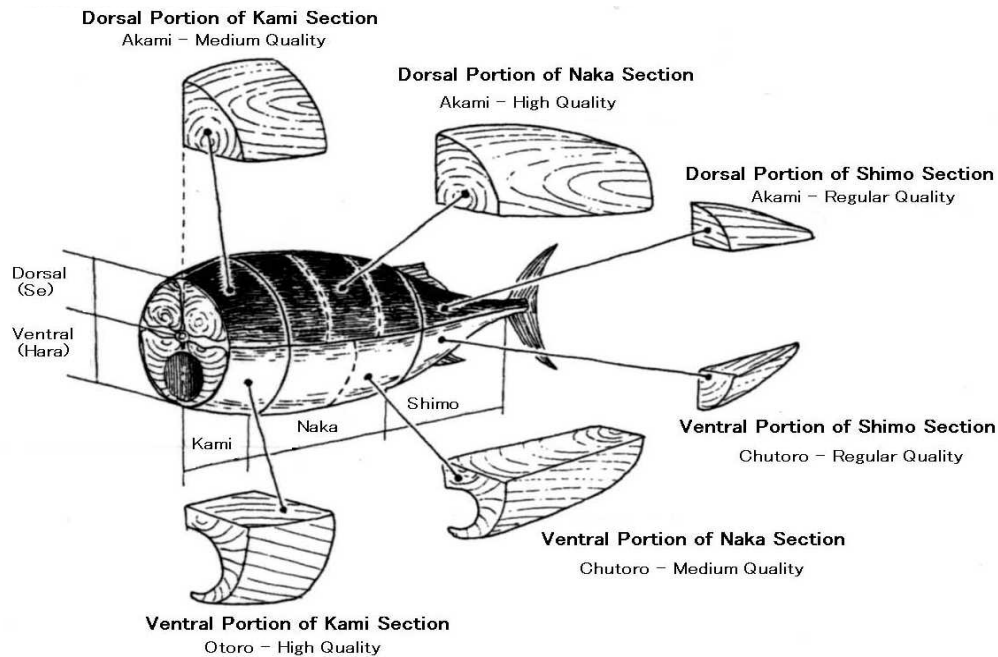


Figure 2.11: The sectioning of a sashimi tuna carcass at the wholesale level (Ueda, 2003).

The three main cuts of a tuna known to consumers are the *Akami* ('red body'), the *Chutoro* ('middle fat'), and the *Otoro* ('large fat') and it was these three cuts that would be the focus of a sensory testing scheme (Fig. 2.12). However, the availability of each of these three cuts is different within a carcass and between individuals. The remaining parts listed below are named in accordance to their proximity (*gishi* and *pa* meaning adjacent) to either bone (*hone*) or blood meat (*Chiai*). *Wakaremi* literally translates as 'separated meat' and refers to its portioning off by the sinuous tissue referred to as *suji*. These portions are not well known to consumers, are too low in volume, and not easily processed for sensory evaluation.

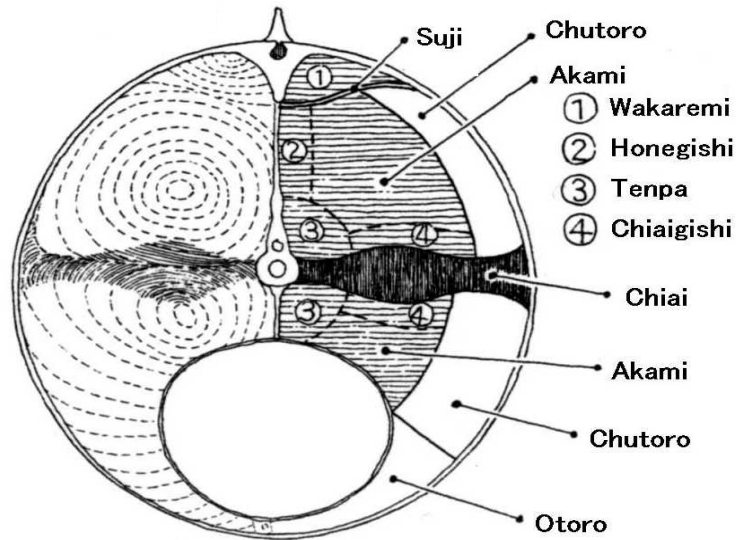


Figure 2.12: The cross-section of the fore portion (*kami*) of a tuna carcass and the various cuts of meat as determined by Japanese wholesalers (Ueda, 2003).

The concentrations of both fat and the red coloured pigment myoglobin are inversely proportional to one another and vary in both the longitudinal and cross-sectional planes of the carcass. The highest concentration of fat and the lowest myoglobin concentration are characteristics of the anterior belly region (*Otoro*), and the reverse relationship can be found in the dorsal tail region (*Akami*). It was necessary to account for these variances in the allocation of sashimi sections to the panellists.

To make sashimi, the loin is first cut into *koro* for each of the *kami*, *naka*, and *shimo* sections (Fig. 2.13). The *koro* are then cut into *saku* (fillets). The upper two *saku* (or inner sections) are cuts of *Akami*, while the lower *saku* near the skin is a cut of *Chutoro*. The *saku* are then cut longitudinally into *sashimi saku*. The diagram illustrates the process for a large Northern bluefin tuna. For Southern bluefin tuna the process stops at the cutting of the first lot of *saku*, and, therefore, the *saku* at point ④ would be referred to as *sashimi saku*.

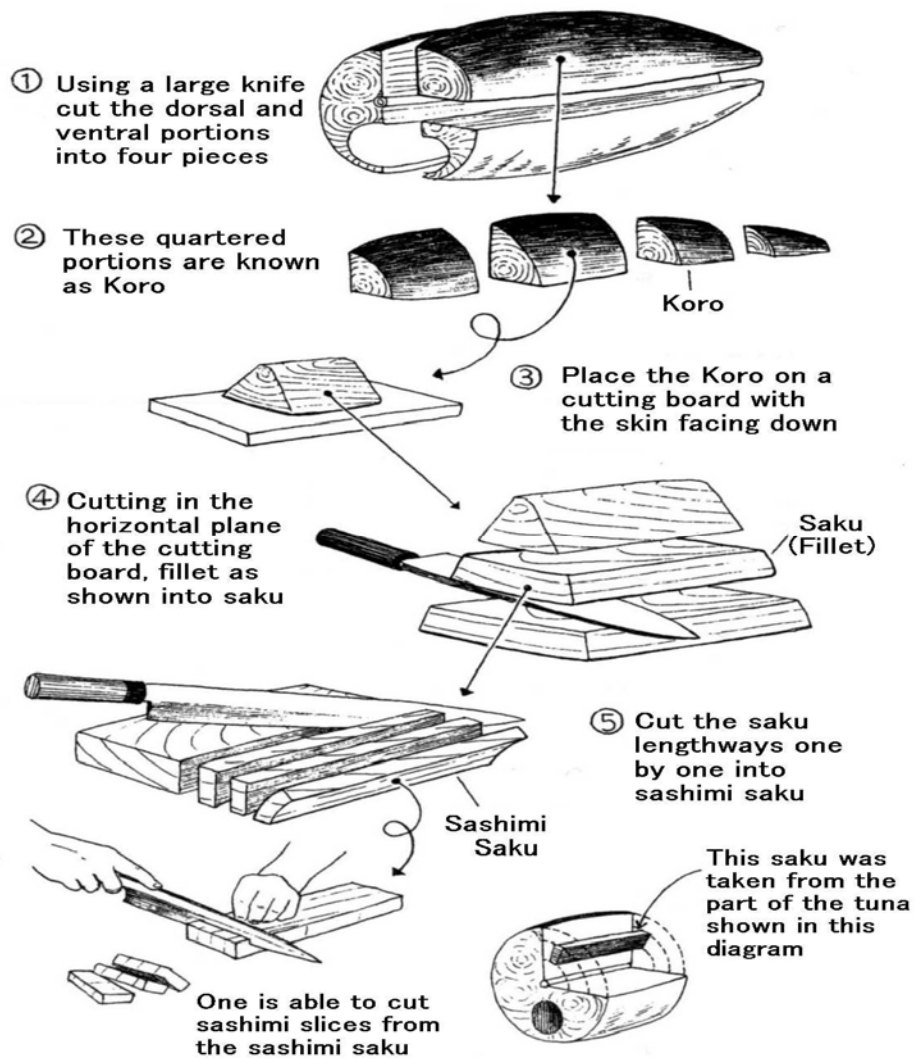


Figure 2.13: The sectioning from a quartered loin (*yotsuware*) of a tuna carcass through to slices of sashimi (Ueda, 2003).

Ventral loins are portioned into the sashimi cuts of *Akami*, *Chutoro*, & *Otoro* (Fig. 2.14). The unused portions include the *Kawagishi* section, which literally means ‘next to the skin’ and refers to the white epidermal tissue between the skin and the muscle. The *Sunazuri* translates as the ‘sand rubbing’ section and refers to its physical location on a tuna’s body, which, being on the belly is closer to the sea floor.

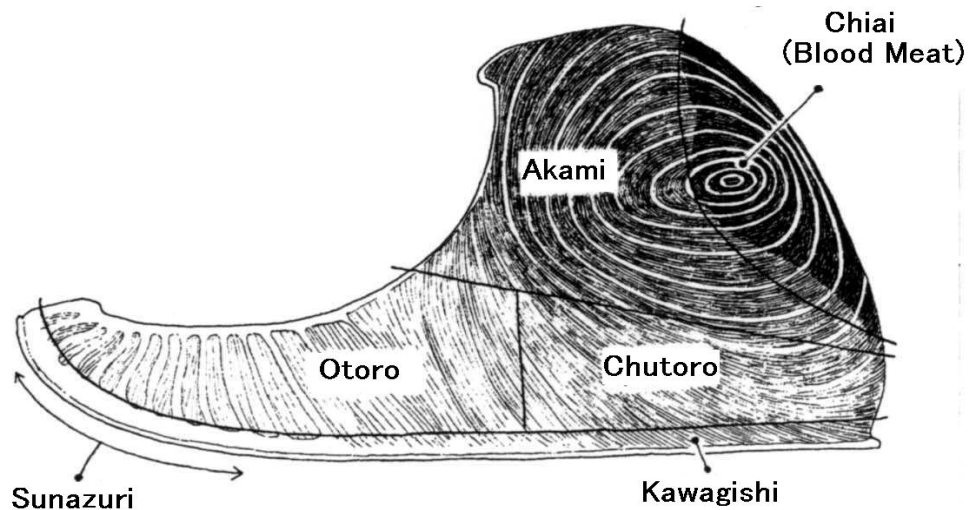


Figure 2.14: The portioning of the ventral loin of a tuna carcass (Ueda, 2003).

The *Otoro* section of a tuna carcass is not only the most valuable cut, it also the lowest in volume of the three cuts on the carcass and has the greatest variation in the concentration of fat in both a longitudinal and cross-sectional plane. As sensory analysis of the *Otoro* of a Southern bluefin tuna carcass had not previously been undertaken it was necessary to run through the sectioning of an *Otoro* portion in order to calculate the yield and number of sashimi slices available for sensory testing, and the degree of variation in the concentration of fat that would need to be taken into account in the allocation of samples to panellists.

Southern bluefin tuna, unlike northern bluefin, possess pleural bones that protrude into the dorsal region of the *Otoro* portion and are an impediment to its sectioning and affect the dimensions of the sashimi slices used in a sensory evaluation. To investigate this and the quantity of product available for sensory and biochemical sampling, cutting trials were carried out (Fig. 2.15).



The belly (hara) loin of the tuna and the removal of the Otoro saku.



The trimming of the gut epithelium and removal of the epidermis.



Slicing and removal of a longitudinal section for biochemical analysis



Slicing up of the dorsal section of the Otoro fillet into sashimi slices.



Slicing up of the ventral section of the Otoro fillet into sashimi slices.



The fully cut up Otoro Sashimi saku into sashimi sections and slices.

Figure 2.15: Investigation into the potential affects of the pleural bones, quantities of flesh available for sampling and the process undertaken to select the sectioning design of the *Otoro* fillet.

Availability of Sample

To calculate the amount of sample available for a sensory experiment yield data was collected from the dorsal loins (Table 2.1) and the ventral loins (Table 2.2) of three fish. The averaged data suggest that a single dorsal loin from a 32.5 kg gilled and gutted tuna would weigh approximately 6.3 kg, and the anterior (*kami*), middle (*naka*) and posterior (*shimo*) portions would weigh approximately 1.73, 3.43, and 1.12 kg respectively. The sashimi fillets (*saku*) from the *naka* portion would approximate 390, 720, and 1020 g for the inner, central, and outer *saku* respectively (inner and central = *Akami*; outer = *Chutoro*).

	Fish Weight	Dorsal Loin	Kami Section	Naka Section	Shimo Section	Inner Saku	Central Saku	Outer Saku	Excess
Fish 1	39.4	7.55	2	3.85	1.7	0.3	0.78	1.104	1.45
Fish 2	33.48	6.6	1.7	4	0.8	0.528	0.903	1.284	1.221
Fish 3	24.6	4.75	1.5	2.45	0.85	0.332	0.488	0.665	0.859
Avg (Kg)	32.49	6.30	1.73	3.43	1.12	0.39	0.72	1.02	1.18

Table 2.1: Yield data from the dorsal loin of three tuna.

An average 29 kg gilled and gutted fish would yield a 5.20 kg ventral loin with an *Akami* and *Otoro* portion approximating 3.3 and 1.8 kg respectively, and yield 800 g of trimmed *Otoro*. The excess column is a measure of the skin and trimmings that were discarded.

	Fish Weight	Ventral Loin	Akami Portion	Otoro Portion	Trimmed Otoro	Excess
Fish 1	29.80	5.50	3.40	2.10	0.85	1.24
Fish 2	24.60	4.40	2.90	1.40	0.65	0.76
Fish 3	33.48	5.70	3.70	2.00	0.94	1.08
Avg (Kg)	29.29	5.20	3.33	1.83	0.81	1.02

Table 2.2: Yield data from the ventral loin of three tuna.

Testing Method

As discussed in chapter one, difference tests provide no indication of the dimension of difference, are designed for mostly homogenous materials, are based more on probability and guessing behaviour than on sensory discrimination, their suitability to the task at hand when compared to descriptive techniques using rating scale responses was considered unsatisfactory. Descriptive rating scales were selected as the method for use by the experienced sensory panel so that instrumental and sensory data could be compared.

The Sensory Descriptors and Instrumental Measures

A full range of sensory descriptors used by Nippon Suisan to evaluate the sensory properties of fish were considered as possible quality descriptors for the flesh of tuna. It was first necessary to translate these terms from Japanese into an English equivalent so they could be understood by the Australian research group, the Australian tuna farming industry, and so that the work could be published in English. Descriptor terms had to be carefully worded so they would convey the same concepts in both the Japanese and the English language. An agreed protocol was developed in joint meetings with Nippon Suisan that met all these criteria and which they considered was meaningful to their company and to the tasters and practical to accomplish. The terms were also chosen to relate to as many of the physico-chemical measures as possible.

Some of the terms they suggested were not used. For example, an attribute commonly scored for in the sensory testing of tuna in Japan is described as



Chi no yo na fumi (血のような風味)

which translates as “bloody flavour”.

Tuna flesh can be spotted with blood

as a result of ruptured capillaries from

extreme stress or physical impacts

during capture and slaughter of the animals, and/or from inefficient bleeding post-slaughter (see inset). The occurrence of *shimi* (literally ‘staining’) or *utare* (literally ‘bruising’) in the sashimi markets of Japan is mostly confined to wild tuna that have been caught by long-line, in a purse-seine net, or by poling. The lower levels of stress during capture and the handling advantages of tuna farming are such that the occurrence of this flesh quality issue is far less in the farmed product, and therefore this quality attribute was not evaluated.

The attributes of redness, brightness, and transparency were chosen as the most important visual stimuli used in the assessment and grading of sashimi tuna by wholesalers and consumers alike. The objective measures of colour and transparency as recorded by a tri-stimulus Chromameter (*Minolta CR-310*), the RGB values from a digital camera image of the flesh, and ratio of myoglobin and metmyoglobin were the instrumental measures expected to be the most predictive indicators of these sensory attributes.

Bad fishy odour was the only purely olfactory descriptor chosen for its potential relationship to fish freshness. Flavour descriptors included bad fishy flavour

and umami. Naturally found in a wide variety of foods and distinct from the four 'basic' tastes (sweet, sour, salty, bitter), umami is characterised as the taste sensation elicited by glutamates (e.g. monosodium glutamate - MSG) and disodium salts of 5' nucleotides (inosine monophosphate – IMP, guanosine monophosphate - GMP, and adenosine monophosphate – AMP) (Fuke & Ueda, 1996). Alone in aqueous solutions umami compounds are not rated by sensory panellists as pleasant tasting, however, when combined with other flavour solutions, even just salt, and as part of foods, they enhance sensory panellists' ratings of overall flavour and preference (Fuke & Ueda, 1996). The amount of IMP in the flesh was to be compared to the scores of umami for the flesh. Bad fishy odour and bad fishy flavour were selected to compare these sensory characteristics with a biochemical freshness indicator (K value) and a lipid breakdown indicator (hydroperoxide). Sourness was the other sensory flavour descriptor and was to be compared to the flesh pH values and lactate concentrations.

The mouth feel descriptors of hardness and fattiness were chosen in order to examine their relationships to the proximate data of percentage moisture and fat in the flesh. Hardness has been defined as the force necessary to attain a given deformation and is considered a primary, mechanical characteristic of texture. Fattiness (often termed oiliness or greasiness) is neither a mechanical or geometrical characteristic, but, along with moisture content, is classified separately (Brennan, 1984). The tenderness measures of elasticity, smoothness, juiciness, and stickiness were not selected as, according to Brown *et al.* (1996), they are multifactorial attributes with ease of fragmentation,

degree of adhesion between fibres, mealiness, and amount of residue considerations, which, if individual assessment strategies are adopted, can lead to variation in subject responses.

The sensory attributes were evaluated by subjects using interval scales and anchors of 'extreme' or 'none' (Fig. 2.16). Panellists were provided with four separate, three-digit coded plates containing a sashimi sample and were asked to provide a score for each sample to a degree of no less than 0.5 of an interval. Therefore, if the panellist perceived a particular sample to lie between interval points 4 and 5 he/she would score the sample as 4.5 in the box provided.

Bight in February, 2003, by the *f.v. Boston Bay* using a purse seine net. They were then transferred to a tow cage for transportation to Port Lincoln, where they were transferred into commercial grow-out cages. Fed a diet of baitfish until the harvest and processing date of May 19, 2003, the eight fish used for this experiment were selected during harvest to have relatively uniform size and weight (average 32.27 kg gilled and gutted with a range of 24.6 to 39.4kg). Following processing at Australian Bight Seafood Pty. Ltd. in Port Lincoln the tuna were trucked in refrigerated conditions overnight to Adelaide and dispatched on Qantas flight QF738 to Sydney at 9:30am (ACST), 20th May, 2003, where they were held in cold storage for 24 hours prior to loading on Qantas flight QF21 departing Sydney at 9:30pm, 21st May, 2003 (AEST) arriving at Narita International Airport in Japan at 7:30am, 22nd May, 2003 (AEST).

The tuna were then transported to the Nittsu Distribution Centre where their coffins were opened, they were re-iced, and the coffins re-sealed for refrigerated trucking to Tokyo Uoichiba Pty. Ltd at the Tsukiji Wholesale Fish Markets in Tokyo. The tuna were taken from their coffins and loined (*yotsuware*) by the wholesaler Sugahei Ltd. at around 4:30am on the 23rd of May, 2003, with individual loins wrapped and placed in sealed Styrofoam boxes with ice as per industry practice. The loins were transported immediately to the Central Research Laboratory of Nippon Suisan Pty. Ltd. for processing (Fig. 2.17).



Figure 2.17: Loining of tuna at Tsukiji Wholesale Fish Market and sample cutting at the Central Research Laboratory of Nippon Suisan, Hatchioji, West Tokyo.

Southern bluefin tuna from the farming industry are a large animal of between 20 and 80kg in weight and 100 to 150cm in length and will often require two people to carry a single carcass. Unlike other species of fish, tuna are regionally endothermic and trap heat in the core of their bodies. This heat energy that stems from aerobic metabolism can create flesh quality problems if activity rates rise significantly during capture and the carcass is not rapidly chilled in ice slurry following slaughter. Using data loggers the carcass temperatures of the tuna used in this experiment were monitored every fifteen minutes from the point of harvest, through shipment, to the time of loining, and during chilled transport to the testing facility in Hatchioji (Fig. 2.18). There was no significant difference in the time-temperature profiles of the tuna between each treatment and therefore temperature was not a confounding factor.

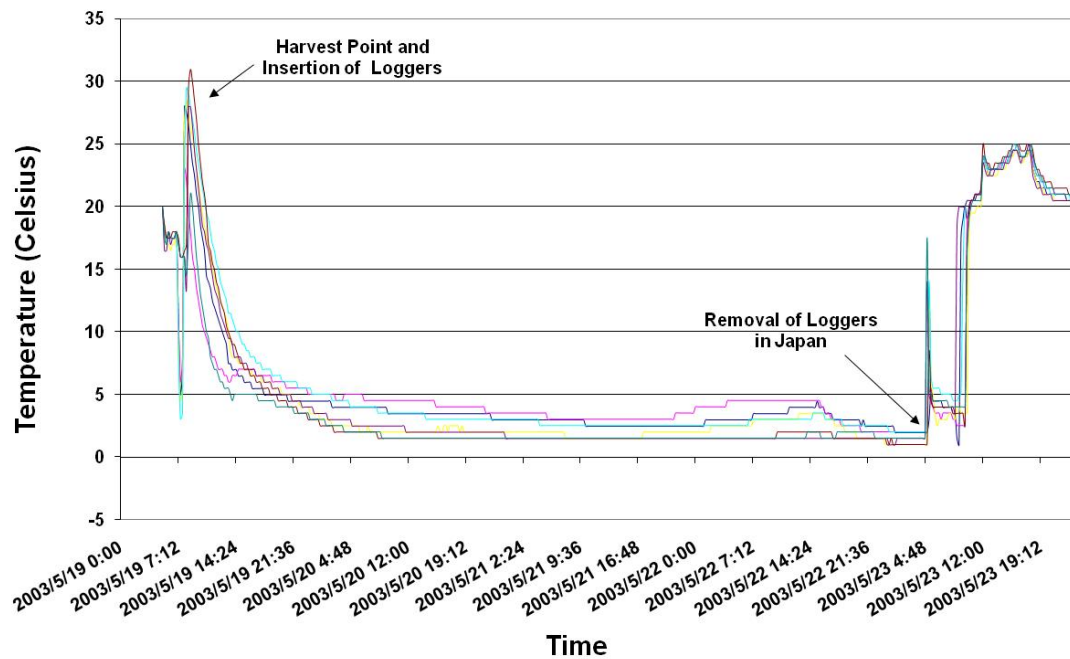


Figure 2.18: The carcass temperature profiles of air-freighted tuna from capture in Port Lincoln, through shipment, to the loining point in Japan.

The facilities and tools of the well-equipped Central Research Laboratory in Hachioji, West Tokyo were used for the processing and preparation of the tuna for sensory evaluation with a total of eight research staff from Nippon Suisan, and four Australian research staff participating. Samples for physico-chemical analysis required weighing and physical measurements to be taken on location and then stored on dry-ice for shipment back to the Tokyo University of Fisheries for further analysis (approximately 5 hours from the time of sampling to the time of storage in liquid nitrogen for later analysis). The time taken to section the sixteen loins (eight for Akami and Chutoro and eight for Otoro) into sashimi slices and record the physical measures of quality (Colorimeter Lab, pH, and digital image) was one and a half hours. Samples, once sliced, were assessed approximately 30 minutes later by subjects.

Southern bluefin tuna are the second most valuable tuna species in the Japanese sashimi market. The average per kilogram price at auction in 2002 at the Tsukiji Wholesale Fish Market was 3,400 Japanese yen. At an exchange rate of 72 Japanese yen per AUD\$1, the value per kilogram equalled just over AUD\$47.00, and would return over \$1650.00 for a 35kg carcass. Any experiment with this species needs to take into account the high cost of an individual carcass.

Subject Qualifications & Availability

The panellists used in the sensory evaluation of tuna all were members of the Central Research Laboratory of Nippon Suisan Pty. Ltd. located in Hatchioji, Western Tokyo. The laboratory's staff participate in over 200 sensory evaluation trials conducted on mostly salmon and yellowtail kingfish in a single year (*pers. comm.* Mori, 2001). As a consequence, all panellists had been screened, were trained in sensory analytical procedures, familiar with the terms and scales employed, as well as the sensory attributes frequently examined in fish flesh. Although not specifically trained to evaluate farmed Southern bluefin tuna, panellists at the laboratory had experience in the grading of the Akami and Chutoro cuts of sashimi tuna.

It is considered 'best practice' to run two sessions in one day to obtain replication in the judgements, provide a measure of consistency, and to minimise exposure to systematic errors. However, running two separate panels, with each subject participating in both, was not possible due to the disruption of the workday of the panellists. Each individual panellist was available for a

single session with a maximum duration of 20 minutes. Consequently the sessions were run as back to back evaluations with up to 50 individuals available to take part.

Experimental Design

One of the major objectives of the trial was to not only develop a sensory analytical protocol for Southern bluefin tuna that was balanced and accounted for within-carcass variations in fat, but to also develop a design amenable to, and statistically robust enough for the analysis of treatment effects and between carcass variations. Such a design would enable the effects of any on-farm or in-chain manipulation to be evaluated by sensory techniques. A number of designs were proposed initially with some involving three fish per treatment, four fish per treatment, or five fish per treatment.

Experienced panellists at Nissui Central Research Laboratory would be able to perform a single sensory evaluation within a 15 second time-frame, which equates to approximately 80 possible sensory evaluations in a 20 minute session. Eight fish 10 sensory attributes equates to 80 individual evaluations.

This enabled us to select a design (four fish per treatment) in which a panellist could sample every fish from each treatment if they were only to examine sashimi slices from a single cut (eg:akami, chutoro or otoro) from the carcasses.

A design was chosen where 24 subjects would examine the Akami and Chutoro cuts from the naka portion of the dorsal loin, and 20 subjects to examine the whole of the important Otoro cut of each fish (Fig. 2.19). This would minimise the effects of the unavailability of some subjects on the day. At 20g per sashimi slice and two slices per panellist, each panellist would require 40g of sample per fish. Each fish would be required to supply 960g of Akami/Chutoro and 800g of Otoro from the naka portion respectively. Biochemical analysis of hydroperoxides (~5g), nucleotides (K Value and IMP) (~5g), lactate (~2g), myoglobin-metmyoglobin ratio (~5g), and proximates (~10g) would require approximately 27g of flesh from each of the three cuts for each tuna with representative slices removed from the Akami and Chutoro cuts and a strip removed from the middle and along the Otoro cut. According to the previous calculations the required amounts for sensory and instrumental analyses would both be available from an average sized tuna.

Sample Presentation, Randomization & Allocation

In Japan, raw tuna is consumed as either sushi or sashimi. Sushi consists of raw pieces of seafood resting on a ball of vinegar flavoured rice with a small amount of a Japanese horseradish, known as wasabi, between the two. Sashimi is raw seafood pieces served on its own. Both are eaten with soy sauce and extra wasabi with the amount depending upon personal preference, if used at all. In Japan, there are five main categories of soy sauce with divisions made according to the ingredients used and the method of production. Constituting 80% of domestic production the most widely used soy sauce in Japan is called Koikuchi. Originating in the Kanto region (East Japan) it is

produced from roughly equal quantities of soybean and wheat. Usuikuchi is a lighter and sweeter soy sauce than Koikuchi and is very popular in the Kansai region (West Japan). In the Chubu region (Central Japan) Tamari soy sauce is richer in flavour and darker in appearance than Koikuchi. The lightest soy sauce is known as Shiro soy and, like Usuikuchi, is also sweet in flavour. Finally, Sashikomi is a soy variety that is brewed in soy sauce rather than salty water leaving it with a very dark appearance and a much stronger and richer flavour than other varieties of soy.

Wasabi is a paste formed by the grating of the root-like stem (rhizome) of the wasabi plant (*Wasabia japonica*) and has a fiery hot flavour that rapidly dissipates in the mouth leaving a lingering sweet taste without a burning sensation. There are two varieties of wasabi – Daruma and Mazuma. Daruma is the more popular of the two producing less ‘heat’ than Mazuma.

With five varieties of soy sauce, each being salty and containing small amounts the naturally occurring flavour enhancer mono-sodium glutamate, it was decided that the sensory evaluation be conducted without soy sauce. As there were two varieties of wasabi both with powerful flavours, it was also left out of the evaluation procedure. The decision to exclude both of these condiments was not based solely on the response variation that they could bring into the equation, but also the procedural constraints of time, sample availability, and subject fatigue.

The sensory evaluation environment of the Central Research Laboratory is purpose-built with subjects able to evaluate samples in a quiet, temperature controlled booth in which samples are passed through a sliding window. The colours are neutral and constant lighting illuminates the individual booths. Sashimi samples of traditional dimension (length = width of four fingers; width = width of two fingers) were presented without condiments to subjects on individual plastic white trays marked with a three digit code.

In a 20 minute long session subjects were able to receive four samples on two occasions (termed Evaluation 1 and Evaluation 2) for a total of eight samples. In a two treatment, four fish per treatment experimental design, it is necessary to select two samples from two fish from each treatment for each evaluation. With such a design there are 36 possible combinations.

<u>Treatment One</u>		<u>Treatment Two</u>		
Fish 1 & Fish 2		Fish 1 & Fish 2		
Fish 1 & Fish 3		Fish 1 & Fish 3		
Fish 1 & Fish 4	×	Fish 1 & Fish 4	=	36 Possible
Fish 2 & Fish 3		Fish 2 & Fish 3		Combinations
Fish 2 & Fish 4		Fish 2 & Fish 4		
Fish 3 & Fish 4		Fish 3 & Fish 4		

Considering only the Akami and Chutoro sections, 24 subjects were available for evaluation, and therefore 12 combinations were deleted randomly from this table (Table 2.3). The possible combinations and the order of presentation of the sashimi sections were then randomized and coded (Table 2.4 & 2.5). The same method of randomization and coding was used for the sashimi sections of the Otoro as used for the Akami and Chutoro.

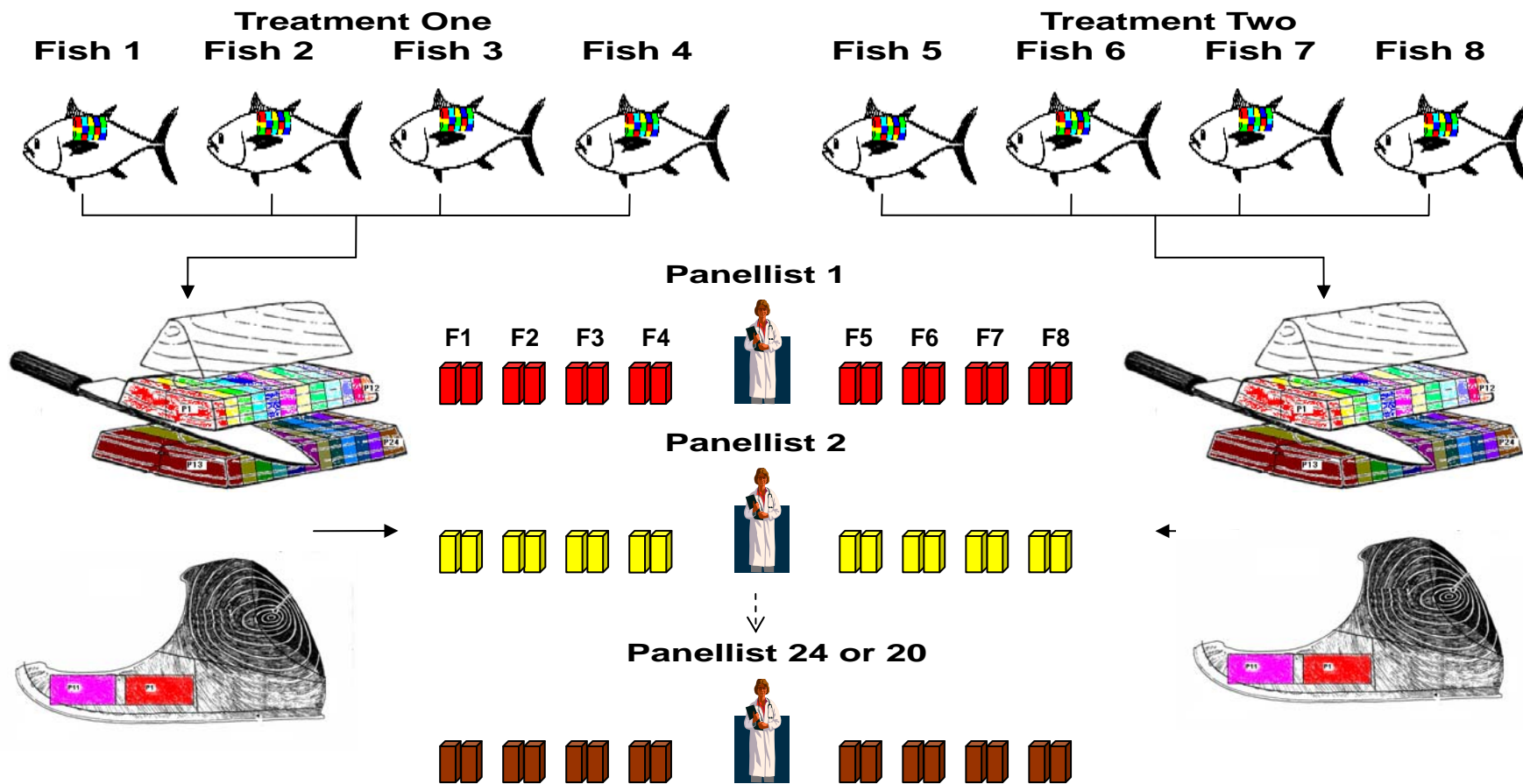


Figure 2.19: Allocation sashimi slices to panellists from a two treatment, four fish per treatment design where each panellists receives to sashimi slices from each fish from either the Akami, Chutoro, or Otoro cut.

○ : Evaluation 1
● : Evaluation 2

○ : Evaluation 1
● : Evaluation 2

○ : Evaluation 1
● : Evaluation 2

Combination No.	Treatment 1				Treatment 2			
	F1	F2	F3	F4	F1	F2	F3	F4
1	○	○	●	●	○	○	●	●
2	○	○	○	○	○	○	○	○
3	○	○	○	○	○	○	○	○
4	○	○	○	○	○	○	○	○
5	○	○	○	○	○	○	○	○
6	○	○	○	○	○	○	○	○
7	○	○	○	○	○	○	○	○
8	○	○	○	○	○	○	○	○
9	○	○	○	○	○	○	○	○
10	○	○	○	○	○	○	○	○
11	○	○	○	○	○	○	○	○
12	○	○	○	○	○	○	○	○
13	○	○	○	○	○	○	○	○
14	○	○	○	○	○	○	○	○
15	○	○	○	○	○	○	○	○
16	○	○	○	○	○	○	○	○
17	○	○	○	○	○	○	○	○
18	○	○	○	○	○	○	○	○
19	○	○	○	○	○	○	○	○
20	○	○	○	○	○	○	○	○
21	○	○	○	○	○	○	○	○
22	○	○	○	○	○	○	○	○
23	○	○	○	○	○	○	○	○
24	○	○	○	○	○	○	○	○
25	○	○	○	○	○	○	○	○
26	○	○	○	○	○	○	○	○
27	○	○	○	○	○	○	○	○
28	○	○	○	○	○	○	○	○
29	○	○	○	○	○	○	○	○
30	○	○	○	○	○	○	○	○
31	○	○	○	○	○	○	○	○
32	○	○	○	○	○	○	○	○
33	○	○	○	○	○	○	○	○
34	○	○	○	○	○	○	○	○
35	○	○	○	○	○	○	○	○
36	○	○	○	○	○	○	○	○

Random No.	Combination No.	Treatment 1				Treatment 2			
		F1	F2	F3	F4	F1	F2	F3	F4
3	1	○	○	●	●	○	○	●	●
1	2	○	○	○	○	○	○	○	○
6	3	○	○	○	○	○	○	○	○
5	4	○	○	○	○	○	○	○	○
2	5	○	○	○	○	○	○	○	○
4	6	○	○	○	○	○	○	○	○
2	7	○	○	○	○	○	○	○	○
5	8	○	○	○	○	○	○	○	○
1	9	○	○	○	○	○	○	○	○
4	10	○	○	○	○	○	○	○	○
3	11	○	○	○	○	○	○	○	○
6	12	○	○	○	○	○	○	○	○
2	13	○	○	○	○	○	○	○	○
3	14	○	○	○	○	○	○	○	○
4	15	○	○	○	○	○	○	○	○
1	16	○	○	○	○	○	○	○	○
6	17	○	○	○	○	○	○	○	○
5	18	○	○	○	○	○	○	○	○
4	19	○	○	○	○	○	○	○	○
1	20	○	○	○	○	○	○	○	○
6	21	○	○	○	○	○	○	○	○
3	22	○	○	○	○	○	○	○	○
5	23	○	○	○	○	○	○	○	○
2	24	○	○	○	○	○	○	○	○
6	25	○	○	○	○	○	○	○	○
4	26	○	○	○	○	○	○	○	○
1	27	○	○	○	○	○	○	○	○
5	28	○	○	○	○	○	○	○	○
3	29	○	○	○	○	○	○	○	○
2	30	○	○	○	○	○	○	○	○
6	31	○	○	○	○	○	○	○	○
3	32	○	○	○	○	○	○	○	○
4	33	○	○	○	○	○	○	○	○
1	34	○	○	○	○	○	○	○	○
2	35	○	○	○	○	○	○	○	○
5	36	○	○	○	○	○	○	○	○

New Random No	Combination No.	Treatment 1				Treatment 2			
		F1	F2	F3	F4	F1	F2	F3	F4
10	1	○	○	●	●	○	○	●	●
22	3	○	○	○	○	○	○	○	○
12	4	○	○	○	○	○	○	○	○
17	6	○	○	○	○	○	○	○	○
3	8	○	○	○	○	○	○	○	○
11	10	○	○	○	○	○	○	○	○
7	11	○	○	○	○	○	○	○	○
8	12	○	○	○	○	○	○	○	○
14	14	○	○	○	○	○	○	○	○
24	15	○	○	○	○	○	○	○	○
18	17	○	○	○	○	○	○	○	○
4	18	○	○	○	○	○	○	○	○
6	19	○	○	○	○	○	○	○	○
23	21	○	○	○	○	○	○	○	○
1	22	○	○	○	○	○	○	○	○
21	23	○	○	○	○	○	○	○	○
19	25	○	○	○	○	○	○	○	○
15	26	○	○	○	○	○	○	○	○
20	28	○	○	○	○	○	○	○	○
5	29	○	○	○	○	○	○	○	○
16	31	○	○	○	○	○	○	○	○
2	32	○	○	○	○	○	○	○	○
9	33	○	○	○	○	○	○	○	○
13	36	○	○	○	○	○	○	○	○


Table 2.3: Combination numbers were ascribed to each of the 36 possible combinations and then further divided into 6 blocks of 6 numbers each (left table). Within the 6 blocks random numbers were ascribed, and the rows numbered 1 and 2 were deleted for each (yellow rows, middle table). New random numbers were ascribed for randomization of subjects within the panel (right table).

○ : Evaluation 1
 ● : Evaluation 2

New Random No	Combination No.	Treatment 1				Treatment 2				Panel No	Saku	Section	Evaluation 1				Evaluation 2			
		F1	F2	F3	F4	F1	F2	F3	F4				T1F1	T1F2	T1F3	T1F4	T2F1	T2F2	T2F3	T2F4
1	22	●	○	○	●	●	○	○	●	P1	Akami	1	T1F2	T1F3	T2F2	T2F3	T1F1	T1F4	T2F1	T2F4
2	32	●	●	○	○	○	●	○	●	P2	Akami	2	T1F3	T1F4	T2F1	T2F3	T1F1	T1F2	T2F2	T2F4
3	8	○	●	○	●	○	●	○	●	P3	Akami	3	T1F1	T1F3	T2F1	T2F3	T1F2	T1F4	T2F2	T2F4
4	18	○	●	●	○	●	●	○	○	P4	Akami	4	T1F1	T1F4	T2F3	T2F4	T1F2	T1F3	T2F1	T2F2
5	29	●	○	●	○	●	○	●	○	P5	Akami	5	T1F2	T1F4	T2F2	T2F4	T1F1	T1F3	T2F1	T2F3
6	19	●	○	○	●	○	○	●	●	P6	Akami	6	T1F2	T1F3	T2F1	T2F2	T1F1	T1F4	T2F3	T2F4
7	11	○	●	○	○	●	○	○	○	P7	Akami	7	T1F1	T1F3	T2F2	T2F4	T1F2	T1F4	T2F1	T2F3
8	12	○	●	○	●	●	○	○	○	P8	Akami	8	T1F1	T1F3	T2F3	T2F4	T1F2	T1F4	T2F1	T2F2
9	33	●	●	○	○	○	●	○	○	P9	Akami	9	T1F3	T1F4	T2F1	T2F4	T1F1	T1F2	T2F2	T2F3
10	1	○	○	●	●	○	○	●	●	P10	Akami	10	T1F1	T1F2	T2F1	T2F2	T1F3	T1F4	T2F3	T2F4
11	10	○	●	○	○	○	○	●	○	P11	Akami	11	T1F1	T1F3	T2F2	T2F3	T1F2	T1F4	T2F1	T2F4
12	4	○	○	●	●	○	○	○	○	P12	Akami	12	T1F1	T1F2	T2F2	T2F3	T1F3	T1F4	T2F1	T2F4
13	36	●	●	○	○	○	●	○	○	P13	Chutoro	13	T1F3	T1F4	T2F3	T2F4	T1F1	T1F2	T2F1	T2F2
14	14	○	●	●	○	○	●	○	○	P14	Chutoro	14	T1F1	T1F4	T2F1	T2F3	T1F2	T1F3	T2F2	T2F4
15	26	●	○	○	○	○	○	○	○	P15	Chutoro	15	T1F2	T1F4	T2F1	T2F3	T1F1	T1F3	T2F2	T2F4
16	31	○	●	○	○	○	○	○	○	P16	Chutoro	16	T1F3	T1F4	T2F1	T2F2	T1F1	T1F2	T2F3	T2F4
17	6	○	○	●	●	○	○	○	○	P17	Chutoro	17	T1F1	T1F2	T2F3	T2F4	T1F3	T1F4	T2F1	T2F2
18	17	○	●	●	○	○	○	○	○	P18	Chutoro	18	T1F1	T1F4	T2F2	T2F4	T1F2	T1F3	T2F1	T2F3
19	25	●	○	○	○	○	○	○	○	P19	Chutoro	19	T1F2	T1F4	T2F1	T2F2	T1F1	T1F3	T2F3	T2F4
20	28	●	○	○	○	○	○	○	○	P20	Chutoro	20	T1F2	T1F4	T2F2	T2F3	T1F1	T1F3	T2F1	T2F4
21	23	○	○	○	○	○	○	○	○	P21	Chutoro	21	T1F2	T1F3	T2F2	T2F4	T1F1	T1F4	T2F1	T2F3
22	3	○	○	○	○	○	○	○	○	P22	Chutoro	22	T1F1	T1F2	T2F1	T2F4	T1F3	T1F4	T2F2	T2F3
23	21	○	○	○	○	○	○	○	○	P23	Chutoro	23	T1F2	T1F3	T2F1	T2F4	T1F1	T1F4	T2F2	T2F3
24	15	○	○	○	○	○	○	○	○	P24	Chutoro	24	T1F1	T1F4	T2F1	T2F4	T1F2	T1F3	T2F2	T2F3

Table 2.4: Combination numbers were sorted according to the new random numbers, and panel numbers, fillet (saku), and sashimi section columns were added (left table). Fish were then colour coded (right table).

Panel No	Saku	Section	Evaluation 1				Evaluation 2			
			1st	2nd	3rd	4th	1st	2nd	3rd	4th
P1	Akami	1	T1F2	T2F3	T2F2	T1F3	T1F4	T2F4	T1F1	T2F1
P2	Akami	2	T2F3	T2F1	T1F3	T1F4	T2F2	T1F1	T2F4	T1F2
P3	Akami	3	T2F1	T1F1	T1F3	T2F3	T2F2	T1F4	T2F4	T1F2
P4	Akami	4	T1F4	T2F4	T1F1	T2F3	T1F2	T1F3	T2F2	T2F1
P5	Akami	5	T1F2	T2F2	T2F4	T1F4	T1F1	T1F3	T2F1	T2F3
P6	Akami	6	T1F3	T1F2	T2F2	T2F1	T2F3	T1F4	T2F4	T1F1
P7	Akami	7	T1F3	T2F2	T2F4	T1F1	T2F3	T2F1	T1F2	T1F4
P8	Akami	8	T2F4	T1F3	T1F1	T2F3	T2F2	T1F2	T1F4	T2F1
P9	Akami	9	T2F4	T2F1	T1F4	T1F3	T1F1	T2F3	T1F2	T2F2
P10	Akami	10	T2F1	T1F1	T1F2	T2F2	T1F3	T2F4	T2F3	T1F4
P11	Akami	11	T2F2	T2F3	T1F1	T1F3	T2F1	T1F4	T1F2	T2F4
P12	Akami	12	T1F2	T2F2	T2F3	T1F1	T2F4	T1F3	T1F4	T2F1
P13	Chutoro	13	T1F3	T1F4	T2F3	T2F4	T2F1	T2F2	T1F2	T1F1
P14	Chutoro	14	T1F1	T1F4	T2F1	T2F3	T1F2	T2F4	T1F3	T2F2
P15	Chutoro	15	T2F3	T2F1	T1F4	T1F2	T2F4	T1F3	T1F1	T2F2
P16	Chutoro	16	T1F3	T2F2	T1F4	T2F1	T2F3	T2F4	T1F1	T1F2
P17	Chutoro	17	T1F1	T2F3	T1F2	T2F4	T2F1	T2F2	T1F4	T1F3
P18	Chutoro	18	T2F2	T1F4	T1F1	T2F4	T1F2	T2F3	T2F1	T1F3
P19	Chutoro	19	T1F2	T1F4	T2F1	T2F2	T1F1	T2F3	T2F4	T1F3
P20	Chutoro	20	T2F2	T2F3	T1F4	T1F2	T1F3	T2F4	T2F1	T1F1
P21	Chutoro	21	T2F2	T1F2	T2F4	T1F3	T1F4	T1F1	T2F3	T2F1
P22	Chutoro	22	T1F1	T2F4	T2F1	T1F2	T2F3	T2F2	T1F3	T1F4
P23	Chutoro	23	T1F3	T2F1	T1F2	T2F4	T1F1	T2F3	T1F4	T2F2
P24	Chutoro	24	T2F1	T2F4	T1F4	T1F1	T1F2	T2F2	T2F3	T1F3



Saku	Section	Evaluation 1				Evaluation 2			
		1st	2nd	3rd	4th	1st	2nd	3rd	4th
Akami	1	812	603	966	188	584	394	522	267
Akami	2	603	267	188	584	966	522	394	812
Akami	3	267	522	188	603	966	584	394	812
Akami	4	584	394	522	603	812	188	966	267
Akami	5	812	966	394	584	522	188	267	603
Akami	6	188	812	966	267	603	584	394	522
Akami	7	188	966	394	522	603	267	812	584
Akami	8	394	188	522	603	966	812	584	267
Akami	9	394	267	584	188	522	603	812	966
Akami	10	267	522	812	966	188	394	603	584
Akami	11	966	603	522	188	267	584	812	394
Akami	12	812	966	603	522	394	188	584	267
Chutoro	13	188	584	603	394	267	966	812	522
Chutoro	14	522	584	267	603	812	394	188	966
Chutoro	15	603	267	584	812	394	188	522	966
Chutoro	16	188	966	584	267	603	394	522	812
Chutoro	17	522	603	812	394	267	966	584	188
Chutoro	18	966	584	522	394	812	603	267	188
Chutoro	19	812	584	267	966	522	603	394	188
Chutoro	20	966	603	584	812	188	394	267	522
Chutoro	21	966	812	394	188	584	522	603	267
Chutoro	22	522	394	267	812	603	966	188	584
Chutoro	23	188	267	812	394	522	603	584	966
Chutoro	24	267	394	584	522	812	966	603	188

Table 2.5: The position of each fish was then randomly assigned (left table) and random three digit codes were then allocated to each fish (right table).

The sashimi fillets (saku) for the Akami and Chutoro were removed and sectioned with panellists 1 to 12 receiving slices of Akami and panellists 13 to 24 receiving slices of Chutoro from the same position from each fish. The Otoro saku was similarly removed and sectioned with panellists 1 to 10 and 11 to 20 receiving the ventral and dorsal sides of the Otoro cut (Fig. 2.20).

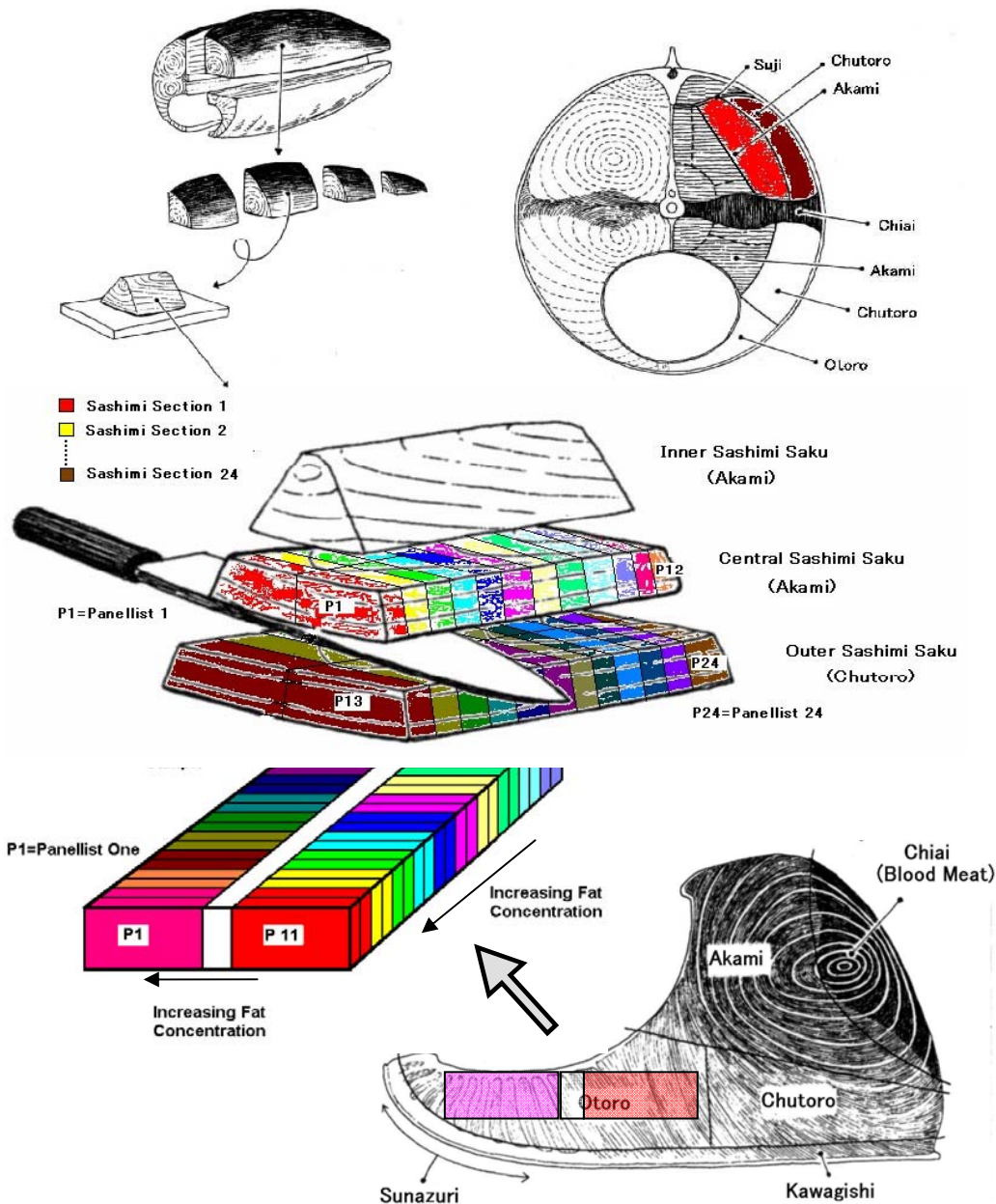


Figure 2.20: Sectioning and position of sashimi slices from the Akami, Chutoro and Otoro

Results & Discussion

Descriptive Analysis – Instrumental Data

Lipid & Moisture

As expected there were significant ($P < 0.05$) differences between the three main cuts of a sashimi tuna carcass in both the lipid and moisture concentrations (Fig 2.21). The Akami, Chutoro, and Otoro contained an average of 1.4, 8.8 and 31.2% of lipid and 70.5, 65.0, and 50.3% moisture respectively. These relative proportions of fat and moisture correlate well to the Japanese nomenclature that describes these cuts – Akami meaning red meat (low fat), Chutoro meaning medium level fat, and Otoro meaning high level fat.

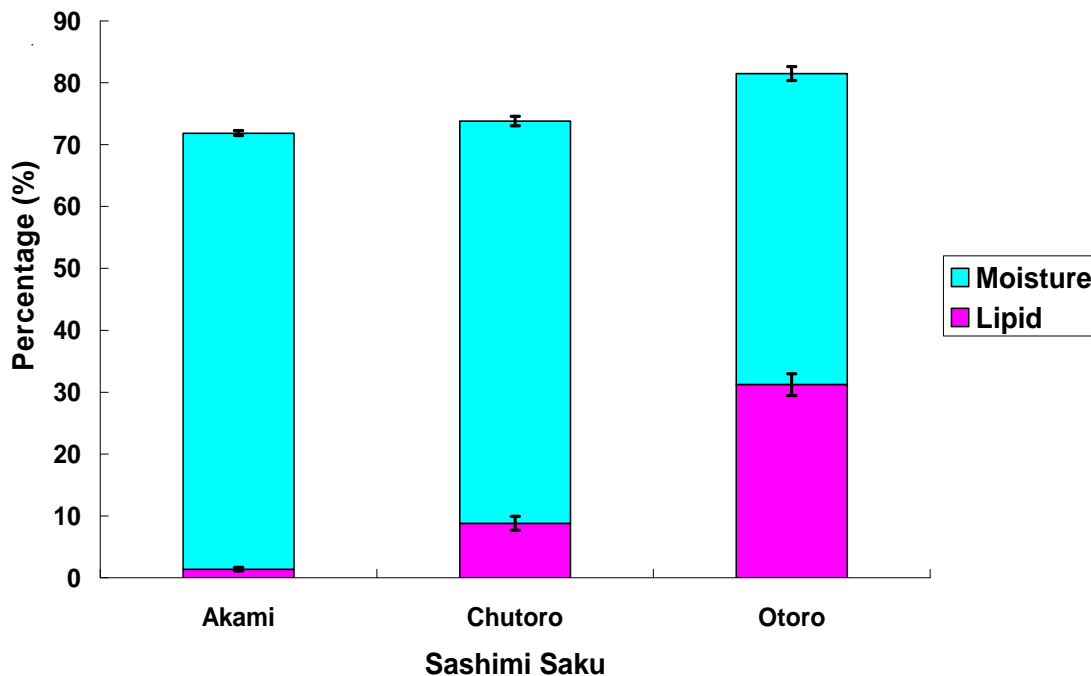


Figure 2.21: Average lipid and moisture concentrations (+/- Std Err) of the three main cuts from the carcasses of farmed Southern bluefin tuna in Japan air-freighted fresh from Australia (n=8; day four post-mortem).

The chemical composition of the Akami fell well within the range for seafood, which according to Jacquot (1961), is similar to terrestrial animals in that 66-84% is water, 15-24% is usually protein, 0.1-22% is lipid. As with other fatty species there is a great deal of variation in the concentrations of fat cells in the different tissues within the body (Borrensens, 1995). The Chutoro is at the extreme ends of the range and the Otoro is outside the normal range.

The chemical composition of aquatic animals however is extremely varied and is impacted upon by the intrinsic factors of genetics, morphology, physiology, or the extrinsic environmental factors related to living, in particular the feeding (Jacquot, 1961). In fish the lipid component is the most variable and according to Jacquot (1961) there are no strict lines of separation between species due to the amount of variation witnessed within a species. Herring, for example, contain between 2% and 22% lipid depending on the time it is caught. In comparison to wild fish however, aquacultured fish exhibit less variation in their chemical composition as the fish farmer has some control over the environment, the feeding rate, the genetic traits of the stock, and/or the composition of the feed (Borrensens, 1995).

The lipid concentrations found in this investigation also correlate well with previous findings for farmed Southern bluefin where the Akami region remains low in lipid for the whole farming season and generally under 5%, the Chutoro was starting its rise

from around 5-10% in the initial months of February and March up to 20-25% during the peak season months of between June and July when water temperatures are at their lowest, and the Otoro is starting to climb from a range of 8-12% to its peak of up to 40-45% during the same period (D'Antignana, unpublished results, 2007). When compared to wild Southern bluefin tuna, where the lipid concentration of the Akami, Chutoro, and Otoro has been reported to range between 0.7~5.4%, 1.6~24.7% and 4.5~32.3% respectively (Matsuda, 1998), the farmed version is on average a far fattier product.

The findings were similar to that of farmed juvenile Northern bluefin tuna in Japan. The dorsal ordinary muscle (incorporating cuts of Akami and Chutoro) of full-cycle cultured Pacific Northern bluefin tuna (*Thunnus orientalis*) contained 70.2% and 71.9% moisture, and 6.3% and 5.6% lipid in pre- and post-fasted individuals respectively. The ventral ordinary muscles (incorporating cuts of Chutoro and Otoro) of the same tuna contained 57.2% and 70.7% moisture and 26.3% and 10.6% lipid in pre- and post-fasted individuals respectively highlighting the large changes in the lipid fraction of the ventral loins relative to the dorsal loins (Nakamura *et al.*, 2006).

With such consistent and significant variations in the concentrations of lipid within a carcass and across a farming season it would be necessary to account for these differences and changes when conducting any sensory testing trial on farmed Southern bluefin.

pH - The terminal pH of the flesh of farmed SBT in Japan ranged from 5.6 to 5.8 with no significant differences recorded between the cuts of Akami, Chutoro, and Otoro (Fig. 2.22). This range of ultimate pH values is consistent with pH values witnessed over the period of a tuna farming season for 285 tuna as measured at the Tokyo Wholesale Markets, Tsukiji, Japan in 2002.

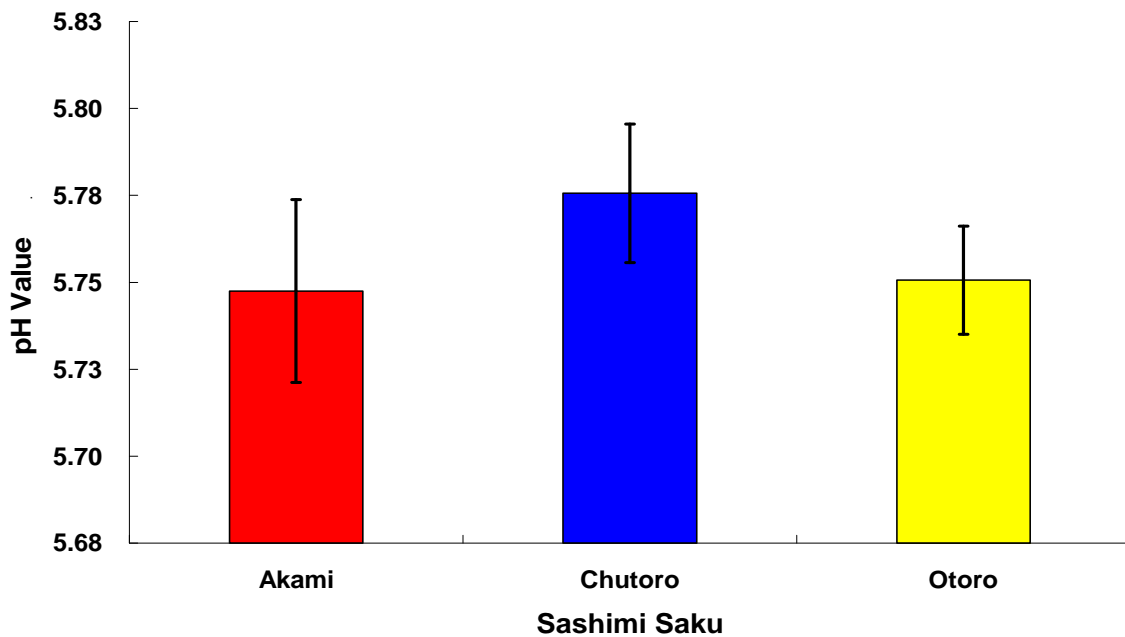


Figure 2.22: The average ultimate pH values (\pm Std Err) of the three main cuts from the carcasses of farmed Southern bluefin tuna in Japan air-freighted fresh from Australia ($n=8$; day four post-mortem).

The pH of tuna muscle decreases gradually after slaughter because of the anaerobic glycolytic pathway in muscle (Nakamura *et al.*, 2006) and the ultimate pH levels found in this investigation are also consistent with the levels reported for other tuna species (pH range of 5.4~5.6), which is lower than species such as cod (pH range of 6.1~6.5), mackerel (pH range of 5.8~6.0), flounder (pH range of 6.5~7 (Gill, 1995; Massa *et al.*, 2005).

Inosine Mono-Phosphate (IMP) & K Value

The concentrations of IMP in the flesh of the carcass differed significantly ($P < 0.05$) between all three cuts (Fig. 2.23). The pattern was a reduction in the concentrations of metabolic nucleotides as one moves from the leaner working muscle (Akami) to the high fat, non-working muscle (Otoro) of the carcass. In contrast, there were no significant differences between the Akami, Chutoro and Otoro in the ratio of the metabolic nucleotides used to form the freshness indicator known as the K value, signifying a similar level of 'freshness' across the carcass. (Fig. 2.25). These levels, however, indicate that the product as a whole is close to reaching its use-by-date for raw consumption (K Value = 20; Ehira, 1976)..

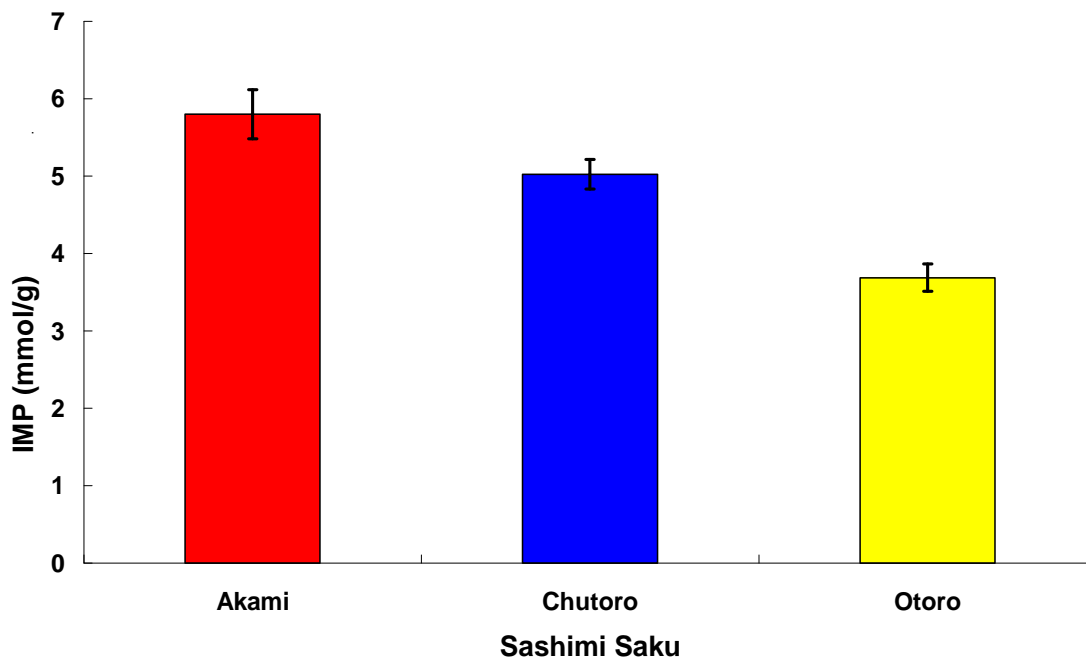


Figure 2.23: IMP concentrations (+/- Std Err) of the three main cuts from the carcasses of farmed Southern bluefin tuna in Japan air-freighted fresh from Australia (n=8; day four post-mortem).

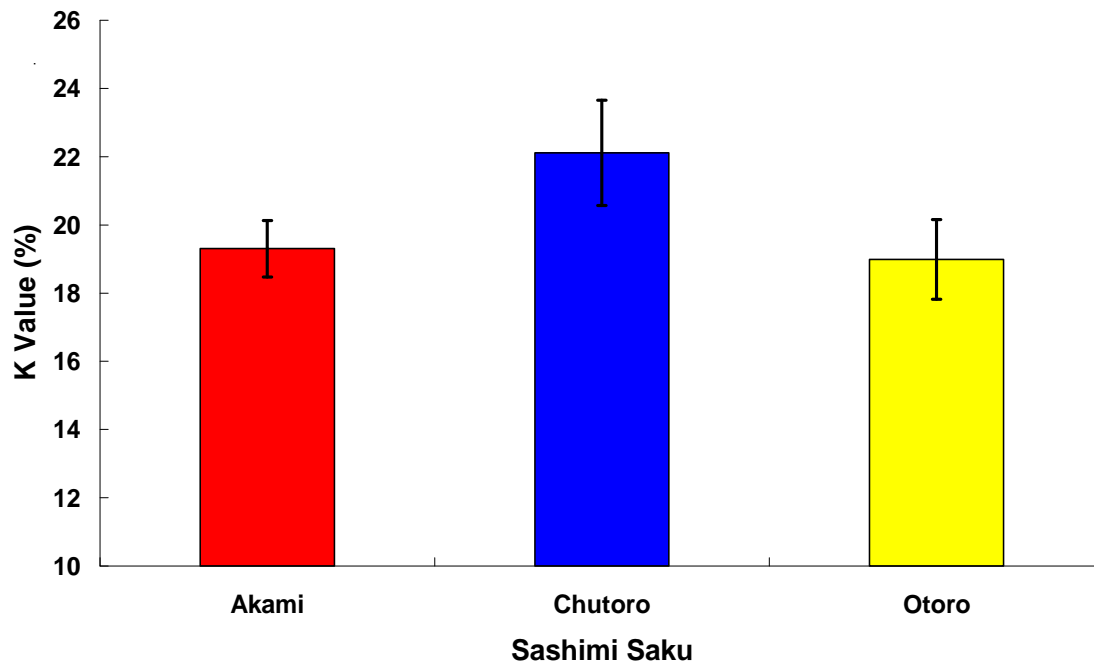


Figure 2.25: K Values (+/- Std Err) of the three main cuts from the carcasses of farmed Southern bluefin tuna in Japan air-freighted fresh from Australia (n=8; day four post-mortem).

In the post-harvest muscle tissue of fish the initial breakdown of ATP normally results in a fast and temporary accumulation of IMP, however the rates and patterns of changes in nucleotides and their related compounds, thus the K values, differ between species, muscle type, and aspects related to handling and storage (Ryder, 1985; Murata & Sakaguchi, 1986; Hattula & Kiesvaara, 1992; Erickson *et al.*, 1997; Guizani *et al.*, 2005; Massa *et al.*, 2005). Overall the IMP concentrations were in the range seen in other species of fish and at day four post-mortem with K Values ranging between 18 and 24 for the three different cuts IMP concentrations were probably in decline. In fresh mackerel (K Value of 9.9) IMP concentrations were

reported to be 10.9 mmol/g (Kuda *et al.*, 2007), a level, according to the authors, reached in raw mackerel one day post-mortem in ice storage. In pink salmon of an average K Value of 28.6, IMP concentrations were found to be 5.33 mmol/g, with Barnett *et al.* (1991) observing IMP levels in premium quality iced pink salmon that ranged between 3.5 and 5.0 mmol/g. In two day post-mortem flounder (*Paralichthys patagonicus*) an IMP concentration of 10.5 ± 0.2 mmol/g was observed to decrease by about 55% during the first 2 days of storage on ice and continued to gradually decline thereafter up to the end of storage (Massa *et al.*, 2005). Ryder (1985) reported gradual declines between 5~3 mmol/g of IMP between days 2~12 post-mortem on ice for Hoki, stating this finding was consistent with jack mackerel (*Trachurus novaezelandiae*), orange roughy (*Hoplostethus atlanticus*).

Sashimi grade Yellowfin tuna 24 hours post mortem recorded a K Value of 15 and, four days later, a K value of 25 (Huang *et al.*, 2006). With starting K values of $17 \pm 4\%$ Guizani *et al.*, (2005) observed a slow but linear rate of increase in the K values ($2.4\%/day$, $r^2 = 0.90$, $p < 0.05$) for Yellowfin tuna stored at 0 °C, and claimed the flesh maintained acceptable shelf life for 12, 5 and 1 day during storage at 0, 8, and 20°C respectively. Mazorra-Manzano *et al.*, (2000) reported a K value of 19.5% for black skipjack two days post mortem and considered the flesh to be sashimi grade during this 48 hour period. However, with struggle stress, storage conditions, and time from capture rarely documented in detail in most of the research conducted into tuna it is difficult to make comparisons between previous findings

and those of the current investigation. This is due to the fact that most investigators can only obtain tuna caught by commercial fisherman. Further, most published data is in Japan and reports on the rates of decline in the K values of frozen tuna that has undergone differing freezing, storage, and/or defrosting methods.

As the K value in dark muscle increases more rapidly than in white muscle due to the catabolic ATP rate (Mazorra-Manzano *et al.*, 2000), the rate of decline of K values may vary between the low lipid, more aerobic Akami cut than the high lipid Otoro cut in tuna white muscle. In the current investigation K values were examined only at day four and examination of the K values of the different cuts after longer periods in storage would be required. Furthermore, no published data in either English or Japanese was found reporting the K values of the fatty portions of bluefin tuna. Moreover, as nucleotide catabolism is arrested during frozen storage, and that more than 70% of Australia's Southern bluefin is frozen, it could be argued (Agustini *et al.*, 2001) that examining K value changes in the Chutoro and Otoro cuts is irrelevant as the quality deterioration of these fatty portions of the tuna carcass would stem from lipid oxidation.

Colorimeter

There were significant ($P < 0.05$) increases in the average L value going from the Akami through to the Otoro (Fig. 2.24). Otoro, being more opaque than the Akami and Chutoro regions reflects more light back into the colorimeter for detection. The

a* values (redness) of the Akami were significantly ($P < 0.05$) lower than those of the Chutoro and the Otoro. This is likely to be an artefact of the greater translucency of the Akami flesh resulting in lower light levels being reflected back to the detector of the colorimeter. The b* values (yellowness) significantly ($P < 0.05$) increased from the Akami to the Otoro.

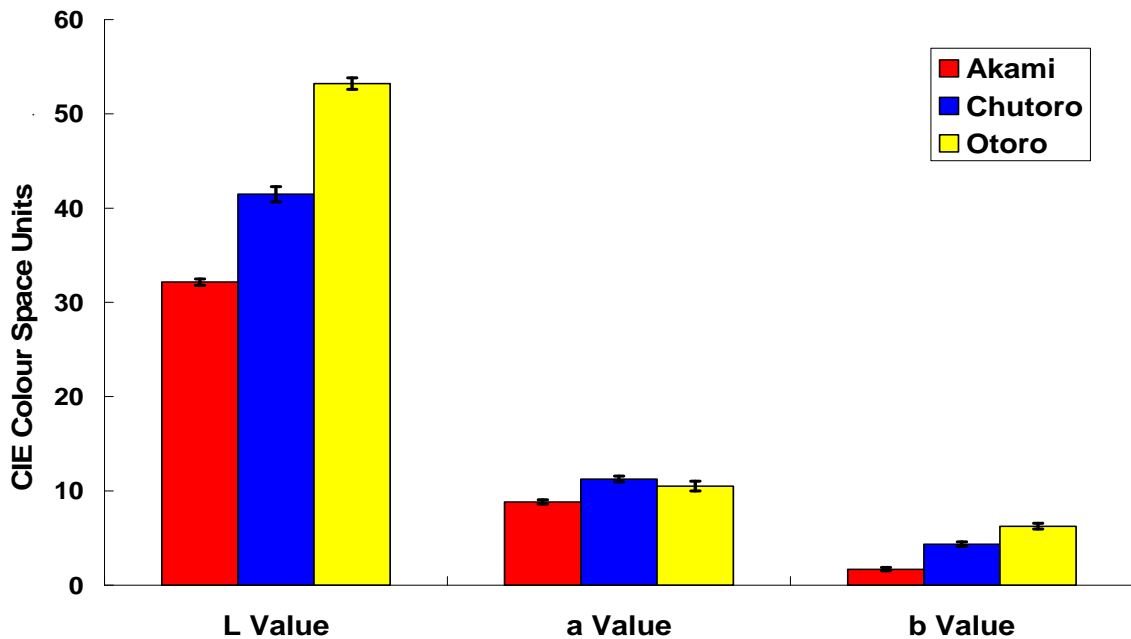


Figure 2.24: CIE Colorimeter L*a*b* values (\pm Std Err) of the three main cuts from the carcasses of farmed Southern bluefin tuna in Japan air-freighted fresh from Australia ($n=8$; day four post-mortem).

The values recorded for L, a*, and b* of 30~55, 8~11, and 1~7 respectively are within the ranges found in other species such as tilapia (43~47, 9~23, 4~8; Li *et al.*, 2008), cod (63~65, -2~-3, ~8; Bjornevik *et al.*, 2003), Atlantic salmon (61~64, 7~10, 6~8; Buttle *et al.*, 2001), rainbow trout (47~55, 1~7, 14~16; Skonberg, 1998), and yellowtail kingfish (55~57, -0.41~1.22, 12~15; Sohn *et al.*, 2005). However, none of these individual species exhibit the range of values recorded here – particularly the L value.

In other tuna species, Yellowfin tuna four days post-mortem recorded L values in the range of 25-33, and a^* and b^* values of 4 and 5 respectively (Huang *et al.*, 2006), and similar to the current investigation's findings for the Akami cut. In pre- and post-fasted, full-cycle farmed Pacific bluefin tuna, Nakamura *et al.* (2006) reported comparable L values in the range of 28.9~35.9 for the dorsal ordinary muscle (Akami-Chutoro), and 42.2~56.2 for the ventral ordinary muscle (Chutoro-Otoro). However, despite similar ranges for the b^* values of the dorsal (0.9~8.7) and ventral (5.2~10.1) muscles of Pacific northern bluefin when compared to the Southern bluefin (1~7) reported here, the a^* values reported by Nakamura *et al.* (2006) at ranges of 30.0~36.1 and 23.2~28.9 for the dorsal and ventral ordinary muscles respectively, were much higher for the Pacific northern bluefin than those recorded here for the Southern bluefin (8~11). An investigation into the CIELAB colour of the derivatives of Northern bluefin tuna myoglobin, the pigment responsible for the red colour of tuna, resulted in a^* values ranging from 43-55 for deoxy-, oxy-, and the carbonyl-myoglobin forms, and 15 for metmyoglobin (Ochiai *et al.*, 1988). Although one could hypothesize that these lower a^* values in Southern bluefin when compared to the Pacific northern bluefin could be due to a higher percentage metmyoglobin content of the Southern bluefin, Nakamura *et al.*, (2006) found percentage metmyoglobin to be higher in three day post mortem samples of the Pacific northern bluefin.

With the colorimeter values significantly different between the various cuts of tuna white muscle it would be necessary to examine all three cuts when investigating treatment effects on the colour properties of the flesh.

Hydroperoxide

The high content of polyunsaturated lipids in fish flesh and its oxidation is a primary cause of quality deterioration (Morrissey *et al.*, 1998; Sohn *et al.*, 2005). The levels of a by-product of lipid breakdown, hydroperoxide, varied significantly ($P < 0.05$) between the three different cuts of a tuna carcass, increasing from the Akami to the Otoro, and correlating to the lipid concentrations of each (Fig. 2.26). In the current investigation a recently developed flow injection analysis (FIA) system coupled with a fluorescence detection system enabled us to determine hydroperoxides in fish muscle at pmole levels during the early stages of lipid oxidation (Sohn *et al.*, 2005b). However, in this early post-mortem period the concentration of hydroperoxides in the lipid fraction of the flesh is believed to be in constant flux, both forming and degrading (Jorgensen, 1995), and therefore it is difficult to comment on whether the levels witnessed here are low, average, or high for each particular cut.

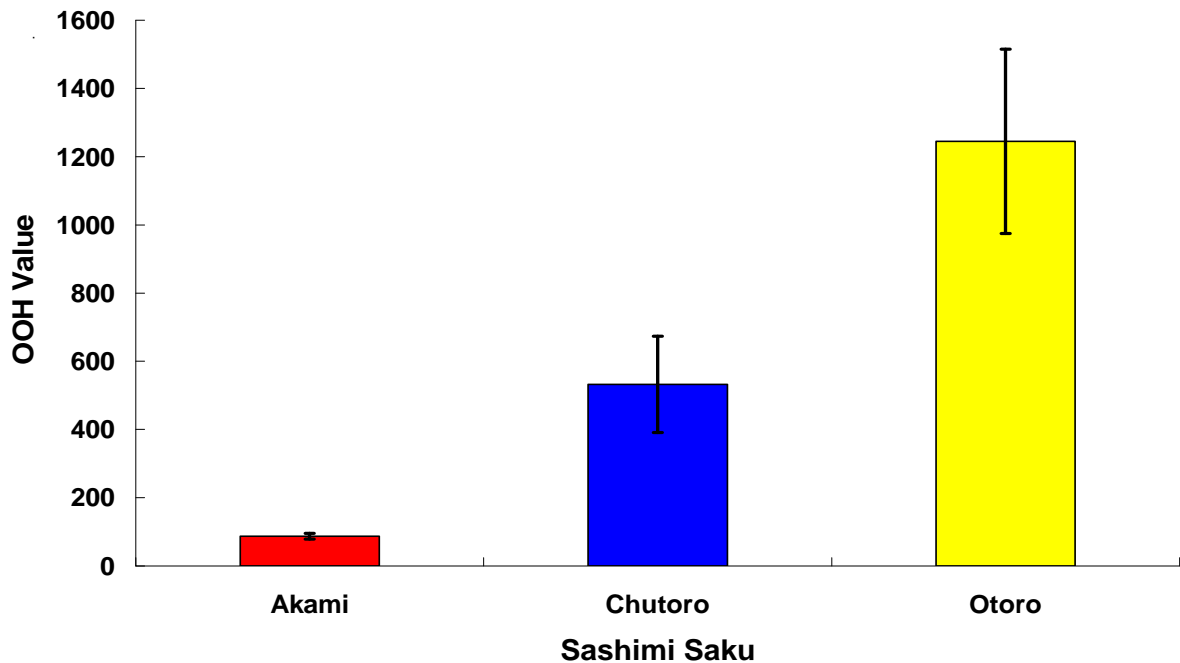


Figure 2.26: Hydroperoxide levels (+/- Std Err) of the three main cuts from the carcasses of farmed Southern bluefin tuna in Japan air-freighted fresh from Australia (n=8; day four post-mortem).

In other species, hydroperoxide levels of minced ordinary muscle measured using the same method were, at day four on ice, reported to be 296, 498, 701, 8076, 8154, 19,084 nmole/5g muscle for yellowtail kingfish, amberjack, Japanese butterfish, Pacific saury, Japanese Spanish Mackerel, and Chub mackerel respectively (Sohn *et al.*, 2005a). The same investigation revealed hydroperoxide levels remained under 350 nmole/5g muscle over two days for the ordinary muscle of yellowtail kingfish, and that the lipid hydroperoxide content in dark muscle was much higher and increased more rapidly when compared to that in ordinary muscle. These results, according to the authors, indicate that lipid hydroperoxide accumulation in the early stage of lipid oxidation differs not only between fish species but also

between ordinary and dark muscle. Further, Ohshima *et al.* (1988) reported that the lipid oxidation in fish muscle was promoted by the autoxidation of myoglobin, suggesting a close relationship between lipid oxidation and myoglobin oxidation. Therefore, with both the myoglobin and lipid concentrations varying greatly between the cuts of Akami, Chutoro, and Otoro in the ordinary muscle of bluefin tuna, it would be necessary to measure these parameters separately when examining treatment effects.

Percentage Metmyoglobin

The colour and metmyoglobin (metMb) content of tuna changes during chilled and frozen storage, and these changes result in the deterioration of meat quality (Nakamura *et al.*, 2006). The percentages of metmyoglobin in the flesh of Southern bluefin five days post-mortem were significantly ($P < 0.05$) different between the Chutoro and Otoro when compared to the Akami (Fig. 2.27) however there was a great deal of variation in the samples percentage ranging from 0~14% in the Akami, 3.5~30% in the Chutoro, and 5~30% in the Otoro cuts. High oxidative pressures and formation of free radicals in the lipid fraction could be triggering the oxidation of the available myoglobin to metmyoglobin in the Chutoro and Otoro cut.

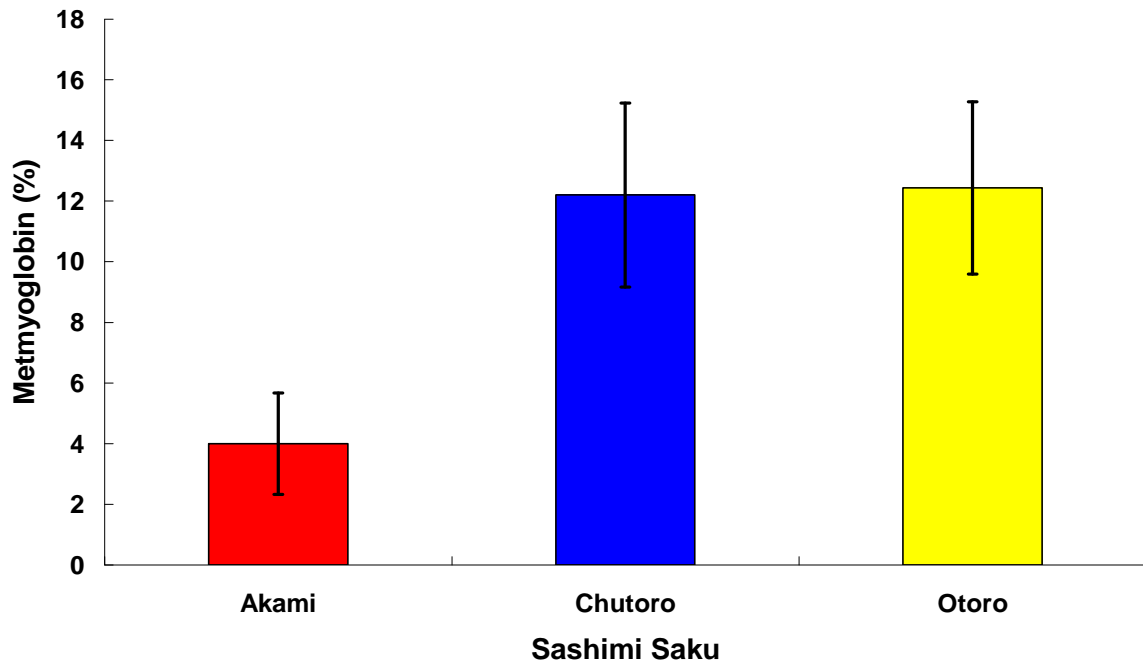


Figure 2.27: Percentages of metmyoglobin (+/- Std Err) in the three main cuts from the carcasses of farmed Southern bluefin tuna in Japan air-freighted fresh from Australia (n=8; day four post-mortem).

In other species, metmyoglobin percentages in the ordinary muscles of Yellowtail kingfish, were reported to be 4.6% at 0 hours, increasing to 30.9% after 48 hours in ice storage (Sohn et al., 2005). In farmed Pacific northern bluefin tuna, percentage metmyoglobin content increased rapidly within the first 24 hours in chilled conditions, and, ranging between 12~38% in the dorsal ordinary muscle (Akami/Chutoro) after 57~81 hours, was higher than reported here for the Akami/Chutoro of farmed Southern bluefin four days post-mortem. In a similar trend, the percentage metmyoglobin in the ventral ordinary muscle (Chutoro/Otoro) of farmed Pacific northern bluefin at 18~28% after 57~81 hours of storage was higher

on average than the percentage metmyoglobin of Southern bluefin after four days post-mortem (Nakamura *et al.*, 2006). As the rate of metmyoglobin formation increases with an increase in storage temperatures, and without the exact storage temperature conditions of the Pacific northern bluefin, as well as the average carcass temperatures at the time of death, it is not possible to make clear comparisons between the two tuna species.

RGB Ratio

Significant differences ($P < 0.05$) between the Akami, Chutoro and Otoro cuts of the carcass for the RGB ratio were recorded (Fig. 2.28). The ratio is a measure of the digitally recorded red value averaged over a 5 by 5 (25) pixel area of the flesh relative to the sum of the other two primaries (green and blue). The increasing adipose tissue and decreasing muscle tissue moving from the Akami to the Otoro cuts, resulted in significantly higher RGB ratios in the cuts with greater concentrations of muscle fibres that contain the red-coloured pigment myoglobin as expected.

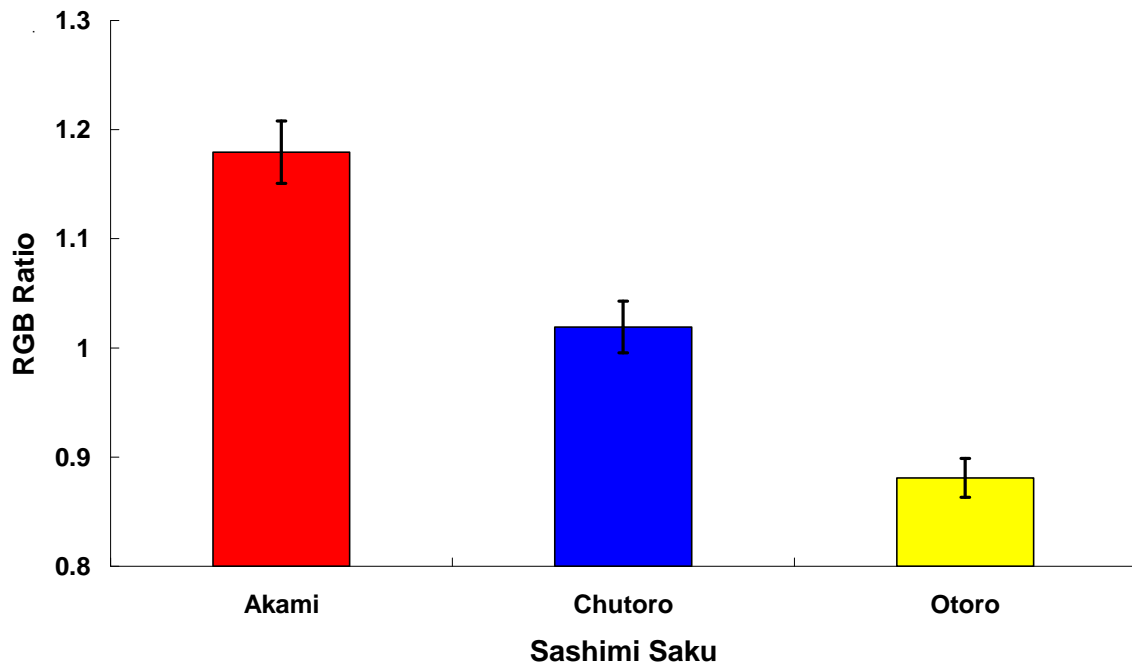


Figure 2.28: RGB ratios (+/- Std Err) of the three main cuts from the carcasses of farmed Southern bluefin tuna in Japan air-freighted fresh from Australia (n=8; day four post-mortem).

Lactate

Significantly ($P < 0.05$) higher levels of lactate in the Akami and Chutoro cuts when compared to the Otoro is a trend expected from the greater breakdown of glycogen in these working muscles (Fig. 2.29).

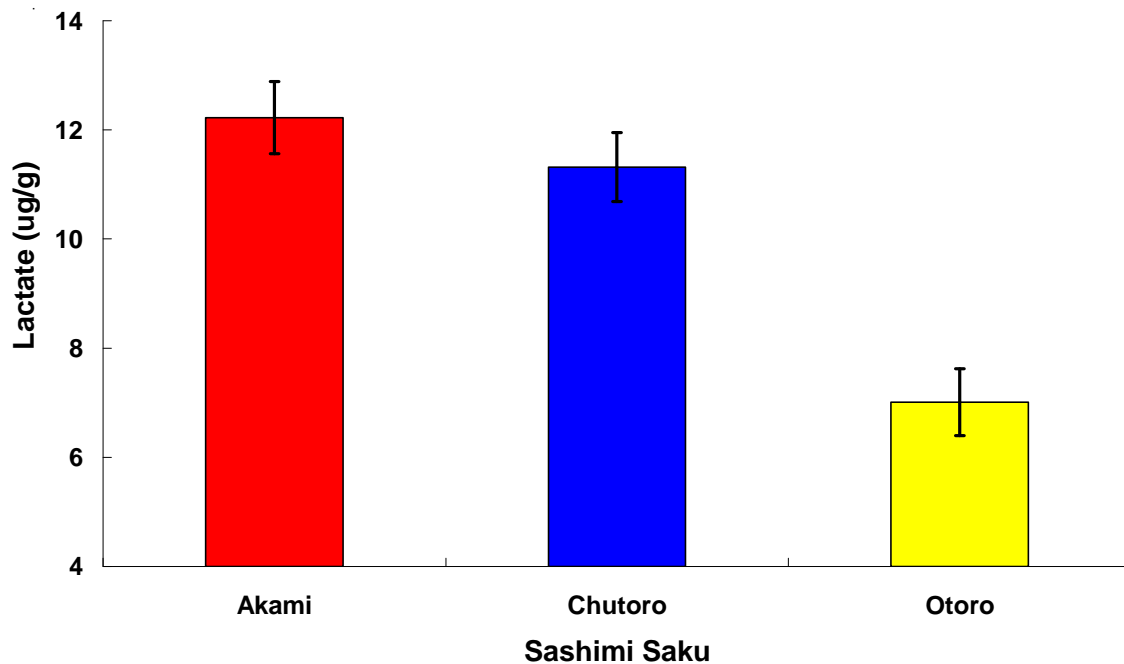


Figure 2.29: Lactate concentration (+/- Std Err) in the three main cuts from the carcasses of farmed Southern bluefin tuna in Japan air-freighted fresh from Australia (n=8; day four post-mortem).

When compared to other species the muscle lactate concentrations are low. Trout that were strenuously exercised for only two minutes recorded an increase in muscle lactate concentrations of 7 to 25 $\mu\text{moles/g}$, and reached a plateau of 32 $\mu\text{moles/g}$ after only seven minutes (Driedzic & Hochachka, 1978). Thomas *et al.* (1999) reported muscle lactate levels of between 17.1 ~25.6 $\mu\text{moles/g}$ in exercised and stressed Atlantic salmon, and in unstressed Atlantic salmon Einen & Thomassen (1998) recorded an average muscle lactate concentration of 5.6 $\mu\text{moles/g}$. In catfish, lactate levels have ranged between 8~20 $\mu\text{moles/g}$ in

normoxic and hypoxic conditions (MacCormack *et al.*, 2006), and in flounder muscle lactate ranged between 20~35 μ moles/g (Barnett & Pankhurst, 1998). At the extreme, Skipjack tuna chased to exhaustion recorded some of the highest concentrations of lactate in all vertebrates at 100-150 μ moles/g of lactate in the white muscle and (Brill, 1996).

The high aerobic enzyme activities (e.g. citrate synthase and α -glycerophosphate dehydrogenase) of tuna white muscle, combined with an exceptionally high specific activity of lactate dehydrogenase (LDH) when compared to ectothermic scombrid fishes and other teleosts, facilitates rapid lactate clearance and recovery of blood lactate concentrations to normal levels following maximal burst activity in a time-frame that is one-fourth to one-twelfth of that recorded for other teleosts (Bushnell & Jones, 1994; Dickson, 1996; Weber & Haman, 1996; Korsmeyer *et al.*, 1996; Mathieu-Costello, 1996).

In the present study, despite significantly higher concentrations of muscle lactate in the working Akami and Chutoro cuts when compared to the comparatively non-working Otoro cut, this trend was not mirrored by the values of ultimate pH for the different cuts. The lower concentrations of lactate in the Otoro cut may have resulted from its oxidation by lipid hydroxide molecules, which are in high concentration in this high-lipid muscle block, or due to lower levels of glycolytic activity (Driedzic & Hochachka, 1978).

Although the three different cuts of tuna white muscle possess different concentrations of the above measured biochemical indicators of flesh quality there is a consistency of metabolic function. Taking into account the moisture levels of 70.0, 65.0 and 50.3% and fat levels of 1.4, 8.8 and 31.2%, the fluid contents/100g total 71.4, 73.8 and 81.5g for each of the three cuts of Akami, Chutoro, and Otoro respectively. As a result, total solids, or dry matter, for each cut equate to 28.6, 26.2 and 18.5 g/100g. Expressing IMP content on a solids basis (5.8mmol IMP/0.286, 5.1 mmol IMP/0.262 and 3.7mmol IMP/0.185g solids), gives 20.2mmol IMP/g of Akami solids, 19.5 mmol IMP/g of Chutoro solids and 19.9 mmol/g of Otoro solids respectively. Further, expressing total nucleotides and lactate concentrations on a solids basis (Table 2.6), reveals that the structural and functioning parts of the three differing cuts that make up tuna white muscle have similar metabolic activities and produce lactate at a similar rate.

Metabolite	Cut	Wet weight basis	Fat-free dry weight basis
IMP mmol/g	Akami	5.8	20.2
	Chutoro	5.1	19.5
	Otoro	3.7	19.9
Total Nukes mmol/g	Akami	7.7	26.8
	Chutoro	7.0	26.7
	Otoro	4.9	26.6
Lactate µg/g	Akami	12.2	42.7
	Chutoro	11.3	43.2
	Otoro	7.0	37.9

Table 2.6: Inosine monophosphate, total nucleotides, and lactate concentrations expressed on a wet weight basis and fat-free dry weight basis for the three cuts of Akami, Chutoro, and Otoro (n=8; day four post-mortem).

If a similar trend exists with other quality related biochemicals, such as total myoglobin, it would validate using any of the three tissues as indicators of redness in any of the other tissues and may justify using the tail cut as the test muscle for all three cuts. There is also greater certainty that time/temperature, and hence storage effects, are proceeding uniformly in these important tissues.

Descriptive Analysis – Sensory Data

Appearance & Odour: Of the sensory descriptors of appearance for the three cuts of the carcass, statistically significant ($P < 0.10$)* differences were evident between the Akami and the Chutoro and Otoro cuts for redness, brightness, and transparency (Fig. 2.30). No significant differences ($P > 0.10$) were detected between the panels for the cuts of Chutoro and Otoro. The higher levels of the colour pigment myoglobin in the Akami results in flesh with a redder and brighter appearance. No significant differences ($P > 0.10$) were evident between the panel scores for Akami, Chutoro and Otoro for bad fishy odour, and as all cuts recorded low values, it indicates little and uniform deterioration five days post-harvest for this sensory descriptor across the carcass.

Flavour and Mouth Feel: No significant differences ($P > 0.10$) between the panel's scores for bad fishy flavour and umami of the Akami, Chutoro, and Otoro, indicating consistency across the carcass for these quality traits (Fig. 2.31). In contrast, the sensory descriptor of sourness scored significantly different averages between all

three cuts of Akami, Chutoro, and Otoro ($P < 0.10$), and probably related to the higher concentration of lactate with the working muscles (Fig. 2.29). The panel's scores for the mouth feel descriptor of fattiness was significantly ($P < 0.10$) higher for the high lipid Otoro when compared to the medium- and low-lipid cuts of Akami and Chutoro respectively. Conversely, the low lipid Akami was scored significantly ($P < 0.10$) harder in texture when compared to the cuts of Chutoro and Otoro.

*Given the low sample and panellist numbers, a heterogeneous testing material, and attempts to avoid a type II error, an alpha level of 0.10 was used for the sensory data (see Bower, 1995; Munoz, 2004).

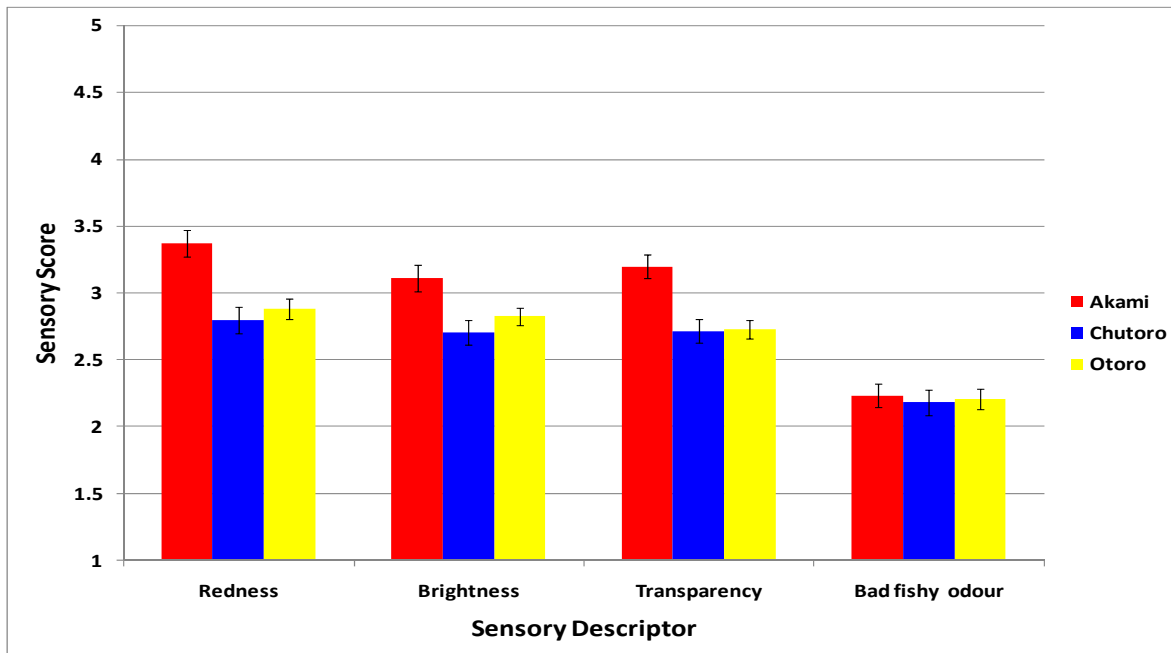


Figure 2.30: Average sensory scores (\pm Std Err) of redness, brightness, transparency and bad fishy odour for the three main cuts from the carcasses of farmed Southern bluefin tuna in Japan air-freighted fresh from Australia ($n=8$; day four post-mortem).

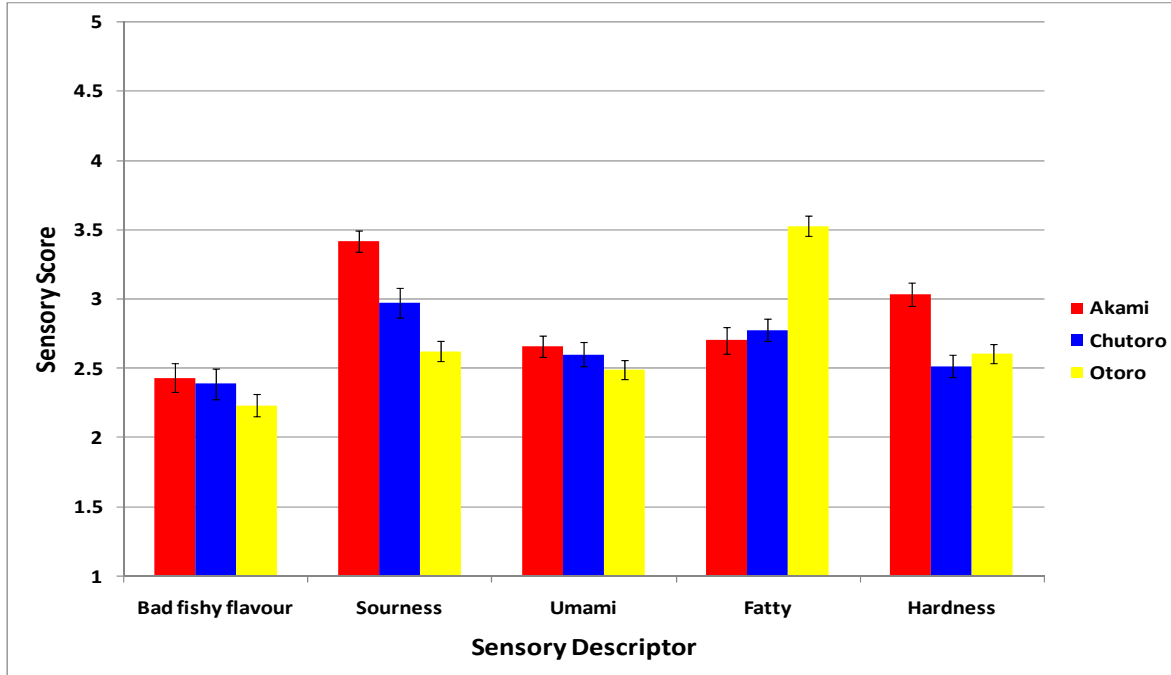


Figure 2.31: Average sensory scores (+/- Std Err) of bad fishy flavour, sourness, umami, fattiness, and hardness for the three main cuts from the carcasses of farmed Southern bluefin tuna in Japan air-freighted fresh from Australia (n=8; day four post-mortem).

Multivariate Analysis of the Instrumental and Sensory Data

Prior to multivariate analysis of the instrumental and sensory data both sets were subjected to data exploration to investigate the normality (skewness and kurtosis) of the data and determine whether data transformations were necessary. All of the sensory variables were normally distributed, however, some instrumental variables returned significant Shapiro-Wilks statistics (L Value, metmyoglobin, K Value, fat, moisture, and hydroperoxide). Of these non-normally distributed instrumental variables the hydroperoxide data was of the most concern due to the units of measurement and the intra-cut variation for the Akami, Chutoro, and Otoro. Examination of the Q-Q plots (Fig. 2.32), normality statistics (Table 2.7), and

skewness and kurtosis statistics confirmed that a log 10 transformation was most appropriate for the hydroperoxide data.

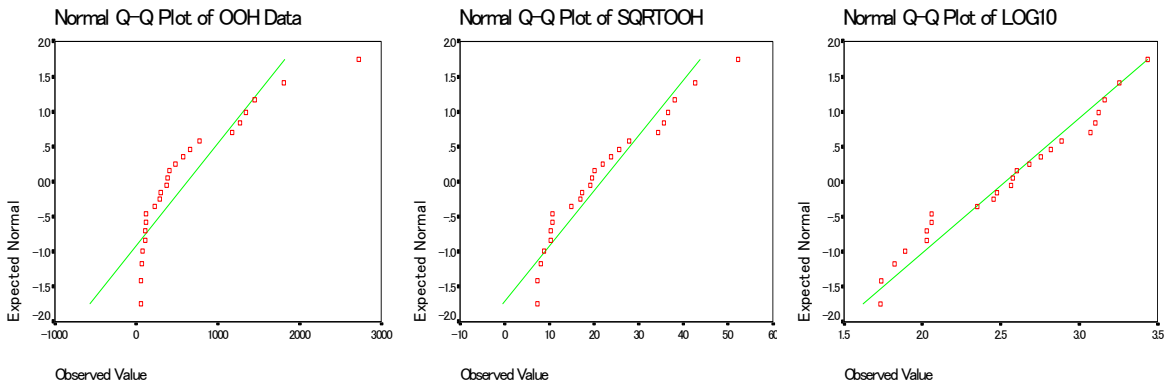


Figure 2.32: Q-Q normality plots for untransformed (left), square root transformed (middle) and log 10 transformed hydroperoxide data (right).

Transformation	Kolmogorov-Smirnov (a)			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Untransformed	.210	24	.008	.798	24	.000
Square Root	.142	24	.200(*)	.912	24	.039
Log 10	.150	24	.175	.949	24	.264

Table 2.7: Normality statistics for untransformed, square root transformed and log 10 transformed hydroperoxide data (* This is a lower bound of the true significance; a: Lilliefors Significance Correction)

Instrumental and sensory data were subject to principal components analysis (PCA; *Unscrambler 7.8*) and two dimensional scatter plots for the main principal components (PCs 1 & 2) together with X-loadings were produced (Fig. 2.33 & 2.34). These bi-plots enable simultaneous interpretation of sample properties (scores) and variable relationships (loadings). The closer together samples lie in the scatter plot, the more similar they are with respect to the two components concerned.

Conversely, samples far away from each other are different from each other. The loadings show the importance of the different variables for the two components specified. Variables with loadings to the right in the plot will be variables which usually have high values for samples to the right in the score plot. Therefore, samples lying in an extreme position in the same direction as a given variable have large values for that variable; samples lying in the opposite direction have low values.

The two dimensional bi-plot of the instrumental data revealed clustering of the samples into the three main cuts of Akami (samples 1A-8A), Chutoro (samples 1C-8C), and Otoro (samples 1OT-8OT) (Fig. 2.33). Further, as one would expect the high fat-associated variables such as percentage lipid (Fat), hydroperoxide concentration (OOH), lightness (L-Value), and yellowness (b Value) are clustering toward the high-fat Otoro samples. And conversely, the low fat (Moisture), red colour (RGB Ratio), and working muscle (IMP and Lactate) associated variables cluster toward the low-fat Akami samples.

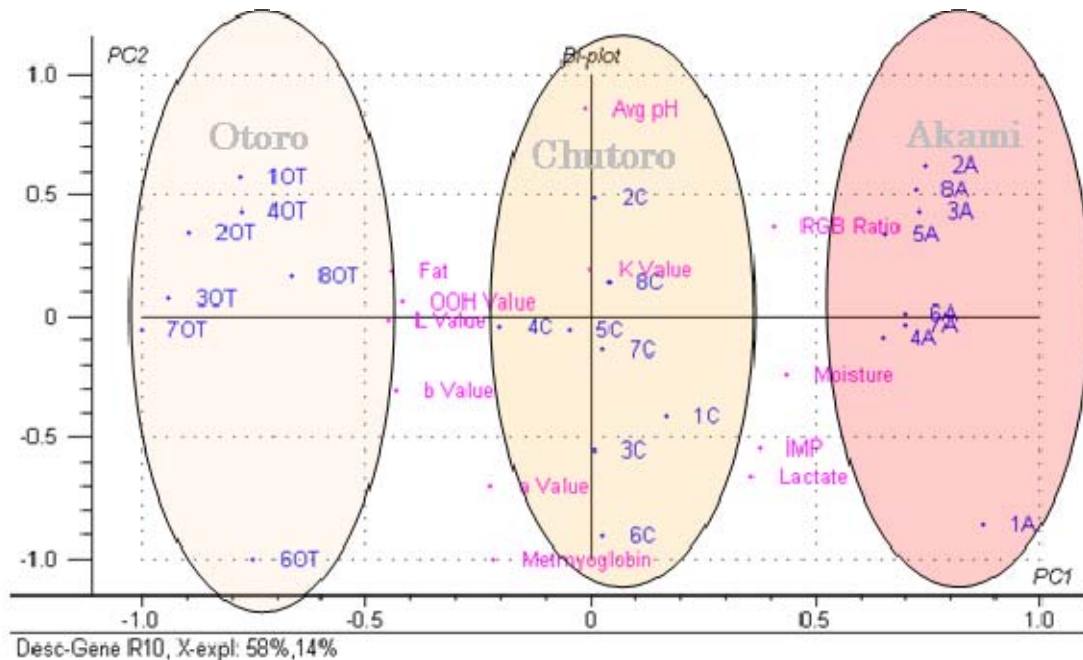


Figure 2.33: Two-Dimensional scatter bi-plot of the loadings of instrumental variables and the scores of Akami, Chuturo, and Otoro flesh samples taken from fresh, farmed Southern bluefin from Port Lincoln, Australia in Tokyo (n=8; day four post-mortem).

The two dimensional bi-plot of the sensory data also revealed clustering of the samples into the three main cuts of Akami, Chuturo, and Otoro (Fig. 2.34). Further, fat-associated variables such as fatty, bad fishy odour and bad fishy flavour are clustering toward the Otoro samples. And conversely, the characteristics of Hardness, Transparency, Redness and Brightness, Sourness associated cluster toward the low-fat Akami samples.

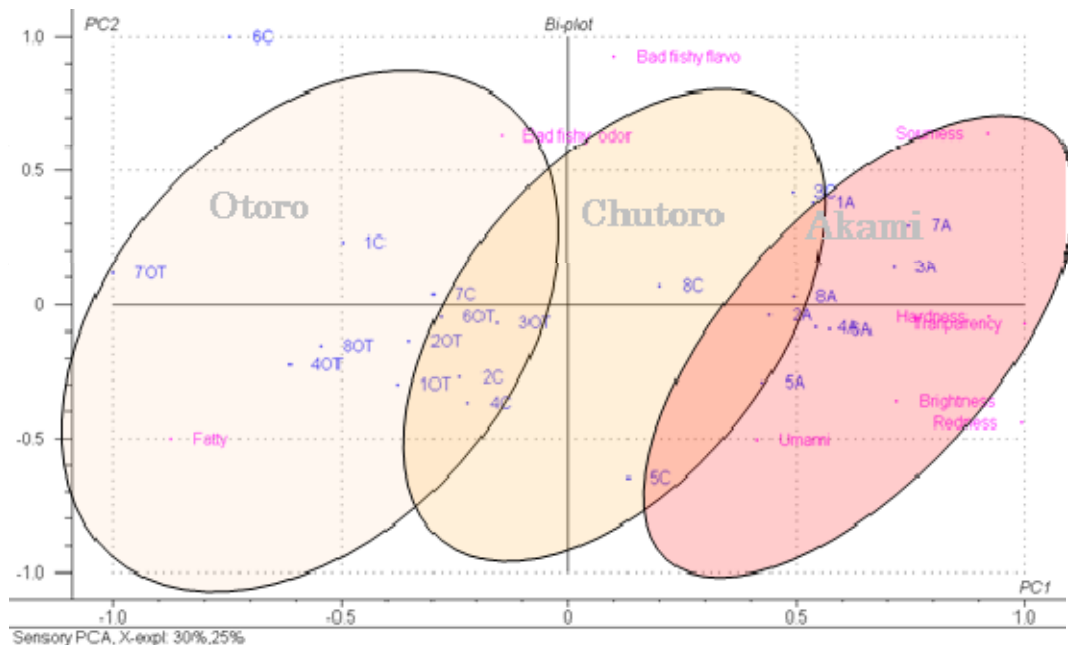


Figure 2.34: Two-Dimensional scatter bi-plot of the loadings of sensory variables and the scores of Akami, Chutoro, and Otoro flesh samples taken from fresh, farmed Southern bluefin from Port Lincoln, Australia in Tokyo (n=8; day four post-mortem).

In order to investigate links and understand the relationships between the instrumental (predictor X) and sensory (response Y) variables the data was analysed by partial least squares (PLSR; *Unscrambler 7.8*) multivariate regression with a $1/S_{dev}$ weighting of predictor variables, and the results presented in a two dimensional scatter plot of X-loading weights and Y-loadings for two main principal components (Fig. 2.35). Predictors projected in roughly the same direction from the centre as a response are positively linked to that response, and conversely, predictors in the opposite direction are negatively linked. Predictors close to the centre are not well represented and cannot be interpreted from those variables.

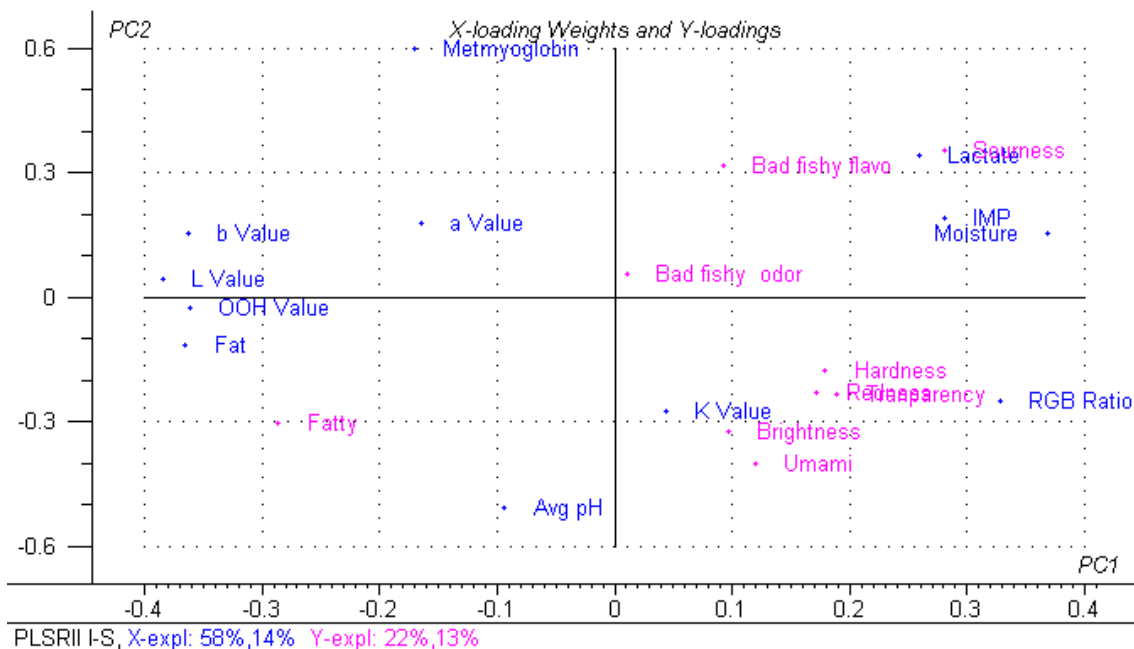


Figure 2.35: Two-Dimensional scatter plot of X-loading weights and Y-loadings of regressed (PLSR) instrumental and sensory data of Akami, Chutoro, and Otoro flesh samples taken from fresh, farmed Southern bluefin from Port Lincoln, Australia in Tokyo (n=8; day four post-mortem)

The instrumental measures associated to fattiness (fat; hydroperoxide (OOH); L value; and b value) are closely and positively linked to the sensory measure of fatty. These variables are negatively associated with the instrumental variables (moisture, inosine monophosphate, RGB ratio, and lactate) and sensory variables (sourness, hardness, transparency, brightness, redness, umami, and bad fishy flavour). Bad fishy odour, being in the centre of the plot is not well represented and cannot be interpreted. The pH values of the flesh and metmyoglobin concentrations are positioned opposite one another and this is consistent with reductions in pH of the flesh increasing the oxidation of myoglobin to metmyoglobin (George & Stratmann, 1954).

PLS scores, represented as T-Scores and U-Scores, of the instrumental and sensory data for the Akami, Chutoro, and Otoro are a summary of the relationship between X (predictor - instrumental) and Y (response - sensory) along a specific model component. T-Scores, which are the new coordinates of the data points in the X-space, are computed in a way that capture part of the structure in X which is most predictive of Y. U-Scores summarize the part of the structure in Y which is explained by X along a given model component. Using the main principal component the data again show clustering in the Akami, Chutoro, and Otoro sections (Fig. 2.36).

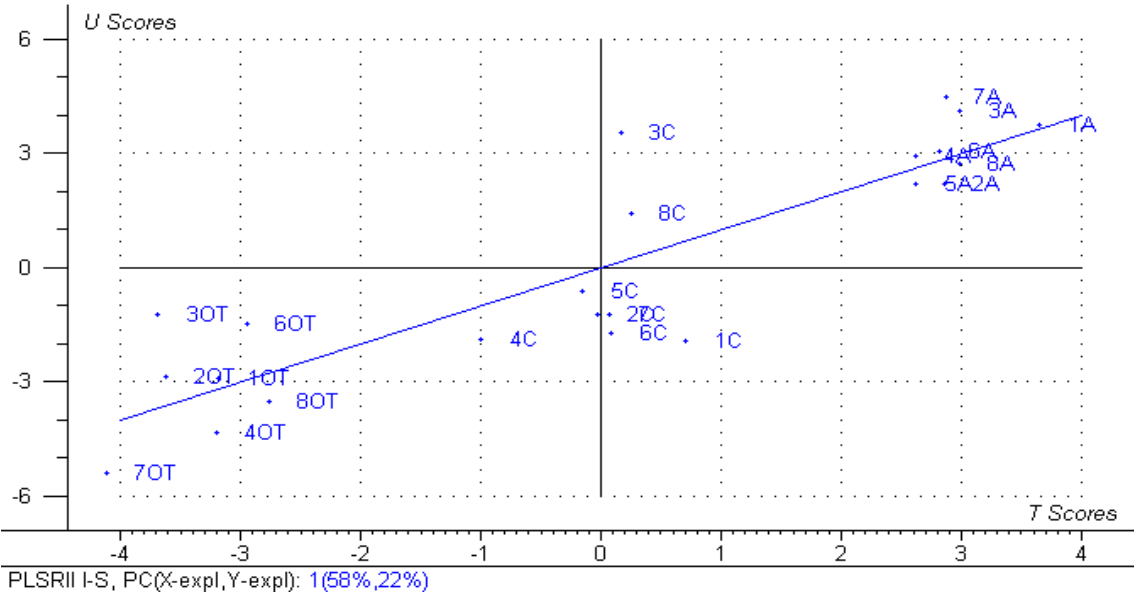


Figure 2.36: U-Scores and T-Scores plot of regressed (PLSR) instrumental and sensory data of Akami, Chutoro, and Otoro flesh samples taken from fresh, farmed Southern bluefin from Port Lincoln, Australia in Tokyo (n=8; day four post-mortem)

The predictive capacity of the instrumental variables to the sensory response variables is too low (E-explained=58%; Y-explained=22%) for practical application

and is greatly influenced by the differing physico-chemical and sensory qualities between the cuts of Akami, Chutoro, and Otoro within the carcass of a tuna. As a result any predictive modelling would require separate examination of these cuts. Following this, predictors with a large regression coefficient and that play an important role in the regression model could be identified, and conversely, predictors with a small coefficient are negligible and the model could be recalculated without these variables.

To improve predictive capacities more sample numbers would be required for each cut of Akami, Chutoro, and Otoro. Using the same experimental design and number of panellists, it would be possible to raise the sample number from eight to twenty-four fish and examine only one of the three cuts. Alternatively, multiple trials examining all three cuts could be conducted over time and the data pooled and the cuts modelled separately. However the potential of missing a quality-related issue in an unexamined cut (e.g. tuna burn in the Akami etc.) or introducing unintentional procedural variation increases with either approach. Therefore it would be preferable to run further trials to gather more sensory and physico-chemical data over the course of a season making it possible to profile the product and the build predictive models. Despite the lack of statistical sensitivity, a balanced experimental design and procedure was developed for the sensory analysis of Southern bluefin tuna - a very useful tool when examining the affects of any on-farm or in-chain manipulation on the sensory qualities of the flesh.

Conclusions

This investigation reports on the first full-carcass, statistically balanced experimental design for the sensory analysis of a bluefin tuna. The method describes the steps and considerations involved from the carcass level to the sashimi slice at the testing booth, and can be adapted to examine larger species of bluefin such as the Pacific and Atlantic northern bluefin.

Probably more than any other species of fish, the white muscle of bluefin tunas, as shown here with farmed Southern bluefin tuna, reveal extreme variation in the proximate composition, and therefore the physicochemical parameters of the three commercial cuts of Akami, Chutoro, and Otoro used for sashimi. Although a consistency in the metabolic function was revealed in the biochemical indicators when examined here on a solids basis, any investigation into the physical properties or sensory qualities of the carcass would require an examination of all three cuts.

However, with this requirement to examine all three cuts within the carcass of a tuna in order not overlook a potentially negative effect of a particular treatment on the quality of the end product, resource requirements to uphold statistical validity increase significantly. To counter this, and to be able to model and predict the sensory qualities of the flesh using physicochemical indicators, multiple investigations throughout the course of the season would be required.

3. VITAMIN SUPPLEMENTATION & THE FLESH QUALITY OF FARMED, FRESH SOUTHERN BLUEFIN TUNA IN PORT LINCOLN & JAPAN

3.1 Introduction

The visual appearance of food is a critical factor in the purchase decision of consumers (Mackinney *et al.* 1966), especially in those products that are to be consumed in the state in which they are purchased. The colour, patterning (visible fat), and lustre of muscle foods are used by consumers as quality and freshness indicators, and therefore influence purchase propensities (Jeremiah *et al.* 1972; Hood & Riordan, 1973; Robbins *et al.* 2003a). The colours of foods have also been shown to influence human appetite, with hues in the red-orange region of the spectrum seeming to, according to Birren (1963), 'arouse the most agreeable sensations'. Bolten *et al.* (1967) stated that consumer demands and expectations regarding the colour and appearance of food commodities are strongly based in culture. In Japan, a country where people are said to "eat with their eyes", the main non-price evaluation criteria for product quality in foods are freshness and taste. In a survey conducted by Sanwa Bank, 88% of the respondents claimed that freshness, ahead of price, was an important criterion for food product selection (U.S. Department of Commerce, 1999).

The colour and appearance of a muscle food, and therefore the freshness and quality indicators for a consumer, are related to the concentration and chemical state of pigments, the physical properties of the flesh, such as the degree of light-scattering, and the levels of intramuscular fat (Renerre, 1990). Of these factors, it is the relative amounts of the three derivatives of a globular haem protein known as myoglobin that primarily determine the surface colour of most muscle foods. Reduced myoglobin (deoxymyoglobin) is purplish in colour and typical of a freshly cut surface; myoglobin, which forms oxymyoglobin in the presence of air, is responsible for the characteristic red colour of meat; and metmyoglobin, which occurs when oxygen binds to the ferric derivative of myoglobin, is brown in colour (Hood & Riordan, 1973). The pre-mortem period, method of euthanasia, and intrinsic (pH, muscle type, animal, age, breed, sex, diet) and extrinsic (temperature, oxygen availability, lighting, microbial growth, storage) post-mortem factors can all affect the colour and the rate of colour deterioration in muscle foods (Kraft & Ayres, 1954; El-Badawi *et al.* 1964; Kopf, 1980; Renerre, 1990 & 2000).

The redox reaction rates which form oxy- and metmyoglobin depend upon the extent of oxygen consumption, myoglobin autoxidation, the presence and concentration of oxidizable lipids, transition metal ions, and the various enzymes that act as either pre-cursors or as catalysts for the production of free radicals in muscle tissue (Xiong, 2000). Protein oxidation is similar to the process of lipid oxidation and involves initiation, propagation, and termination type free radical

chain reactions that lead to the formation of undesirable protein free radicals, polymers, and protein-lipid complexes (Xiong, 2000). The autoxidation of myoglobin occurs through a combination of two mechanisms. At high oxygen concentrations direct dissociation of the neutral superoxide radical from oxymyoglobin dominates, with the reaction catalysed by decreasing pH. At low oxygen concentrations autoxidation occurs by a bimolecular reaction between molecular oxygen and deoxymyoglobin containing a weakly coordinated water molecule (Brantley *et al.* 1993).

In the presence of lipids, reactive oxygen species promote a cascade of chemical reactions that form not only free radicals but also lipid peroxides, and secondary lipid oxidation breakdown products. These molecules can act as promoters of myoglobin oxidation, and damage cellular membranes, proteins, and nucleic acids, thus promoting breakdown of the muscle post-mortem (Decker *et al.* 2000). Alternatively, *in-vitro* studies have shown that the autoxidation of oxymyoglobin results in the formation of met haem proteins and superoxide radicals which dismutase to produce hydrogen peroxide that, following interactions with metmyoglobin, can form active species that promote lipid oxidation (Renerre, 2000; Gorelik & Kanner, 2001).

In living tissues, utilization of oxygen and the associated formation of reactive oxygen species and free radicals within the cellular matrix are necessary for

biological function. In order to combat the harmful effects of these oxidative by-products a biphasic multi-component antioxidant defence system is functional in both the aqueous and lipid fractions of skeletal muscle (Decker *et al.* 2000). The defence system includes components that scavenge free radicals, inactivate peroxides and reactive oxygen species, chelate prooxidative metals, and quench secondary lipid oxidation products that can cause cellular deterioration (Decker *et al.* 2002).

The mechanism by which these biphasic scavengers are able to inactivate the free radicals and reactive oxygen species is electron donation. Two of the most effective groups of these hydrogen-donating free radical scavengers are the hydroxyl group (ascorbate and phenolics) and the sulfhydryl group (glutathione, cysteine, and lipoic acid) which react with lipid peroxy and lipid alkoxy radicals to form free radical scavenger radicals (Decker *et al.* 2000).

The efficacy of these free radical scavengers and antioxidants is dependent upon their hydrogen bond energies, resonance delocalization, and susceptibilities to autoxidation (Decker *et al.* 2000). The most effective antioxidants are those with low reduction potentials and high resonance stabilization which produce low energy antioxidant radicals that do not readily react with oxygen to form peroxides (Buettner, 1993; Decker *et al.* 2000). Alpha-tocopherol (vitamin E; $E^{0/}$ = 500mV) and ascorbate (vitamin C; $E^{0/}$ = 282mV) have lower reduction potentials than

polyunsaturated fatty acids ($E^{0/}$ = 600mV) and therefore do not promote the oxidation of unsaturated fatty acids (Buettner, 1993; Decker *et al.* 2000). Vitamin C with its lower reduction potential than vitamin E, is able donate a hydrogen atom to alpha-tocopherol radicals, and re-generate the molecule to its active form (Buettner, 1993; Decker *et al.*, 2000). Phenolic compounds, according to Decker *et al.* (2000), are the most effective free radical scavengers because of their ability to accept substitution groups and delocalise the energy gained from the addition of the hydrogen atom throughout the phenolic ring.

Photoactive sensitizers, the superoxide anion, and some peroxide compounds are known oxidation intermediates that influence lipid oxidation in an indirect manner by interacting with metals or oxygen to form free radicals and reactive oxygen species (Decker *et al.* 2000). The reduced state of transition metals can form the highly reactive hydroxyl radical via the decomposition of hydrogen peroxide, which is undesirable as it is able to oxidise almost all biomolecules. However, skeletal muscle also contains the enzymes catalase and glutathione peroxidase that catalyse the conversion of hydrogen peroxide to water and oxygen, thereby removing the substrate for hydroxyl formation by transition metals. Unlike catalase, glutathione peroxidase also decomposes lipid peroxides, and is the only antioxidant enzyme in skeletal muscle known to be responsive to the dietary supplementation of selenium (Decker *et al.* 2000).

In muscle foods these oxidative processes lead to an accumulation of undesirable colours and flavours, and therefore are of major economic cost to retailers and, eventually, consumers. At two different levels within the cell, both vitamin C and glutathione peroxidase in the cytosol, and vitamin E at the lipid membrane, function to protect biological membranes from oxidative damage, and therefore, help to preserve desirable colour, flavour, and texture in post-mortem flesh.

With the colour stability of muscle foods dependent upon the redox chemistry of the myoglobin protein and oxidizable lipids, Faustman and Wang (2000) noted that any mechanisms that can directly or indirectly impede the oxidation of oxymyoglobin, promote the reduction of metmyoglobin, and neutralize lipid peroxy and alkoxy radicals will prolong the colour shelf life of meat products. Indeed, a number of studies have investigated the effectiveness of the dietary supplementation of antioxidants to slow the formation of these detrimental oxidative agents and extend the shelf-life of muscle foods (Mortensen & Skibsted, 2000).

Dietary supplementation with vitamin E and vitamin C, either in combination or in isolation, has been shown to inhibit lipid oxidation and/or extend the colour shelf life of pork (Dirinck *et al.* 1996; Houben & Gerris, 2002; O'Sullivan *et al.* 2002), turkey (Gorelik & Kanner, 2001; Nam *et al.* 2003), veal (Granit *et al.* 2001), broiler meat (Guo *et al.* 2003), rabbit (Lo Fiego *et al.* 2004), lamb (Macit *et al.* 2003a,b), and beef (Okayama *et al.* 1987; Arnold *et al.* 1992; Liu *et al.* 1996; Wheeler *et al.* 1996;

Roeber *et al.* 2001; Houben *et al.* 2002; Stubbs *et al.* 2002; Djenane *et al.* 2003; Robbins *et al.* 2003b; Realini, 2004).

Furthermore, in finfish aquaculture the dietary supplementation of vitamin C and E has been shown to reduce lipid oxidation and/or extend the colour shelf life of sea bass (Gatta *et al.* 2000), juvenile and adult turbot (Stephan *et al.* 1995; Tocher *et al.* 2002; Ruff *et al.* 2003), Atlantic halibut (Ruff *et al.* 2002; Tocher *et al.* 2002), Atlantic salmon (Scaife *et al.* 2000; Hamre *et al.* 2004), sea bream (Tocher *et al.* 2002), rohu (Sau *et al.* in press), rainbow trout (Frigg *et al.* 1990; Chaiyapechara *et al.* 2003), yellow perch (Lee & Dabrowski, 2003), and on treated fillets of Norwegian herring (Hamre *et al.* 2003a).

The advent of the global tuna aquaculture industry in the 1990's saw a new product with higher and more consistent levels of fat supplied to the sashimi tuna markets of Japan. Farmed tuna have been well received in Japan as this important quality trait (fat) is highly variable in the wild product. Farmed tuna, however, have gained a reputation in the wholesale market and with restaurateurs of having a comparatively short colour shelf-life. This reduction in shelf-life may stem from increases in the oxidative pressures in the flesh that can lead to the oxidation of myoglobin, or from reductions in the concentrations of myoglobin in the flesh owing to the reduced activity rates of the animals in the grow-out pens. In addition, the Australian tuna farming industry faces the pre-existing notion that Southern bluefin tuna, as a

species, has a characteristically short colour shelf-life when compared to other species of tuna (Taguchi, *pers. comm.*). The benefits of dietary vitamin supplementation to counteract the negative effects of the oxidative processes and potentially extended the colour shelf-life of the product has a sound scientific basis and a strong commercial impetus.

Research into the effects of dietary antioxidants in farmed tuna commenced in Port Lincoln in 1999 centring on the delivery of vitamins C and E, fortifying each into a prototype pellet developed for use in the South Australian Research and Development Institute's experimental tuna farm. Concurrently the tuna farming industry also began trialling the use of vitamin powders. The delivery and incorporation of vitamins into the flesh of farmed Southern bluefin tuna via this pellet was proven to be a valid approach (Thomas *et al.* unpublished data). However, at the time of writing the Southern bluefin tuna farming industry is reluctant to substitute the use of wild-caught bait fish for pelleted feeds. Of the vitamin delivery methods used by commercial operators the most commonly practiced method involves the sprinkling and mixing of a vitamin premix powder with defrosted baitfish prior to dispersal into the tuna pens. This practice comes at a cost to the tuna farming companies and the validity of the approach to extend colour shelf-life is anecdotal (Warland, D. *pers comms*).

3.2 Aims & Objectives

The main objective of this study was to establish if the industry practice of sprinkling and shovel-mixing of vitamin pre-mix powders on to defrosted baitfish prior to feeding is effective in raising the levels of vitamins E and C in the flesh of tuna. Further aims included comparing the levels of vitamins E and C and selenium in the flesh of wild and farmed tuna, and to investigate whether the colour shelf-life of the flesh is extended in tuna fed a comparatively higher quantity of vitamins via this method.

3.3 Materials & Methods

Vitamin Pre-Mix Powder: A commercially available dietary premix powder (*TGA Tuna Premix* supplied by *Ridley Pet Products*) with varying vitamin E and C was ordered for the trial by the tuna farming company (*Blaslov Fishing*). The low level vitamin premix powder contained 25mg/kg and 30mg/kg of vitamin E and C (acetate form), and the high level vitamin premix powder contained 100mg/kg and 60mg/kg of the vitamins E and C respectively. There was no additional selenium in the premix.

Experimental Design: In January 2002, two weeks were spent on board the fishing support vessel *Nanci* in the south-eastern part of the Great Australian Bight to collect core flesh samples from a total of fifteen line-caught wild Southern bluefin tuna to examine the base levels of vitamins E, C and selenium. The flesh samples

were stored in liquid nitrogen following removal. A school of tuna from the same location were then captured using a purse seine net, transferred into a towing cage, and towed from the fishing ground to Port Lincoln. The captive tuna were fed defrosted baitfish mixed with the low level vitamin pre-mix powder for the duration of the two week tow and then transferred into grow-out pens upon arrival.

Four tuna grow-out pens were divided into two treatments - two pens (pens 1 and 4) for low vitamin, and two pens (pens 2 and 3) for high vitamin supplementation. The stocking densities in each pen were approximately equal but due to commercial constraints the information cannot be revealed. Tuna were fed twice daily with the morning feed consisting of frozen blocks of baitfish placed into a feeding cage inside the grow-out pen, and the afternoon feed consisting of defrosted baitfish shovelled into the pens until satiation. It was during the afternoon feed that approximately one kilogram of either the low or high level vitamin pre-mix was shovel-mixed in with 400-500 kg of defrosted baitfish (~2g/kg). A control grow-out pen with tuna receiving baitfish with no vitamin pre-mix supplementation was not available for this trial.

Harvest 1: A total of 31 tuna (16 from the low vitamin and 15 from the high vitamin treatments) were harvested on the 15th of March, 2002 and two core samples were removed from each fish. One core sample was immediately placed in liquid nitrogen for the analysis of vitamins E and C, and selenium. The second core sample was

used in an 8 day, 9 person (6 male: 3 female) colour shelf-life panel at the Lincoln Marine Science Centre in Port Lincoln. On each day following the panellists assessment of colour the samples were then analysed for their pH and CIE L*a*b* values. The harvested tuna were processed and trucked overnight to Adelaide, loaded onto Qantas flight QF738 to Sydney and then transferred onto Qantas flight QF21 to Japan departing on the evening of the 16th of March, 2002. In Japan, a section of the tail from all 31 fish was removed at a distribution centre near Narita International Airport on the afternoon of the 17th of March, 2002 and were used in a 3 day, 7 person (3 male: 4 female) shelf-life trial at the Tokyo University of Fisheries. The tuna were of approximately equal size and position within the harvest in an attempt to account for the possible effects of size and harvest stress on the colour shelf life of the samples. Data loggers, programmed to record temperature every fifteen minutes, were inserted into the bleed cut regions of the tuna carcass at the harvest point, and on the inside and outside of the tuna coffins during processing. The loggers were removed at the distribution centre near Narita International Airport.

Harvest 2: A second harvest occurred on the 12th of April, 2002. On this occasion 15 tuna (8 from the low vitamin and 7 from the high vitamin treatments) had two core flesh samples removed immediately post harvest, one core for biochemical analyses and the other core for physical analyses and an 8 day, 6 person (3 male: 3 female) colour shelf-life assessment in Port Lincoln as per harvest one. The

harvested tuna were processed and trucked overnight to Adelaide, loaded onto Singapore Airlines flight SQ230 to Singapore the following day and then loaded onto Singapore Airlines flight SQ998 to Japan departing on the evening of the 13th of April. The tuna were then trucked and placed in cold storage at the Tsukiji Wholesale Fish Market in Tokyo. Prior to sale the tuna were intercepted on the auction floor where tail sections were removed on the 15th of April and analysed by a 4 day, 7 person (4 male: 3 female) colour shelf panel at the Tokyo University of Fisheries. As per harvest 1, the tuna were of approximately equal size and position within the harvest in an attempt to account for the possible effects of size and harvest stress on the colour shelf-life of the samples. Data loggers, programmed to record temperature every fifteen minutes, were also inserted into the bleed cut regions of the tuna carcass at the harvest point, and on the inside and outside of the tuna coffins during processing. The loggers were removed prior to auction on the 15th of April.

Sampling & Measurement

Core Sampling: samples were removed from the bleed cut and stored in either liquid nitrogen for vitamin and mineral analysis or in a constant temperature refrigerated environment for colour shelf life examination in Port Lincoln. In Japan redundant sections of the tails of each tuna were removed from between the 3rd and 4th finlet from the caudal peduncle (as per industry practice for flesh quality grading by Japanese technicians in Port Lincoln). These sections of the tail were then used

for colour panel assessments at the Tokyo University of Fisheries (see Methods 2.1).

Vitamin & Mineral Analysis: Vitamin's E and C, and the mineral selenium were analysed as per section 2.2.9 in the Methods Chapter.

pH: The pH of the flesh was measured from the core samples in Port Lincoln by calibrated hand-held meters using surface probes (*Eutech Instruments Model Cyber Scan pH 310*).

Instrumental Colour Assessment: CIE L*a*b* measurements were recorded directly from the core flesh samples in Port Lincoln (see Methods 2.2.2).

Panel Colour Assessment: In order to assess the effects of experimental treatments on the colour shelf-life of farmed Southern bluefin tuna a ranking system was developed in 1999 for use by panellists at the Lincoln Marine Science Centre (LMSC) (Thomas, *in press*). The ranking system, which comprises of seven ranks, attempts to describe the colour changes that occur in tuna flesh cores whilst kept under cellophane wrap in constant temperature conditions of 4 degrees Celsius for several days (see Table 3.1). Colour ranks were assigned to core samples by nine panellists over eight days in Australia and seven panellists over three days in Japan using tail cuts. This ranking system was translated into Japanese for use at the

Tokyo University of Fisheries (now called the Tokyo University of Marine Science and Technology) in Japan. During assessments samples were randomly numbered and presented to panellists on standard white tiles under constant lighting conditions. All panellists at the Lincoln Marine Science Centre had been trained in the assessment of the colour and colour changes of raw tuna flesh, with a majority of panellists participating in colour assessments since 1999. Students at the Tokyo University of Fisheries were also instructed and trained in the use of the ranking system. All panellists had been previously screened for vision defects at the LMSC, and tested at the Tokyo University of Fisheries for use in previous colour assessment trials. Panellist's measurements were recorded on paper and the numbers of panellists used varied depending on the trial (details of which can be found in the chapters themselves). Samples were not evaluated in replicate and agreement assessed in either trial due to time constraints and the labile nature of the sample potentially confounding results and enhancing oxidation and colour change – blooming tuna can change colour in a matter of minutes.

0 = Red meat (initial sample) 赤の肉 - はじめの色
1 = Bright red meat (bloomed) 明るい赤
2 = Change in colour (i.e. a dulling of the bloomed meat) ちょっと鈍くなった (まだ赤)
3 = Either: a significant darkening of the meat or margins beginning to brown 肉はちょっと暗くなったか、きわは茶色になっている
4 = Either: a significant darkening of the meat (i.e. heading towards red/black) or brownness spreading from the margins/general colour change of sample 肉は暗くなったか、きわからサンプルの真ん中の方に茶色になっている
5 = Either: a blackening of the meat or an overall browning of the meat but, for either, still with a pink/red undertone 肉は黒くなっているか、サンプル全部的に茶色になっている
6 = Either a black sample or a brown/green sample (no red/pink to be seen) サンプルは黒くなったか、茶色または緑になった (赤の色見えない)

Table 3.1: Colour ranking criteria of core samples of the flesh of fresh, farmed Southern bluefin tuna

Statistical Analyses

One-Way ANOVA (*SPSS Version 11.5 for Windows*) was used to statistically analyse the vitamin E, C and selenium data, and the pH, L*a*b* and averaged ranks from the colour shelf panellists.

3.4 Results

Vitamin Analysis: No trends and no significant differences ($p < 0.05$) were found in the core sample concentrations of vitamin E between both the wild and the farmed treatments, and the high and low vitamin treatments in both the March and April harvests (Fig. 3.1).

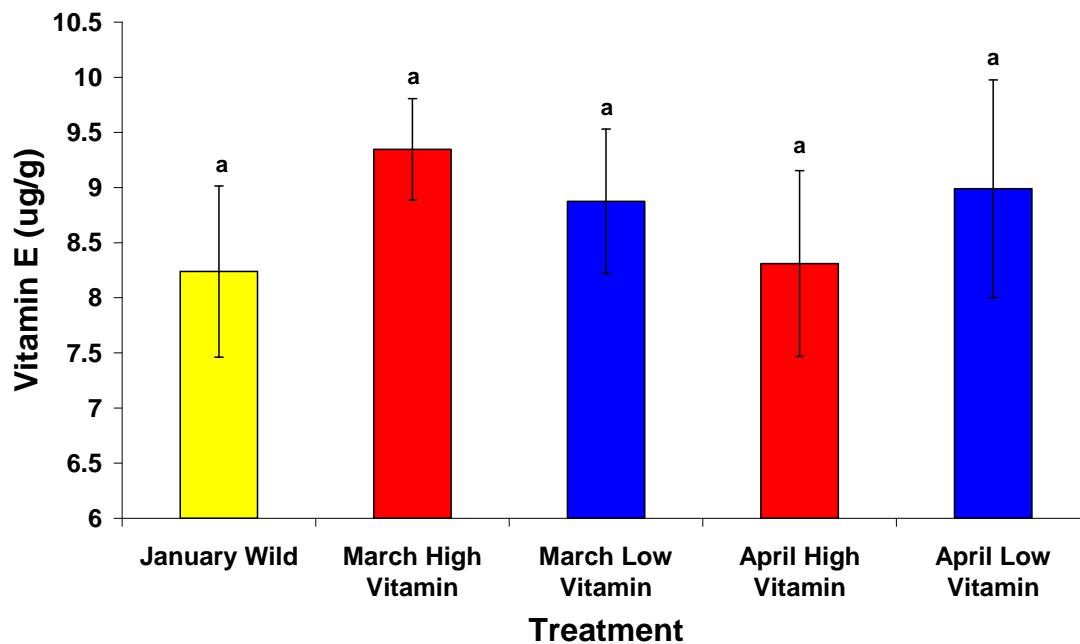


Figure 3.1: Vitamin E concentrations (mean \pm SEM) in flesh samples from wild Southern bluefin tuna caught in January (Wild; $n=15$), and in flesh samples from farmed Southern bluefin tuna fed low and high levels of vitamin supplements that were harvested in March (Harvest 1; High $n=15$, Low $n=16$) and in May (Harvest 2; High $n=10$, Low $n=10$) of the 2002 farming season in Port Lincoln (different letters indicate statistical significance $p < 0.05$).

For vitamin C, the concentration in the wild tuna was significantly higher ($p < 0.05$) than all the farmed treatments except the high vitamin treatment from the March harvest ($p = 0.096$). No significant differences in vitamin C concentrations were found between the high and low vitamin treatments of both the March and April harvests, however, vitamin C concentrations from the samples of the March high vitamin treatment were significantly higher than vitamin C concentrations of the samples from both the high and low vitamin treatments of the April harvest thus indicating a trend of decline (Fig. 3.2).

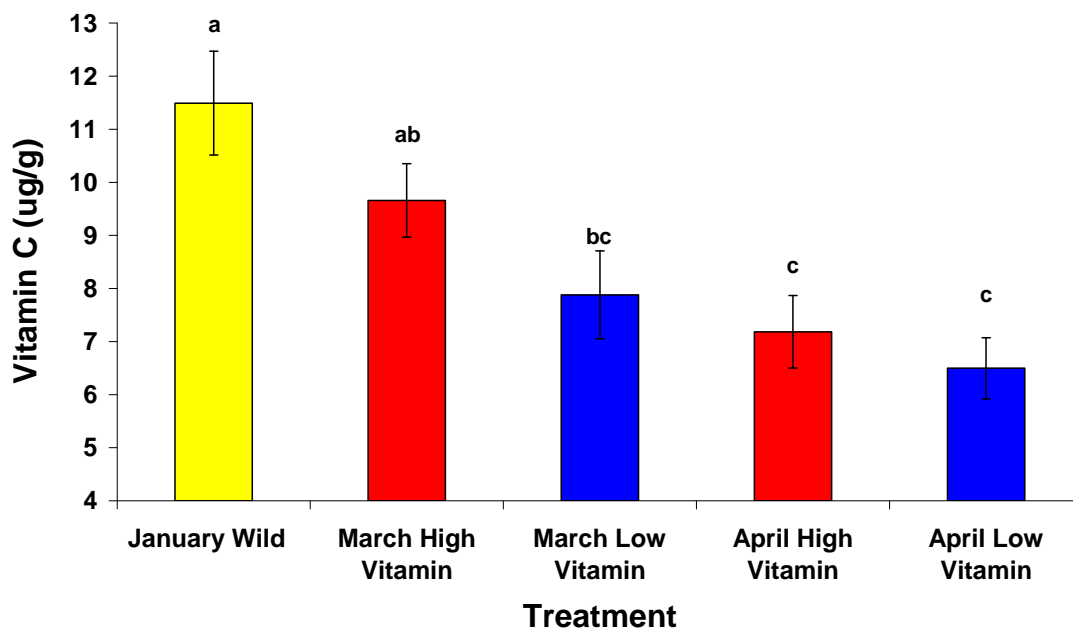


Figure 3.2: Vitamin C concentrations (mean \pm SEM) in flesh samples from wild Southern bluefin tuna caught in January (Wild; $n=15$), and in flesh samples from farmed Southern bluefin tuna fed low and high levels of vitamin supplements that were harvested in March (Harvest 1; High $n=15$, Low $n=16$) and in May (Harvest 2; High $n=10$, Low $n=10$) of the 2002 farming season in Port Lincoln (different letters indicate statistical significance $p < 0.05$).

The concentrations of selenium in the core flesh samples from wild caught tuna were significantly higher than all the farmed treatments. As observed with vitamin C, the selenium concentrations tended to decrease with grow-out time and there was no significant difference between low and high vitamin diet treatments (Fig. 3.3).

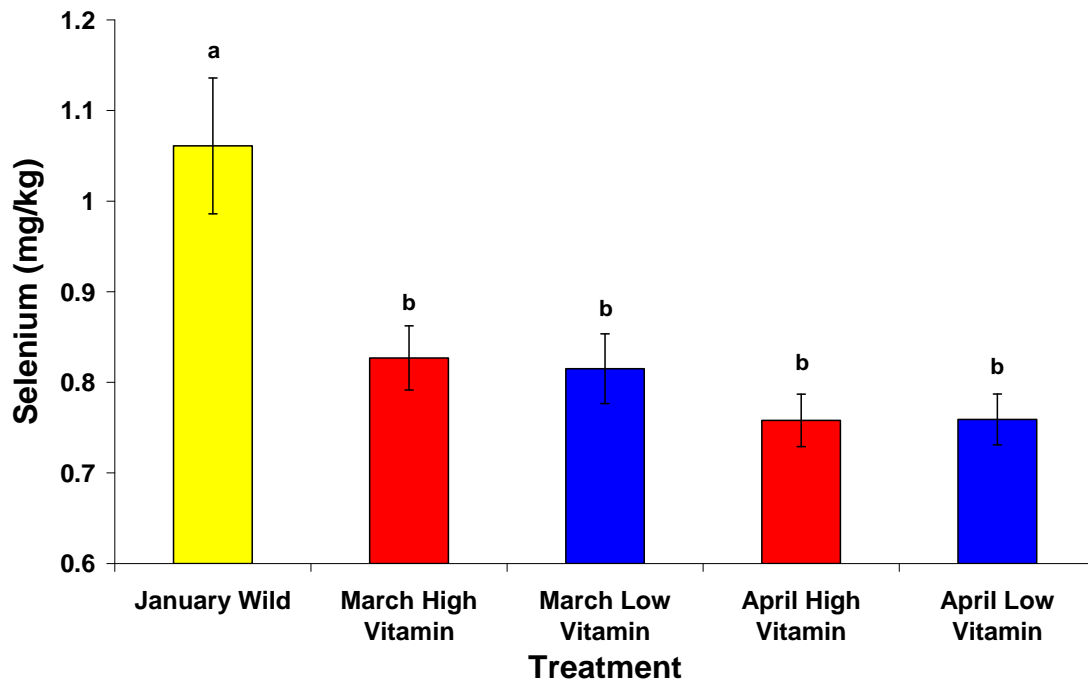


Figure 3.3: Selenium concentrations (mean \pm SEM) in flesh samples from wild Southern bluefin tuna caught in January (Wild; n=15), and in flesh samples from farmed Southern bluefin tuna fed low and high levels of vitamin supplements that were harvested in March (Harvest 1; High n=15, Low n=16) and in April (Harvest 2; High n=10, Low n=10) of the 2002 farming season in Port Lincoln, Australia (different letters indicate statistical significance $p < 0.05$).

pH: The surface pH values of samples over the eight day sampling period were in the normal range for tuna flesh with maximum and minimum values of 6.12 and 5.59, and 5.88 and 5.63 pH units for the March and April harvests respectively. No significant differences ($p < 0.05$) were found to exist over the eight day storage period in March between the high and low vitamin treatments (Fig. 3.4a). In contrast, the surface pH of samples from the high vitamin treatment was significantly higher than the surface pH of the low vitamin samples for all days except Day 5 during the April trial (Fig. 3.4b).

Colorimeter: The ranges of recorded CIE $L^*a^*b^*$ colour measurements were consistent with tuna flesh colour recordings and trends observed in previous trials. The opacity of the flesh increased with time as revealed by an increase of the CIE L^* values over the eight day sampling periods in March and April (Fig. 3.5a and Fig. 3.6a). CIE a^* values decreased (Fig. 3.5b and Fig 3.6b) whilst b^* values increased (Fig. 3.5c and Fig. 3.6c), and were consistent with the gradual darkening and colour changes of the flesh over the sampling period. No significant differences were recorded ($p < 0.05$) in the CIE $L^*a^*b^*$ measurements between the vitamin treatments in either March and April.

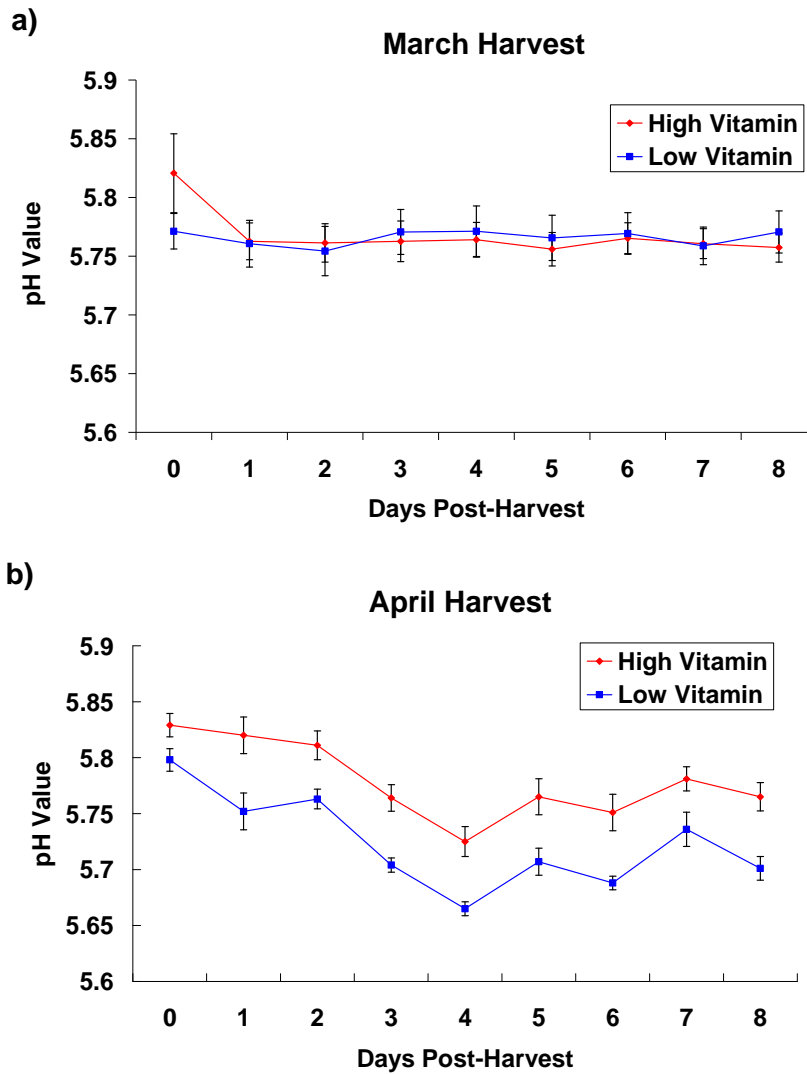


Figure 3.4: Surface pH values (mean +/- SEM) of core flesh samples of tuna fed high and low vitamin supplementation from January until harvest in (a) March and (b) April 2002 in Port Lincoln. Samples were stored on white tiles under cellophane wrap in constant temperature conditions (4°C) for an eight day period.

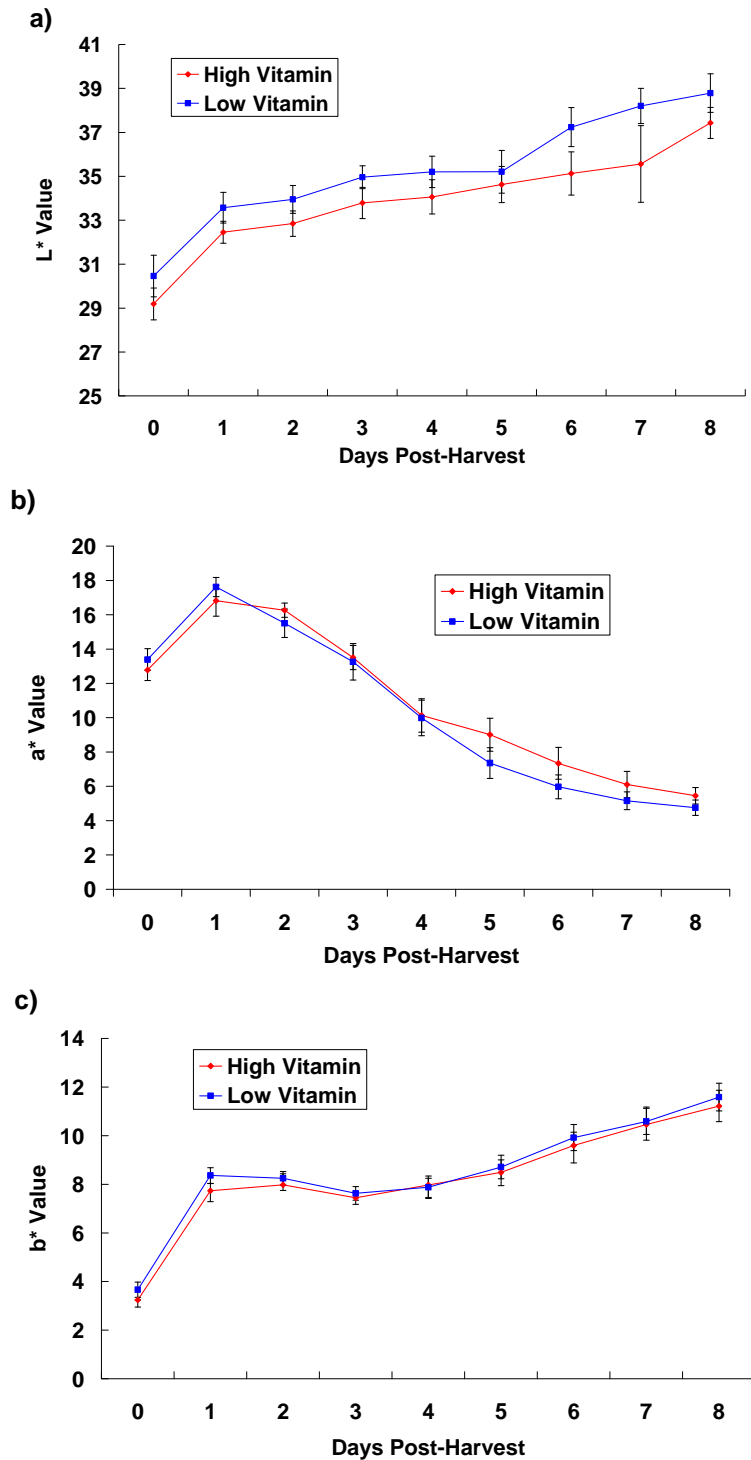


Figure 3.5: Chromameter L*a*b* values (mean +/- SEM) recorded from the core flesh samples of Southern bluefin tuna fed high and low vitamin supplementation from January until harvest in March 2002. Samples were stored on white tiles under cellophane wrap in constant temperature conditions (4°C) for an eight day period.

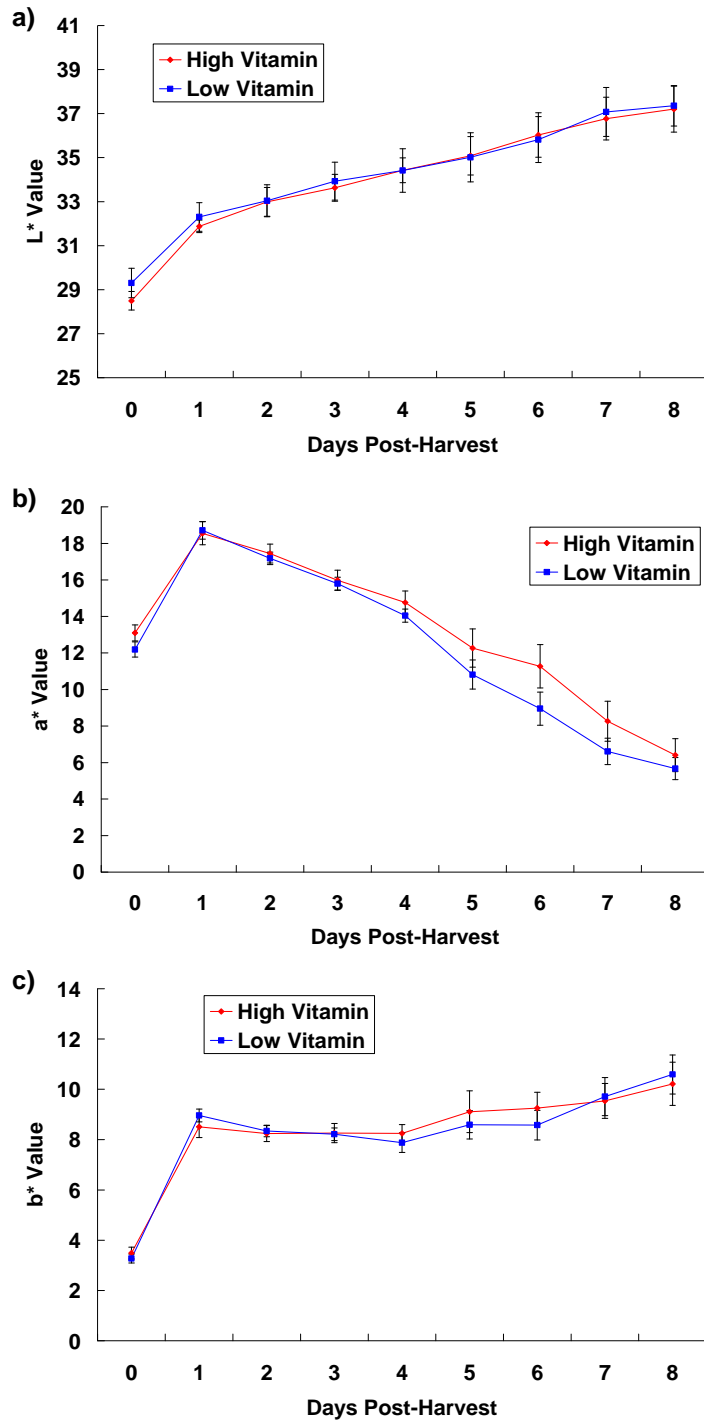


Figure 3.6: Chromameter L*a*b* values (mean +/- SEM) recorded from the core flesh samples of Southern bluefin tuna fed high and low vitamin supplementation from January until harvest in April 2002. Samples were stored on white tiles under cellophane wrap in constant temperature conditions (4°C) for an eight day period.

Panellist Colour Rankings - In March, significant differences ($p < 0.05$) in the averaged Port Lincoln colour panel rankings of the core samples from the high and low vitamin treatments were recorded on Days 3, 5, 6, and 8 ($p = 0.032$; 0.043 ; 0.011 ; 0.002). Colour rankings recorded on Days 0, 1, 2, 4, and 7 were not significantly different ($p = 1.000$; 0.510 ; 0.065 ; 0.081 ; 0.053). On each day of sampling the average colour ranks for the high vitamin treatment were lower than those of the low vitamin treatment indicating that these samples remained a more desirable red colour than samples from the low vitamin treatment (Fig. 3.7). In Japan, significant differences between the average colour panel rankings of the tail cuts from the high and low vitamin treatment were recorded on Days 2 and 3 ($p = 0.000$; 0.015). A non - significant result was recorded on Day 1 ($p = 0.088$). The colour rankings of the tail cuts from the high vitamin treatment were on average lower than the colour rankings of the tail cuts from the low vitamin treatment indicating that dietary vitamin supplementation in Port Lincoln was able to prolong redness in the flesh of tuna removed in the market in Japan (Fig. 3.8).

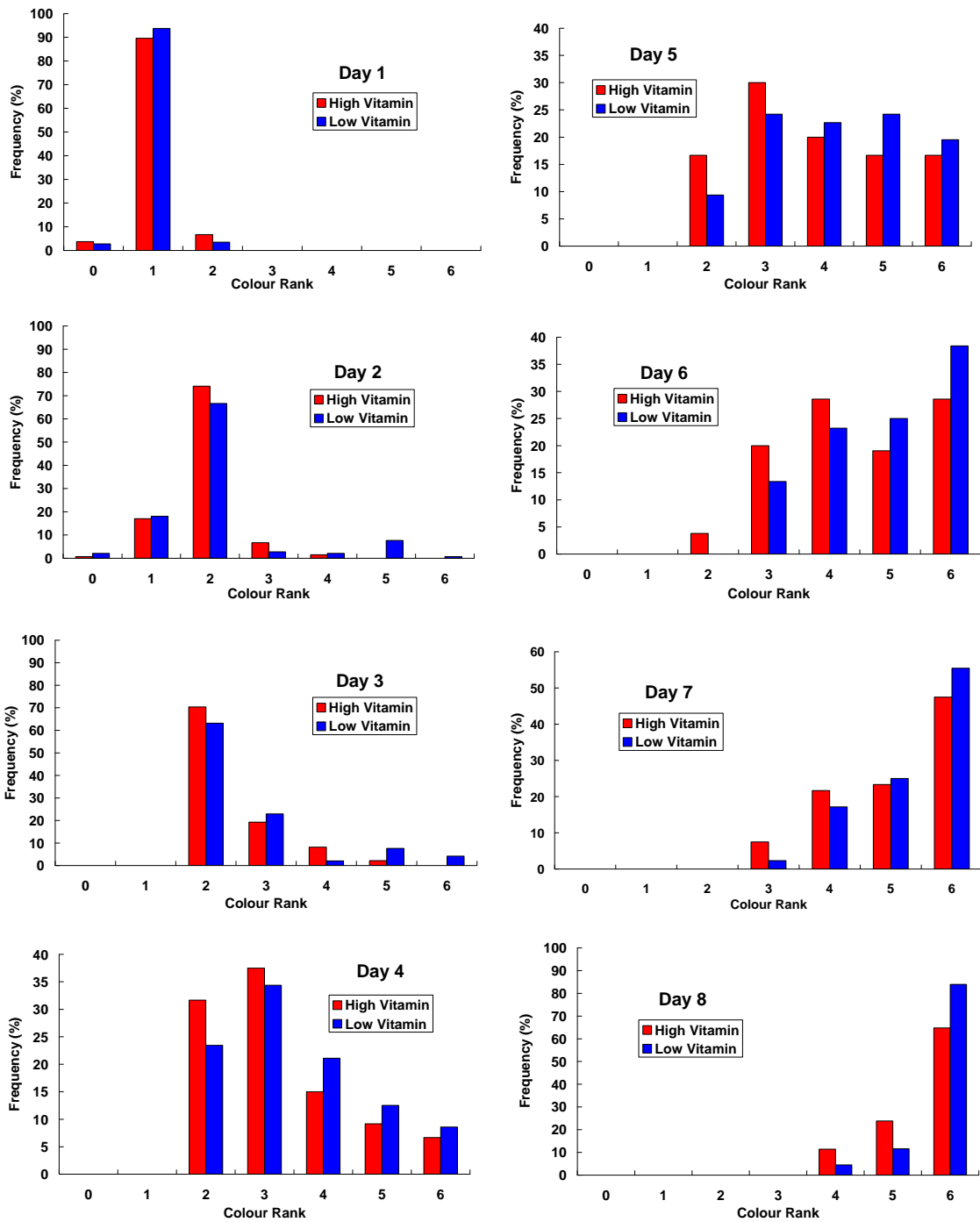


Figure 3.7: Colour rank frequencies from an eight-day evaluation by a panel of nine people of the colour of core flesh samples taken from farmed Southern bluefin tuna fed low and high levels of vitamin supplements and harvested in March 2002 (High n=15, Low n=16) in Port Lincoln.

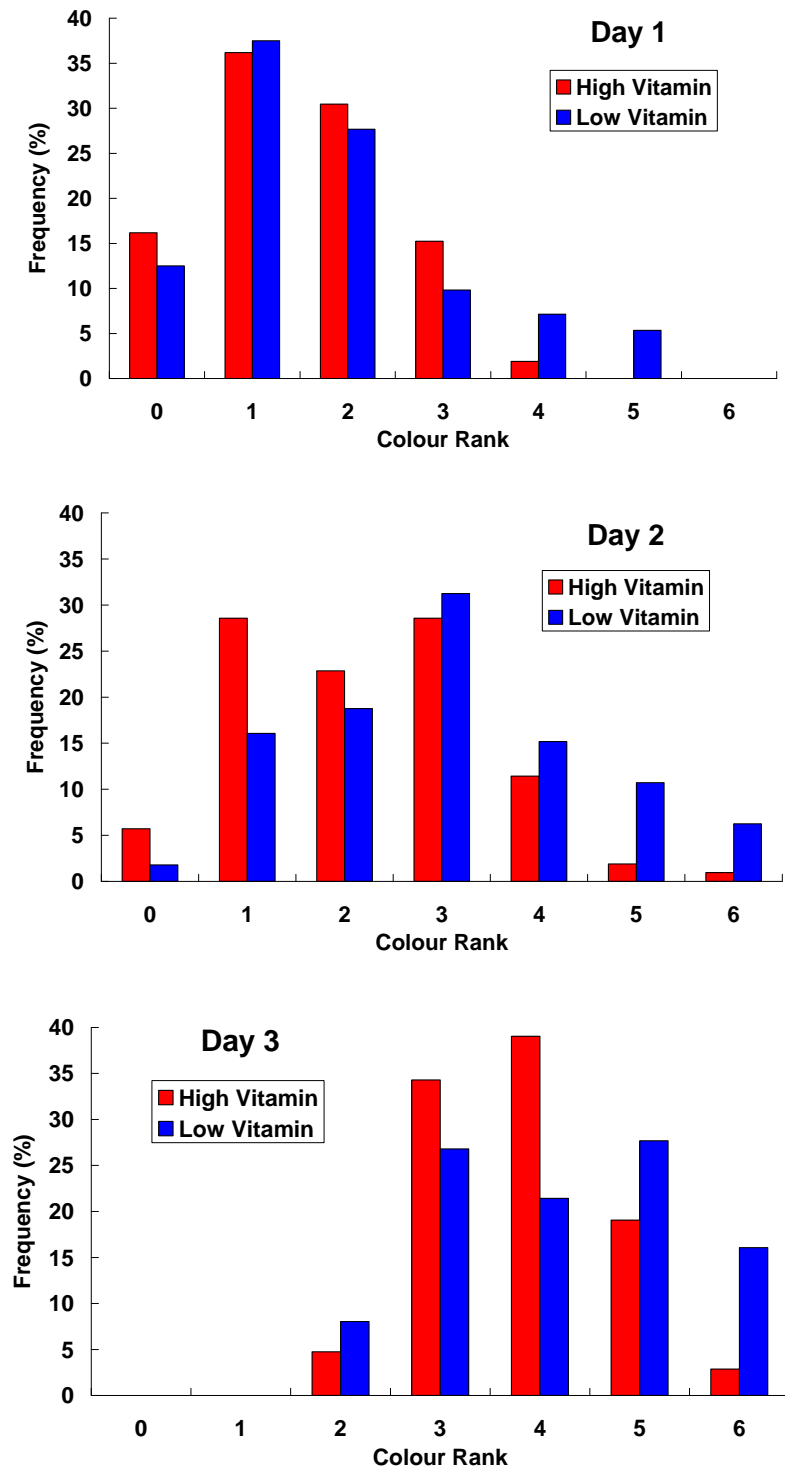


Figure 3.8: Colour rank frequencies from a three-day evaluation by a panel of seven people of the colour of tail cut samples taken from farmed Southern bluefin tuna fed low and high levels of vitamin supplements and harvested in March 2002 (High n=15, Low n=16) in Tokyo, Japan.

In April in Port Lincoln significant differences between the averaged colour panel rankings of the core samples from the high and low vitamin treatments were detected on Days 3, 6, 7, and 8 ($p = 0.033; 0.014; 0.000; 0.001$). Non-significant differences were recorded on Days 0, 1, 2, 4, and 5 ($p = 1.000; 1.000; 0.190; 0.743; 0.146$). As with the average colour rankings from the core samples during March in Port Lincoln the mean colour ranks from the April harvest for the high vitamin treatment were lower than those of the low vitamin treatment on all days after Day 0 indicating that these flesh samples were a more desirable red colour for longer (Fig. 3.9).

The April result in Japan also re-affirmed the March result with high vitamin samples recording significantly lower average colour panel rankings on all four days of the trial ($p = 0.029; 0.034; 0.002$ and 0.001) (Fig. 3.10).

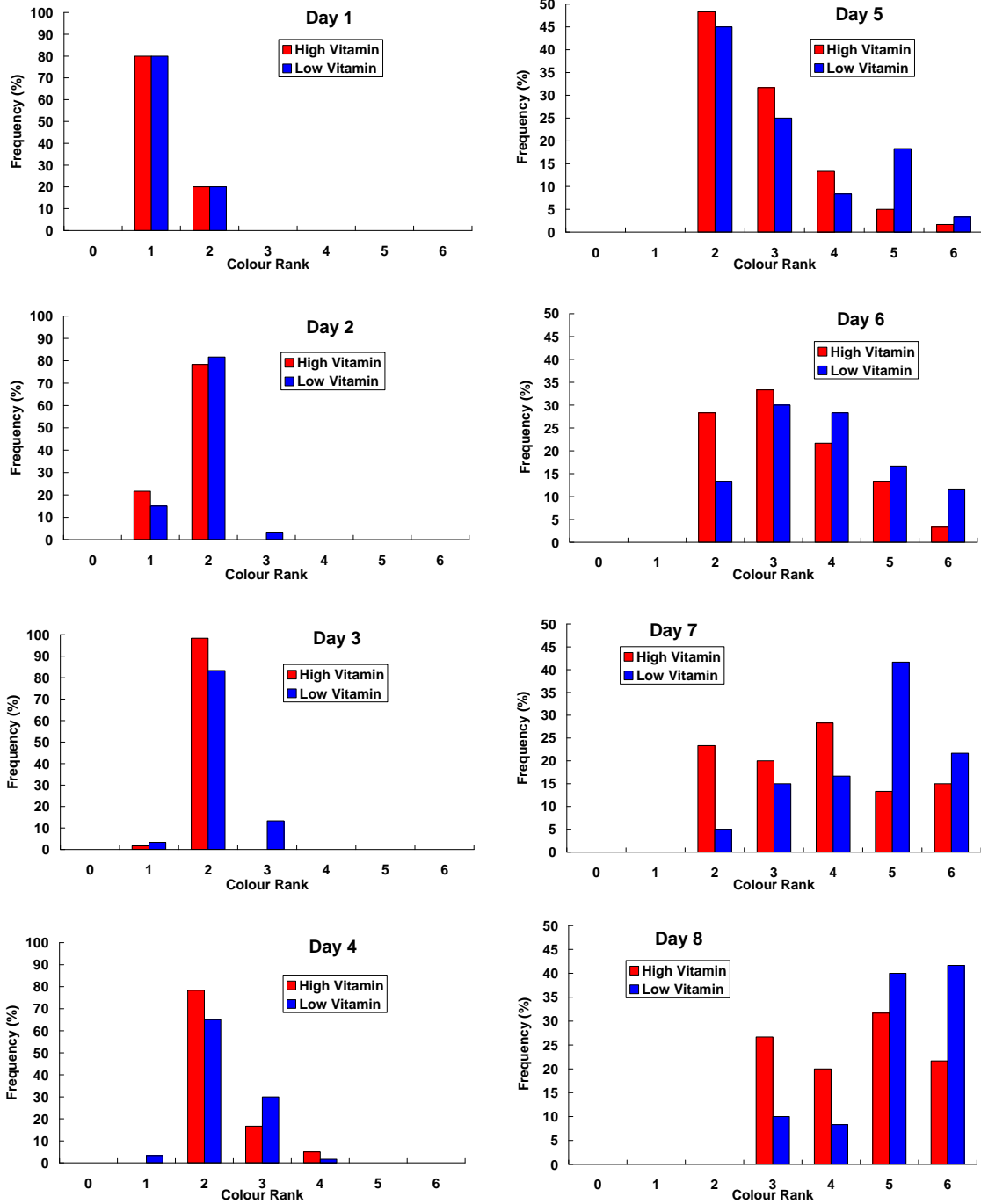


Figure 3.9: Colour rank frequencies from an eight-day evaluation by a panel of six people of the colour of core flesh samples taken from farmed Southern bluefin tuna fed low and high levels of vitamin supplements and harvested in April 2002 (High n=15, Low n=16) in Port Lincoln.

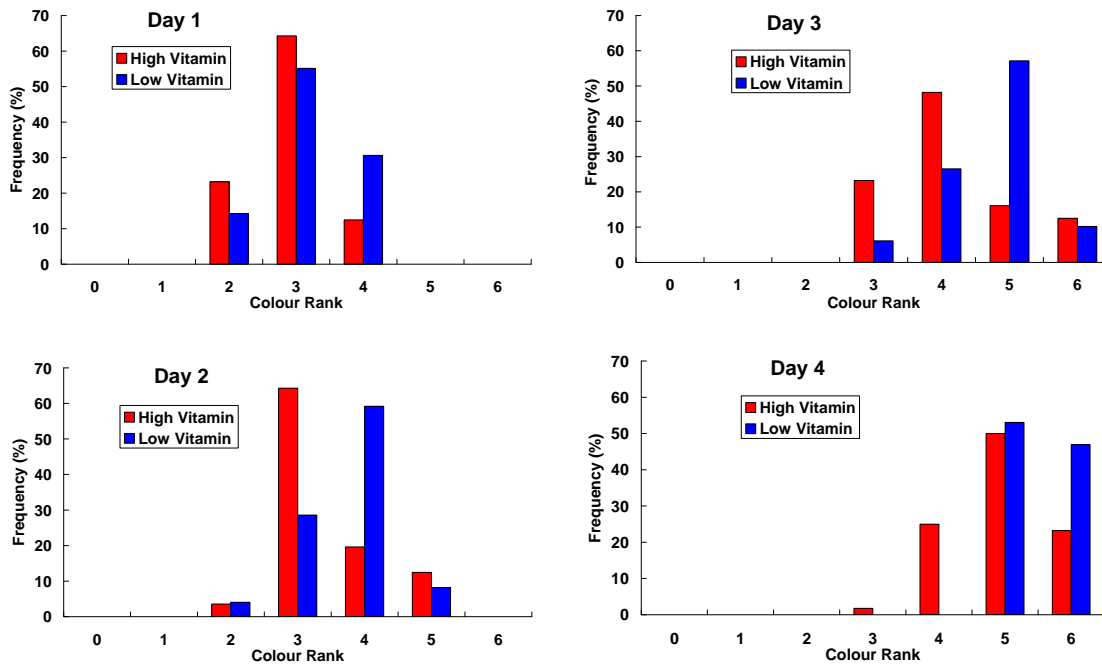


Figure 3.10: Colour rank frequencies from a four-day evaluation by a panel of six people of the colour of tail cut samples taken from farmed Southern bluefin tuna fed low and high levels of vitamin supplements and harvested in April 2002 (High n=7, Low n=8) in Tokyo, Japan.

Time-Temperature and Cage Effects

The time-temperature profiles logged in the carcass and from inside and the outside of the tuna coffins were characteristic of air-freighted tuna sent from Port Lincoln to Japan for both the March and April trials. No significant differences were recorded in these time-temperature profiles between the high and low vitamin treatments on both occasions. There were also no significant effects of cage between the treatments on all parameters measured in both the March and April harvests.

3.5 Discussion

Analyses of the vitamin concentrations of core flesh samples returned non-significant differences between all treatment/month combinations for Vitamin E, and between the high and low vitamin treatments for both the March and April harvests for Vitamin C. These results suggest the sprinkling of vitamin powders over defrosted baitfish at the highest concentrations specified in this study was either an ineffective delivery method of vitamins to the flesh of farmed tuna, the dosage of vitamins E and C was too low to either elevate or maintain the respective levels in the flesh, or a combination of both of these factors. The core samples used in this study consisted of Akami and Chutoro flesh with a lean to medium-level of fat which may have masked results for the fat-soluble vitamin E. The high-fat portions of the belly flap (Otoro) were not available for sampling due to the extremely high value of this cut and the fact the tuna were from a commercial operation and destined for the Japanese auction markets. The vitamin E levels from these flesh core samples however, did not significantly increase or decrease over the sampling period when compared to the samples removed from wild tuna.

Of most concern to the industry would be the significant and gradual reduction in the concentrations of Vitamin C from tuna caught in the wild compared to the farmed tuna harvested in April. Vitamin C, with its lower reduction potential, is able to recycle and replenish vitamin E at the lipid membrane-fat free mass interface (Packer *et al.* 1979; Leung *et al.* 1981; Buettner, 1993). In finfish, evidence of this

sparing effect of Vitamin C on Vitamin E has been reported in mackerel (Peillo *et al.* 1998), tilapia (Shiau & Hsu, 2002), and yellow perch (Lee & Dabrowski, 2003). It is therefore plausible that the levels of vitamin C were in decline in the flesh of farmed tuna in order to recycle and maintain the levels of vitamin E in an increasingly lipid environment.

It is lipid accumulation, combined with overall growth that is the primary objective of tuna aquaculturists. As a consequence, farmed tuna are fed a baitfish diet often high in lipid, twice a day, until satiation. Dabrowski (1990) hypothesized that metabolic rate induced by feeding was the primary factor regulating the vitamin C requirements of fish. Merchie *et al.* (1997) expanded upon this notion and claimed that fingerlings and juvenile fish, with their higher metabolic rates, would require higher dietary vitamin C levels to sustain optimal growth and physiological condition. Tuna, with the highest metabolic rates of all finfish (Fitzgibbon *et al.*, 2007), and farmed Southern bluefin tuna still in a juvenile stage, would be most susceptible to the feed induced regulation of, and requirements for, vitamin C.

Apart from a potential decline in the colour stability of the product in Japan, the trend, if continuing on further during an extended grow-out period, may lead to vitamin C deficiencies detrimental to biological function, and therefore, the performance of the animals in a culture environment. Mild dietary deficiencies of vitamin C in finfish aquaculture have been shown to impair growth rates, feed

conversion ratios, protein efficiency ratios, conditioning, and reduce immune function (Lee *et al.* 1998; Adham *et al.* 2000). Vitamin C, via its role of hydroxylating proline to hydroxyproline for collagen synthesis, is also an indispensable component in the formation of connective tissue and the skeletal matrix of fish (Wilson & Poe 1973; De Silva & Anderson, 1995). As a result, severe deficiencies in vitamin C can lead to structural deformities of the spinal column such as scoliosis and lordosis, as well as other afflictions including lens cataracts, exophthalmia, anorexia, haemorrhaging, and mortality (Aguirre & Gatlin, 1999; Wang *et al.* 2002; Lee & Dabrowski, 2003). Additionally, Koshio *et al.* (1997) postulated that vitamin C may act as a modulator of neurotransmission in fish, and that deficiency of this essential vitamin affected the schooling behaviour of ayu – a complication that would have interesting implications for schooling fish in cages during times of low visibility. Fingerlings and juvenile fish are most susceptible to the negative effects of vitamin C deficiencies, and although the more serious afflictions noted here are not prevalent in the farmed Southern bluefin tuna industry at present, extended grow-out periods of these juvenile fish may increase that risk.

Teleostean fish, such as tuna, are unable to synthesize vitamin C and must rely completely on exogenous sources (Dabrowski, 1994). If the reduction of vitamin C in the flesh of farmed tuna is not mirrored in their wild counterparts due, for example, to seasonal factors such as prey availability/switching, or water temperature cues, a dietary deficiency in vitamin C must therefore exist in farmed tuna whether the direct

or indirect cause for the discrepancy. If direct, the baitfish being fed to farmed tuna must be comparatively low in vitamin C to that of the vitamin C content of the live feeds consumed by wild tuna. If the exogenous supply of vitamin C in the diets of both farmed tuna and wild tuna are equal then the discrepancy must be associated with the farming process. Rises in oxidative pressures coinciding with increasing lipid levels in the flesh during the season, or chronic stressors associated with the farming process (e.g. confinement, water clarity, stocking densities) may be taxing the vitamin C reserves of the farmed animal. Whether the former or the latter scenario, supplementation of vitamin C is required to meet the dietary shortfall and/or the demands placed on the tuna during its culture phase should the current feeding and husbandry practices continue.

Tuna on commercial farms are fed a combination of baitfish species about 50% of which is imported into Australia in frozen containers. Vitamin C, and its variable forms are relatively unstable compounds (Gadient & Fenster, 1994; Marchetti *et al.* 1999). Hamre *et al.* (2003b) noted that ascorbic acid treatment was able to reduce the lipid oxidation in herring fillets frozen at -30°C for up to nine weeks, from which point on vitamin C had no antioxidant effect. The authors also claimed that the fifty percent reduction in vitamin C witnessed in the first two weeks of frozen storage was mostly likely attributable to the initial freezing process. Baitfish used by the industry has often been stored for periods of up to 9 months at temperatures that often fluctuate but average around -20°C prior to use. Fitzgerald & Bremner (1994)

noted that lipid oxidation had occurred in pilchards used by the industry after only one month of storage, which would negatively affect the vitamin status of the baitfish, and therefore both the macro- and micro-nutrient value of the diet. Indeed in a study carried out recently (Carragher *et al.*, 2007) reported significant reductions in vitamin C and E in fresh and frozen storage, and especially during 2-3 day thawing of local baitfish.

Also alarming was the significant reduction of the essential mineral selenium in the flesh samples of farmed tuna when compared to their wild counterparts. Despite the reduction, the levels reported here for farmed Southern bluefin tuna are similar to the average selenium concentration of 0.743 mg/kg reported by Plessi *et al.* (2001) for wild Northern bluefin tuna (*Thunnus thynnus*). The average concentration of 1.06 mg/kg of selenium in the flesh of wild Southern bluefin tuna may indicate significantly higher levels, and/or selenium demands, of Southern bluefin tuna when compared to its northern cousin.

Selenium protects an organism against organic and inorganic mercury via metabolic interaction (Plessi *et al.* 2001) and the reduction in the concentration of selenium in the flesh of farmed tuna interestingly coincides with significantly lower levels in the concentration of mercury found in the flesh of farmed Southern bluefin tuna (Padula *et al.* 2003).

If the decline in selenium is not natural (seasonal) there may be concern for the cultured animal. Halver *et al.* (2004) reported losses of up to 20% of carcass tissue selenium in smolt Chinook salmon during 30 hours of confinement stress in barges. Wild SBT are captured, towed for up to two weeks in confined conditions, and then transferred into pens that are located in a vastly different environment to that of their catching ground. The associated stressors of these farming processes may be contributing to the reductions in carcass selenium levels witnessed in the present study. Bell *et al.* (1985) showed that blood vitamin E concentrations and glutathione peroxidase activities were significantly lowered in selenium deficient salmonids, therefore weakening antioxidant defences. Combined vitamin E and selenium deficiency has been associated with muscle degeneration in ruminants (McDowell *et al.* 1996), and Kirchgessner *et al.* (1995) reported that selenium-deficient pigs had significantly lower glutathione peroxidase activity, daily food intake, and daily weight gains than control pigs. Alternatively, dietary supplementation of selenium has been linked to increases in the quality and shelf-life of pork and poultry via improvements in the integrity of the cellular membrane and increases in selenium-dependent glutathione peroxidase activity (Mahan & Kim, 1999; Downs *et al.* 2000). Farmed tuna may therefore also benefit from the dietary supplementation of selenium.

Partly as a consequence of the present study, and in order to ascertain whether there are vitamin and mineral deficiencies in the baitfish being fed to tuna on

commercial farms, nutritional profiling of the baitfish is currently underway within the research sub-program. Micronutrient content of baitfish can vary due to season (Šatović & Beker, 2004), and storage time and temperature (Hamre *et al.* 2003b). Knowledge of these factors and data on the nutritional value of available baitfish on a species basis, combined with the time-temperature history of the baitfish will enable the industry to procure bait and employ storage practices that better meet the dietary requirements of the tuna in their cages.

In the area of dietary supplementation not much is known regarding the micro-nutrient requirements of many species of fish (Kaushik, 1997), and, as well as interspecies differences, needs are known to vary with age, size, and physiological state (Lochmann *et al.* 1999). In the present trial a vitamin inclusion rate of 2g of a vitamin premix powder to 1 kg of baitfish was employed. However, the commercial delivery method examined here made it impossible to quantify the concentrations of supplemented vitamins that were ingested by the tuna as the amount of vitamin powder that remained attached to the baitfish during application, distribution, following impact with the water surface, and while submersed prior to ingestion was unquantifiable. According to the United States National Research Council (NRC, 1993) dietary requirements of vitamin C, vitamin E and Selenium for fish range from 25-50 mg/kg, 50-100 IU/kg, and 0.25-0.3 mg/kg respectively. These values were based on investigations into rainbow trout, Chinook salmon, European sea bass, and red drum, all of which have lower activity and metabolic rates than tuna. For

vitamin E, Stephan *et al.* (1995) suggested a feeding rate of vitamin E five to ten times higher than nutritional requirements, especially when the fish produced will undergo frozen storage, which to date approximates 70% of the production volume of Southern bluefin tuna farming industry. According to Woodward (1994), however, estimates of dietary vitamin requirements are exaggerated, and the author argues that a number of factors (the use of purified ingredients in experimental diets, the stability of the chemical forms investigated, and the lack of standardised methods) has resulted in an over estimation of the dietary requirements of vitamins for fish. Woodward (1994) suggests that the requirements of most water-soluble vitamins would not differ between cold-water species such as trout and warm-blooded fish species. Contrastingly, Shearer (2000), in a review of 46 papers, stated that, apart from cases of methodological error, the inappropriate use of broken-line analysis and analysis of variance consistently underestimated micronutrient requirements by a factor of between two and five.

The developmental moist pellet has been shown to be an effective vitamin delivery medium on many trials both in research and in commercial SBT farming environments, yet, as the pellet is still in development and not widely accepted by the industry, frozen baitfish remain the preferred feed. In an effort to potentially improve the delivery of vitamins and mineral pre-mixes via baitfish the use of a binder such as gluten was proposed and trialled on the South Australian Research and Development Institute's tuna research farm during the 2003 season. This

method proved highly successful in raising the flesh concentration of vitamin C (Thomas *et al.* unpublished data), and may help the tuna industry raise the levels of vitamin C in commercial tuna using frozen baitfish as feed.

The reduction of vitamin C and selenium in the flesh of farmed tuna early in the culture season is of concern, however, it is impractical to investigate whether the decline is mirrored in wild tuna as a response to seasonal cues as they migrate from the fishing grounds around the time the tuna farming season commences. It is evident from the current literature that there are flesh quality benefits to be gained from vitamin and mineral supplementation, nonetheless, a great deal more research into the nutritional profiles of the baitfish fed to the tuna, the dietary requirements of farmed tuna, and delivery methods is required. In any case, the declines in vitamin C and selenium, and static vitamin E levels support the notion that supplemental concentrations of these beneficial vitamins and mineral be increased.

Of the instrumental data collected in Port Lincoln, CIE L*a*b* colour measurements did not differ statistically between the high and low vitamin treatments over each eight-day sampling period in March and April. The CIE L*a*b* colour system is based on reflectance spectra. Tuna flesh, as with many food types, is a relatively translucent and labile matrix that does not possess consistent reflectance properties and homogenous pigmentation (Mackinney *et al.* 1966; Little & Mackinney, 1969). Absorbance, scattering and internal transmission of the

incidence light affects the ratio of light returned to the photocell, and it is from this proportion of light, relative to the standard, that colour is enumerated. The eye however, does not distinguish between the relative contributions of light scattering and absorption, evaluates the composite visual appearance (Mackinney *et al.*, 1966). As a result, where the eyes of panellists may detect differences in the composite visual appearances of samples, reflectance colorimeters may not. This potential divergence between the sensory and the instrumental measurement of 'colour' was evidenced in the present trial. In contrast to the CIE L*a*b* data, the panellist evaluations of colour in Port Lincoln in both March and April yielded significant differences between the low and high vitamin treatments. Similar findings have been reported in colour stability trials on beef (Gatellier *et al.* 2001), mince (Houben *et al.* 2002), and pork (O'Sullivan *et al.* 2002) where significant differences in the colour of samples seen by panellists were not corroborated by instrumental measures. According to O'Sullivan *et al.* (2002) the 'human' result is more important owing to the fact that the purchase of meat is driven primarily by visual sensory evaluation, and the authors question the validity of using, and the over-reliance on instrumentation to mimic human observation.

The flesh pH levels between treatments in Port Lincoln were not significantly different during the sampling period in March but were statistically significant for the majority of the April sampling period. The pH of skeletal muscle is one of the most significant post-mortem parameters that influence the quality of muscle foods during

storage. The pH of flesh affects both the chemistry of myoglobin and the development of proteolytic microorganisms (Lo Fiego *et al.* 2004). Low pH values favour the oxidation of myoglobin (Faustman & Cassens, 1990) and thus the pH of the flesh samples from the low vitamin treatment were significantly lower than the pH of the samples from the high vitamin treatment in April could explain the significant difference between treatments detected by the colour panel. The higher average pH values recorded from the flesh samples in March when compared to April could be linked to the significantly higher vitamin C concentrations recorded at this earlier stage in the farming season. Vitamin C can be metabolised to oxalic acid, which, as an inhibitor of glycolysis, maintains higher levels of pH in post-mortem flesh (Kremer *et al.* 1999 in Lo Fiego *et al.* 2004). The significant differences in pH between the high and low vitamin treatments in April, as opposed to March, may have resulted from higher levels of accumulated glycogen in the flesh later in the season.

In contrast to almost all of the instrumental data, the colour panel ranking data showed significant differences between the low and high vitamin treatments during March and April in both Port Lincoln and Japan. Where significant, the samples from the high vitamin treatment were redder in colour than the samples from the low vitamin treatment, thus signifying both the beneficial effects of vitamins on the colour stability of sashimi tuna, and the validity, if only limited, of the delivery method under investigation. It is worth noting that although some non-significant results

interrupt the data sets, an alpha value of 0.10 would produce significant results for the duration of trials in both Australia and Japan in March.

Nevertheless, given no increase in the flesh concentrations of vitamin E, and gradual declines in the concentrations of vitamin C, substantial improvements in flesh colour stability could result from significantly increasing the concentrations of these effective vitamins and minerals in the flesh of farmed tuna. It must be stressed that although the panellists used in the sensory assessment of colour were trained they were not trained extensively and repeatedly with the product due time and resource restraints and therefore the results, it could be argued, would be strongly linked to the ranking capabilities of consumers in differentiating the colour of the flesh of sashimi tuna.

3.6 Conclusions

Vitamin supplementation has been categorically proven to aid in the colour retention of the flesh of commercially relevant farmed Southern bluefin tuna with low to medium levels of fat both in Australia and in Japan. Whether improvements in the colour stability extended into the fattier portions of the flesh cannot be elucidated from this trial.

Whether or not the reduction in the concentration of vitamin C and selenium in the flesh is a natural decline, due to a dietary shortfall, or otherwise associated with the

farming process needs to be determined. Further research into the nutritional profiles of baitfish that are currently fed to farmed tuna, the affects of species, location, storage temperature, and storage time will help to improve the quality and supply of not only the micronutrient but also the macronutrient fraction of the baitfish fed to the tuna in Port Lincoln. This in turn may improve the quality attributes and colour stability of the product in Japan.

In the future, research into alternative delivery methods such as vascular infusion and the spraying of loins, or other antioxidants such as tocotrienol, taurine, carnosine, and rosemary powder, may prove beneficial to the colour stability of tuna flesh as they have in beef (Sanchez-Escalante *et al.* 2001; Djenane *et al.* 2002; Djenane *et al.* 2003b).

The results of this trial suggest the instrumental measurement of colour using tri-stimulus reflectance meters may not be suited to translucent, labile, uneven, and heterogeneously pigmented tuna flesh. It may be possible to psychophysical scale the instrumental scatter coefficients over time, but where available, panel assessment of colour is preferable.

4 COMMERCIAL LEVEL HARVEST STRESS AND IT'S CONSEQUENCES ON THE PHYSICO - CHEMICAL AND SENSORY FLESH QUALITIES OF FARMED SOUTHERN BLUEFIN TUNA IN JAPAN

4.1 Introduction

The harvesting of farmed Southern bluefin tuna involves the setting and running of a small seine net through a tuna pontoon in an effort to confine a quantity of tuna that will meet estimates of market demand (or orders) at any one time. The seine net is divided into two areas, a shallow area holding the tuna for the first half of the harvest, and a deeper area holding the tuna for the second half of the harvest (Fig. 4.1).

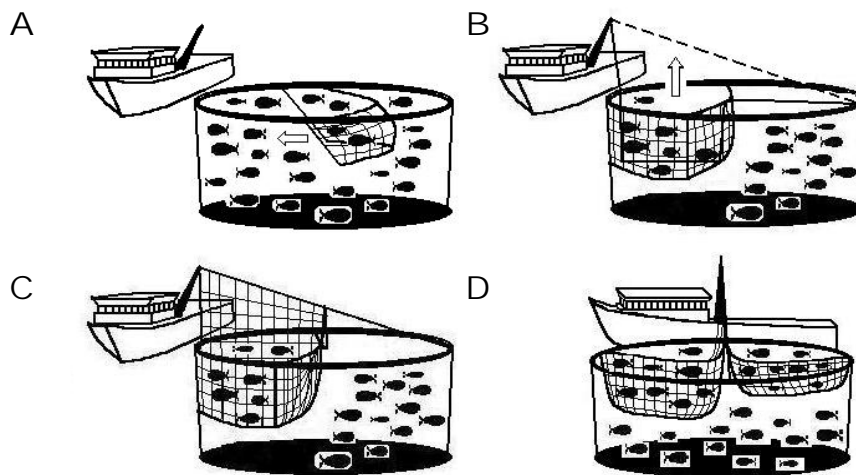


Figure 4.1: A typical harvesting method of farmed Southern bluefin tuna using a seine net to capture a desired number of tuna (A), the attachment of a dividing line (B), the raising of the dividing line (C), resulting in a holding area (deeper left side) and a harvesting area (shallower right side) with approximately equal numbers of tuna in each (D).

The tuna in the harvesting (shallow) portion of the divided seine net are caught by hand using divers who grab the fish by the tail, quickly turn the fish upside-down to



disorient them, and lead them by the isthmus that separates the gill cavities to a platform for killing (see inset). The tuna are immediately spiked through the brain, bled, cored and wired (iki jime method), gilled and gutted, and then hung in ice slurry.

The duration from being caught by a diver to ice slurry ranges from between 2 to 5 minutes. As the tuna are being harvested the volume of water that confines the fish is reduced by a gradual drawing up of the seine net to confine the tuna so they can be captured as efficiently as possible.

The numbers of tuna harvested in a single day and the frequency of harvesting events from a typical pontoon varies greatly according to the destination market and the size of the tuna farming operation. A typical harvesting strategy for the air-freighted, fresh market sees between 100 and 250 fish harvested each day, continuously (weather and market condition dependent) over a one to two week period until the pontoon is emptied. For the frozen market as many as 1200 tuna per day can be harvested from a single pontoon with the main limiting factor being the freezing capacities of the processing plants on land or on-board freezer ships. In this way it may only take 2-3 days to empty a pontoon. Tuna destined for the fresh markets of Japan are normally harvested in quantities to fill the unit load devices (ULD) of aircraft. A typical shipment of fresh tuna to Japan comprises two ULD's –

an AVA container (Rate Class 8) and a pallet with netting (Rate Class 5) that hold 20 and 55 tuna coffins respectively (see SCM Chapter section). A single tuna coffin has an external dimension of 1420 mm by 410 mm by 310mm and is designed to hold two tuna under 35kg (gilled and gutted weight) or a single tuna of over 35kg. Therefore the number of tuna harvested for a single ULD will vary according to the individual weights of the fish caught during a harvest. On average, between 110 and 140 tuna are harvested for a single shipment to Japan.

Stress is a commonly used word to describe a disturbance of sorts but a universal definition has proven elusive, prompting Seymour Levine (1985) to declare *"I am not certain whether one who undertakes this task (of defining the concept of stress) either has an enormous ego, is immeasurably stupid, or is totally mad"*. Nevertheless, during the harvest of Southern bluefin tuna the actions of confinement, pursuit, and capture to the point of death are all commonly recognised 'stressors' for the animal. The setting of the seine net and the confining of the tuna ready for harvest takes approximately 15-20 minutes and all tuna are harvested in approximately 45 to 70 minutes by work units of divers and cutters closely cooperating with each other. Within this time frame there is a minimum and maximum confinement stress of approximately 20 minutes and 90 minutes respectively. Capture stress is defined here as the duration that a tuna is pursued by divers and can range from less than 60 seconds to 35 minutes. Struggle stress is defined here as the period between a tuna being actually caught by a diver till the

point of euthanasia and is usually no more than 60 seconds. Individual tuna can react differently to being handled by divers and how they are handled and their level of exhaustion. Direct contact with the gills often irritates the animals and they will struggle more with the diver (Fig. 4.2).



Figure 4.2: Tuna calmly (possibly exhausted) being led to the killing platform (left) and struggling with a diver (right) (Source Kyodo News).

Fish are considered to be exhausted when they are no longer capable of burst activity although they may be capable of lower energy swimming - an ability all too important for ram jet ventilators as they need to move in order to aerate their gills (Milligan, 1996). Exercise, especially to exhaustion, can result in severe metabolic, acid-base, and endocrine disturbances in fish, and the ability of a species to cope with such physical demands places limits on the overall performance of the animals in both wild and in culture environments where stressors can be frequent (Milligan, 1996).

The stress response in fish, according to Iwama *et al.* (2004), can be classified into three categories - primary, secondary and tertiary responses. The primary response is neuroendocrine in nature and is comprised of the adrenergic response and the hypothalamo-pituitary-interrenal (HPI) response which results in the rapid release of catecholamines and cortisol respectively. In bony fishes, catecholamines, primarily adrenaline and noradrenaline, are released from the chromaffin cells situated throughout the head kidney, the walls of the posterior cardinal vein, and from the extremities of adrenergic nerves (Sumpter, 1997; Iwama *et al.*, 2004). Plasma corticosteroids, including the physiologically important cortisol, are secreted from the inter-renal tissue of the anterior kidney in response to a cascade of hormones that commences with corticotrophin - releasing hormone (CRH) secreted by the hypothalamus and adrenocorticotrophic hormone (ACTH) secreted by the pituitary gland (Sumpter, 1997; Iwama *et al.*, 2004).

The secondary response refers to the hormone-activated biochemical and physiological effects of stress and their associated impacts on a number of metabolic pathways that result in the alteration of the blood chemistry and haematology (Iwama *et al.*, 2004). One of the most commonly used secondary stress response indicators in animals, including fish, is the concentration of plasma glucose - the main energy supply for the brain, gills, and muscles during the peak energy demand. The processes of glycogenolysis and gluconeogenesis that

produce glucose in the liver are actuated by the presence of adrenaline and cortisol and that have a short- and long-term action respectively (Iwama *et al.*, 2004). In fish the Emden-Meyerhof-Parnas pathway of glycolysis yields energy via the catabolism of glucose, or glycogen derived glucose, to pyruvate which is then oxidised to CO₂ and H₂O via the citric acid cycle in aerobic conditions, or converted to lactate by lactate dehydrogenase in anaerobic conditions (Driedzic & Hochachka, 1978). The intensity and time of burst activity influences the rate of depletion of glycogen via anaerobic catabolism and the accumulation of lactate in white muscle, which, can be extremely rapid (Driedzic & Hochachka, 1978). During severe energy demands, such as those during a stress event, the rate of utilization of glycogen can be up to 3 times higher in red muscle when compared to white muscle on a per weight basis (Johnston and Goldspink, 1973 in Driedzic & Hochachka, 1978).

Tertiary responses to stress are at the whole body level where reproductive capacities, growth, and the overall fitness of an animal or a population of fish can be compromised (Barton, 2002; Iwama *et al.*, 2004).

The secondary bio-chemical responses to stress, and their effects on the post-mortem qualities of meat, have been the focus of much research in terrestrial livestock industries. Acute pre-slaughter stress can result in flesh with a higher than normal ultimate pH, which, according to Faustman and Cassens (1990), results in meat that has a greater water holding capacity and a tighter structure that reduces

the rate of oxygen diffusion, pigment oxygenation, and decreases the amount of reflected light from the surface giving the meat a darker and drier appearance. Increased mitochondrial activity at higher pH levels can result in elevated oxygen consumption in muscle tissue, which, according to those authors, can accelerate darkening. Alternatively a rapid pH drop in the flesh of stressed animals increases protein denaturation raising the level of insoluble proteins that, with consequent water loss, changes the way light is reflected from the surface and therefore the lightness, hue and chroma of the flesh resulting in light-cutting meat (Warriss & Brown, 1987).

Poor handling of pigs prior to slaughter has been shown to result in higher muscle temperatures, faster rates of pH decline, and flesh with high levels of drip, that is pale in colour, is soft, and exudative (PSE) (Briskey, E.J., 1964; D'Souza *et al.*, 1998; Juncher *et al.*, 2001), or dark, firm, and dry (DFD) flesh from those animals with low initial glycogen levels which is characterized by a high ultimate pH.

High levels of pre-slaughter stress have also been shown to negatively affect the tenderness of lamb (Geesink *et al.*, 2001), the flesh qualities of reindeer meat (Malmfors & Wiklund, 1999), the palatability of beef (Jeremiah *et al.*, 1988), the texture of rabbit (Jolley, 1990), and the appearance of poultry meat (Debut *et al.*, 2003),

The metabolic disturbances associated with exhaustive exercise are greater in fish than they are in mammals (Milligan, 1996), and ante-mortem stress has been shown to elevate K value - an index of nucleotide metabolism related to sensory assessment of freshness (MAFF, 1989; Izquierdo-Pulido *et al.*, 1992; Lowe *et al.*, 1993), palatability (Tejada & Huidobro, 2002), and, via ATP depletion, initiate the onset of rigor mortis and result in a softer and a more tender muscle (Ando *et al.*, 1992; Izquierdo-Pulido *et al.*, 1992 ; Nakayama *et al.*, 1994; Sigholt *et al.*, 1997; Thomas, *et al.*, 1999; Roth *et al.*, 2002). Contrary to this, Skjervold *et al.* (2001) reported that the earlier onset and resolution of rigor in Atlantic salmon (*Salmo salar*) due to the stress of ante-mortem crowding, resulted in a firmer flesh. This led Skjervold *et al.* (2001) to hypothesize that ante-mortem stress affects the firmness of salmon in opposite ways depending on the severity and duration of the stress with short-term stress leading to muscle softening similar to PSE-meat and long-term stress leading to increased muscle firmness similar to DFD-meat as seen in mammal meat.

According to Erikson *et al.* (1997a) levels of IMP are retained longer in the muscles of unstressed fish delaying the formation of hypoxanthine and resulting in lower K values for fish stored in ice over several days. The affects of high levels of ante-mortem muscle activity as simulated via the post-mortem electro-stimulation of the flesh of rainbow trout resulted in fillets with shorter times to rigor, that were significantly lighter, less red, and that were also more susceptible to gaping (Robb

et al., 2000). Eels that exhibited fewer aversive actions during slaughter had flesh that was firmer, redder, and exhibited reduced rates of lipid oxidation and loss of freshness (Morzel & van de Vis, 2003). Pink salmon (*Onchorhyncus gorbuscha*) that were stressed by tagging and confinement had reduced muscle weight and an increase in liver weight concomitant to a considerable mobilisation and transport of specific fatty acids from the muscle to the liver (5.9% liver lipid in controls and 10.4% in tagged and confined fish) (Love, 1980). The energy demands of stress can temporarily arrest osmoregulation in fish also causing a weight increase and a weight decrease in fresh water and marine fish respectively as they take in or lose water (Love, 1980).

The response to stress is also species-specific with great variations in the chemistry of the blood, muscle, and liver witnessed between species (Love, 1980). Tuna, when compared to other species of fish, are extraordinary in that they are the fastest swimmers, are among the largest of fishes, have the warmest bodies and highest metabolic rates, and are exceptional in their biochemistry with unique haemoglobins and the highest concentrations of lactate, glycogen, and actomyosin (Stevens & Neill, 1978). In tuna, activity generates heat faster than it is conducted away and the body temperature of these species appreciates substantially with the effect of speeding up enzymatic processes making further energy available (Zharov, 1965 in Love, 1970).

One of the well known tuna flesh quality issues on the Japanese sashimi markets is termed *yake* (burn) and refers to meat that is dull, opaque, pale in colour, rough in texture and therefore has limited or even no value as sashimi. The actual causes are uncertain but are commonly believed to be the result of high temperatures and the accumulation of lactic acid in the muscle during the stress of capture (Fig. 4.3; Ohta *et al.*, 2004; Caill-Milly *et al.*, 2001). Watson *et al.* (1988) proposed that *yake* in tuna is caused by the post-mortem activation of enzymes known as calcium-activated proteases and that high blood catecholamine levels directly relates to the activity of these enzymes. The point at which the flesh of a tuna is considered to be suffering from *yake* is not clearly defined but it is commonly an arbitrary decision made on the markets by wholesalers and/or intermediate wholesalers when a fish is core-sampled and finally loined.

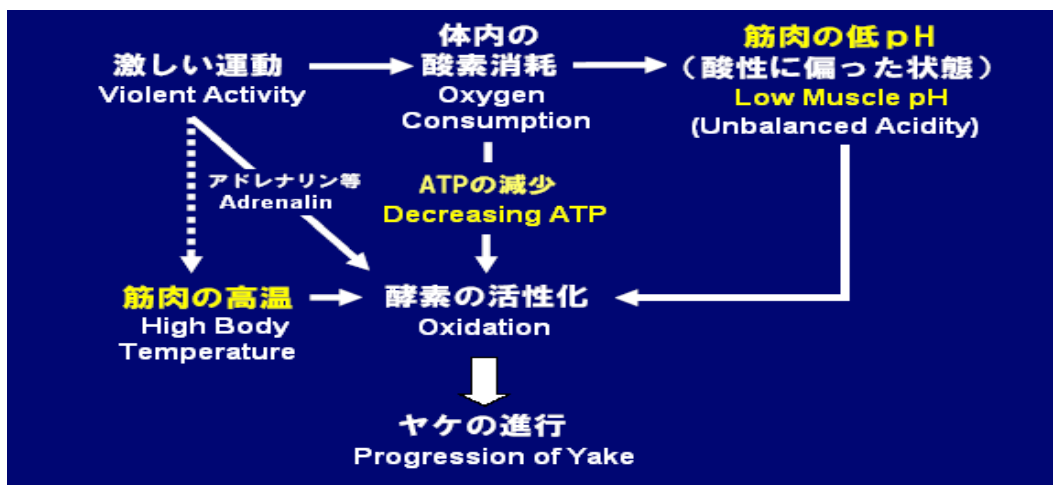


Figure 4.3: Proposed pathway for the causes and onset of 'yake' (burn) in tuna (image translated from a presentation by Ohta *et al* during the Symposium of the Okinawa Prefectural Fisheries Research and Testing Facility at the Fisheries Assembly of Okinawa, 16th October, 2003.)

If the flesh of a tuna is considered to be suffering from yake then an insurance claim is submitted to recover costs. Although yake is not generally considered a problem in the sale of farmed Southern bluefin tuna on the markets in Japan it is possible any physical and biochemical changes accompanying the stress response witnessed during a commercial tuna harvest in Australia may affect the perceivable qualities of the flesh in Japan. Although the effects may not quite be to the point of being termed yake (i.e. unfit for raw consumption), it may exhibit 'mild' degrees of yake thus reducing its value. It has been these possible effects of harvest stress on the flesh qualities of the final product that have been of interest to both the industry and researchers alike since the advent of tuna farming.

4.2 Aims & Objectives

In aquaculture the affects of stress on growth, reproductive physiology, and the immunocompetence of fish have been well documented. Although very important factors they are production focussed and it must not be forgotten that the final product of most aquaculture industries is a food item with appearance, flavour, texture and storage characteristics that compete with other items of food. That being the case it is therefore very important to examine the effects of harvest stresses on the properties of the flesh of farmed SBT. To do this, experiments were devised to examine the biochemical and sensory properties of the flesh of farmed SBT, from fish caught at the start of harvest and those caught at the end of harvest. Assessments of flesh quality were conducted on samples retained in Port Lincoln and from the remainder of the fresh carcass after it was air-freighted to Japan.

4.3 Methods

At the commencement of a commercial harvest on May 19, 2003 four tuna were temporarily removed from the harvesting line of a commercial operator for blood sample collection, flesh core removal, insertion of a data logger pre-programmed to record temperature every 15 minutes, and tagging. The selected tuna were of approximately equal size (average weight 32.7 kg gilled and gutted) due to the fact that the relative amount of red muscle mass as a proportion to total mass decreases in larger tunas and, as a result, any size variation could impact the aerobic scope and rates of lactate clearance between individuals (Korsmeyer & Dewar, 2001).At

the end of the same harvest, which was approximately 50 minutes in duration, four tuna (average weight 31.8 kg gilled and gutted) were removed for the same process. The initial and final four tuna constituted the Start of Harvest and End of Harvest treatments for this experiment respectively.

The tuna were processed at Australian Bight Seafoods in Port Lincoln and trucked in refrigerated conditions overnight to Adelaide and loaded on Qantas flight QF738 to Sydney at 9:30am (ACST), May 20, 2003, where they were held in chilled storage for 24 hours prior to loading on Qantas flight QF21 departing Sydney at 9:30pm, May 21, 2003 (AEST) arriving at Narita International Airport in Japan at 7:30am, May 22, 2003 (AEST). The tuna were then transported to the Nittsu Distribution Centre where their coffins were opened, re-iced, and re-sealed for refrigerated trucking to Tokyo Uoichiba Pty. Ltd. at the Tsukiji Wholesale Fish Markets in Tokyo.

The tuna were loined by the wholesaler Sugahei Ltd. at around 4:30am on the 23rd of May, 2003, with the individual loins wrapped and placed in sealed Styrofoam boxes with ice as per industry practice. The loins were transported immediately to the Central Research Laboratory of Nippon Suisan Pty. Ltd., Hatchioji, Tokyo, where they were sectioned into their Akami, Chutoro and Otoro portions and then further sectioned into sashimi slices on that day for sensory testing by 12, 10, and 18 person panels respectively. Panellists at the Hatchioji laboratories have been

trained in the processes of sensory evaluation, routinely examine seafood products every day and are extremely familiar with the quality attributes and descriptors often employed in the assessment of raw fish.

The sensory descriptors examined included brightness, redness, transparency, bad fishy odour, bad fishy flavour, sourness, umami and hardness. Interval scales from 1 through to 5 with anchoring terms were used by panellists to express their perceptions. The anchoring terms were chosen to reflect the intensities of the selected sensory descriptors and ranged from either extremely weak (redness, transparency, umami), extremely dark (brightness), extremely soft (hardness) or not present (sourness, bad fishy odour, bad fishy flavour), to the antonyms of extremely strong (redness, transparency, umami, sourness), extremely bright (brightness), extremely firm (firmness) or extreme (bad fishy odour and bad fishy flavour) (see Methods: Fig. 2.16).

Physical indicators of quality included measures of CIE Lab using a *Minolta CR-310* colorimeter and RGB ratio analysis of images taken with a *Minolta DiMAGE 5* digital camera prior to sensory assessment at the Central Research Laboratories of Nippon Suisan, Hatchioji, Japan. At the same time flesh samples were taken from slices adjacent to those used for sensory evaluation, wrapped in aluminium foil and frozen in solid CO₂ for transport to the laboratory and transferred to liquid nitrogen for later analyses Biochemical measures recorded and analysed from the flesh

samples taken in Japan included pH (prior to freezing), percentage metmyoglobin, nucleotides, hydroperoxides, and lactate (see Methods 2.2).

Sensory data was examined by Principal Components Analysis (*Guideline + 7.8*) and SPANOVA (Split Plot Analysis of Variance; *Genstat*) with treatment and interaction effects examined at the panellist, sashimi fillet, and individual fish level. Biochemical data was analysed by one-way ANOVA (Analysis of Variance; *SPSS*).

4.4 Results

Time-Temperature Profiles

The data logger time-temperature profiles from the point of insertion in Port Lincoln, through transit, and to the point of removal in Japan were not significantly different ($P < 0.05$) between the Start and End of harvest treatments (Fig 4.4).

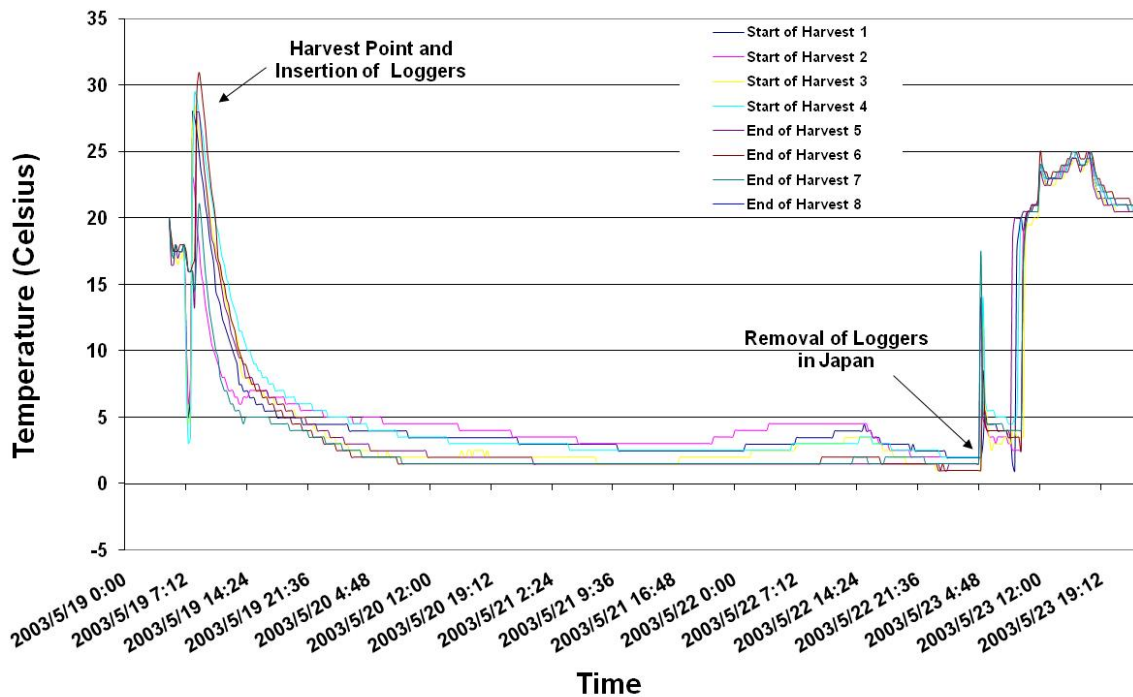


Figure 4.4: Carcass time-temperature profiles of farmed Southern bluefin tuna from the Start and End of a harvest in May of the 2003 farming season in Port Lincoln air-freighted to Tokyo, Japan (Start n=4, End n=4).

Physico-Chemical Analysis

The average plasma pH values at harvest were not significantly different between the Start and End of Harvest treatments ($P>0.05$; Fig. 4.5).

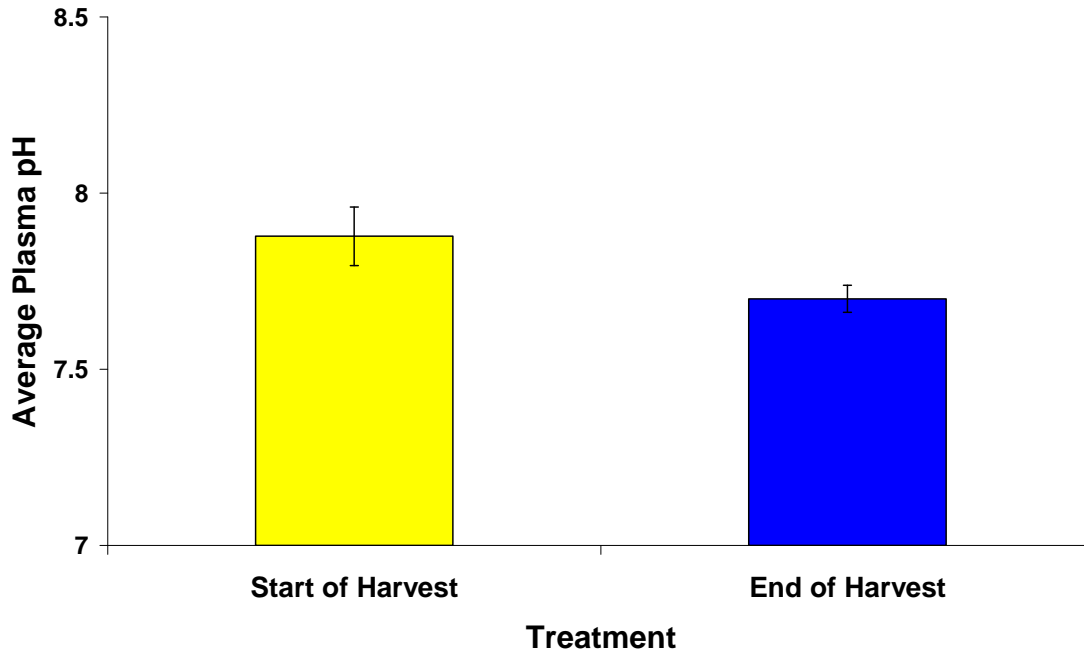


Figure 4.5: Plasma pH values (mean \pm SEM) of the blood of farmed Southern bluefin tuna from the Start and End of a harvest in May of the 2003 farming season in Port Lincoln (Start $n=4$, End $n=4$; 6~7 hours post-mortem).

No trend and no significant differences ($P>0.05$) were found between the Start of Harvest and End of Harvest treatments within and between the cuts of Akami, Chutoro and Otoro for ultimate flesh pH values (Fig 4.6).

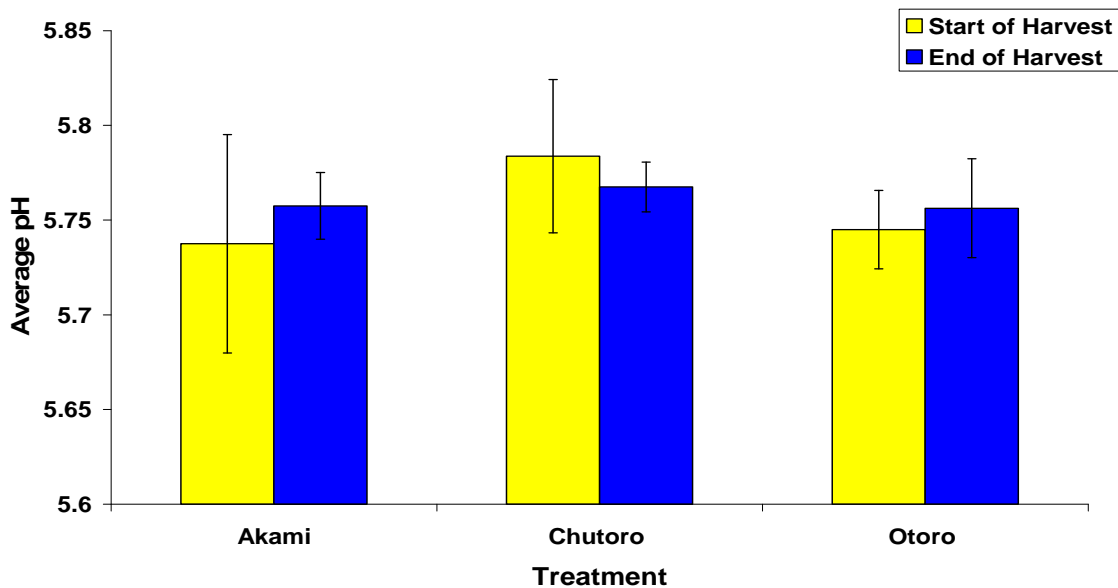


Figure 4.6: Ultimate pH values (mean \pm SEM) recorded from the surface of flesh samples of the Akami, Chutoro and Otoro cuts of farmed Southern bluefin tuna in Japan from the Start and the End of a harvest in May of the 2003 farming season in Port Lincoln (Start $n=4$, End $n=4$; day four post-mortem).

No significant differences ($P>0.05$) between the Start of Harvest and End of Harvest treatments for the flesh lactate concentrations were recorded for the cuts of Akami, Chutoro and Otoro (Fig. 4.7). No significant differences ($P>0.05$) were also found between the Start of Harvest and End of Harvest treatments within and between the cuts of Akami, Chutoro and Otoro for the freshness indicator K value (Fig 4.8).

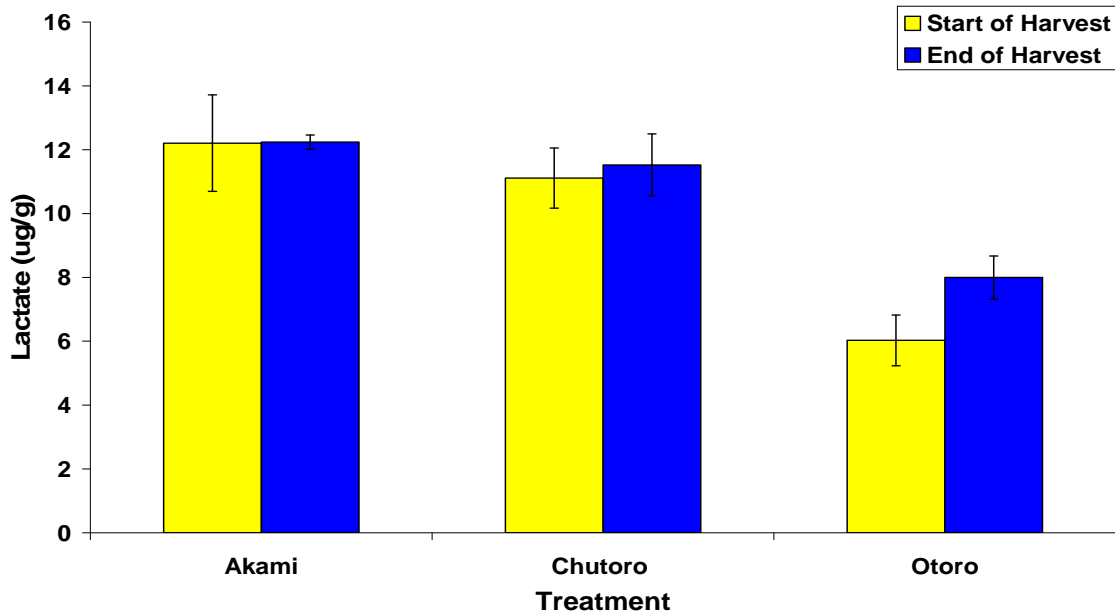


Figure 4.7: Lactate concentrations (mean +/- SEM) from the muscle samples of the Akami, Chutoro and Otoro cuts of farmed Southern bluefin tuna in Japan taken from the Start and End of a harvest in May of the 2003 farming season in Port Lincoln (Start n=4, End n=4; day four post-mortem).

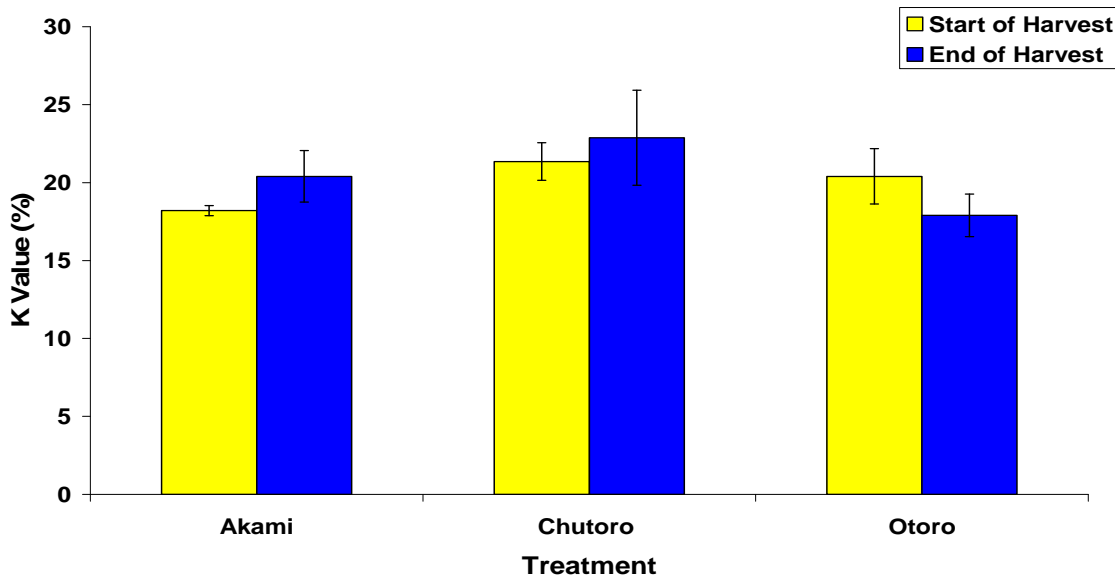


Figure 4.8: Percentage K values (mean +/- SEM) recorded from muscle samples of the Akami, Chutoro and Otoro cuts of farmed Southern bluefin tuna in Japan taken from the Start and End of a harvest in May of the 2003 farming season in Port Lincoln (Start n=4, End n=4; day four post-mortem).

The concentration of the nucleotide inosine monophosphate (IMP) was significantly higher ($P < 0.05$) in the muscle samples of the Akami cut of fish from the Start of Harvest when compared to the End of Harvest treatment (Fig 4.9). The remaining Chutoro and Otoro cuts did not have significantly different concentrations of IMP between the Start and End of Harvest treatments respectively.

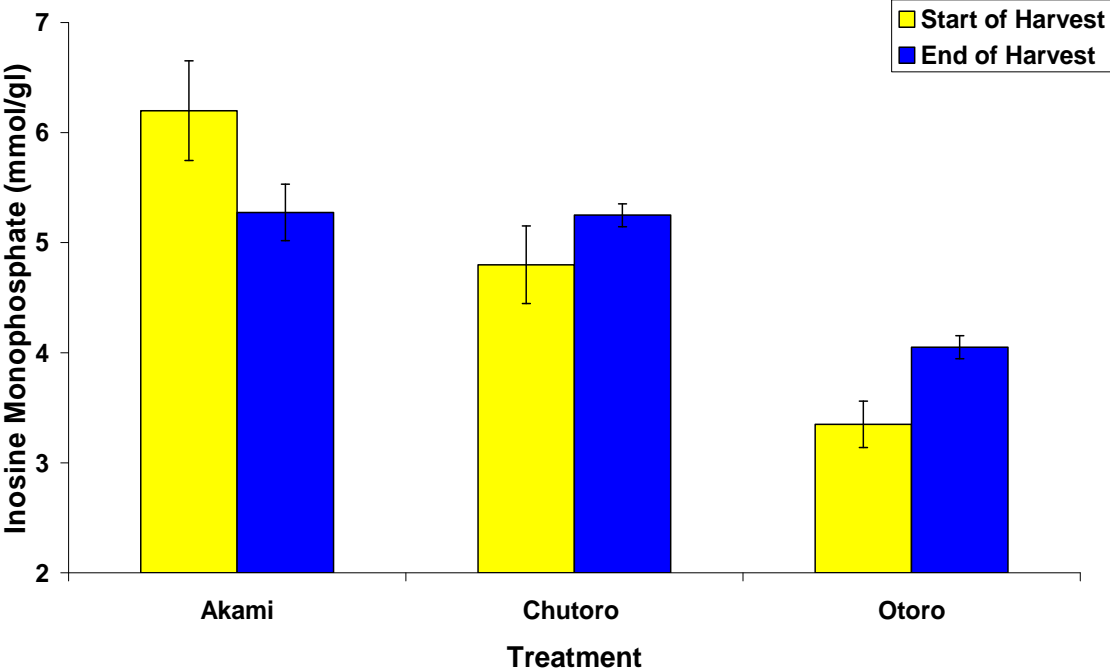


Figure 4.9: Inosine monophosphate concentrations (mean \pm SEM) from the muscle samples of the Akami, Chutoro and Otoro cuts of farmed Southern bluefin tuna in Japan taken from the Start and End of a harvest in May of the 2003 farming season in Port Lincoln (Start $n=4$, End $n=4$; day four post-mortem).

No significant differences ($P>0.05$) between the Start and End of Harvest treatments for the biochemical indicator of rancidity, hydroperoxide (OOH), were recorded for the cuts of Akami, Chutoro and Otoro (Fig. 4.10) but the levels found were highest in the fattier cuts (Akami < Chutoro < Otoro).

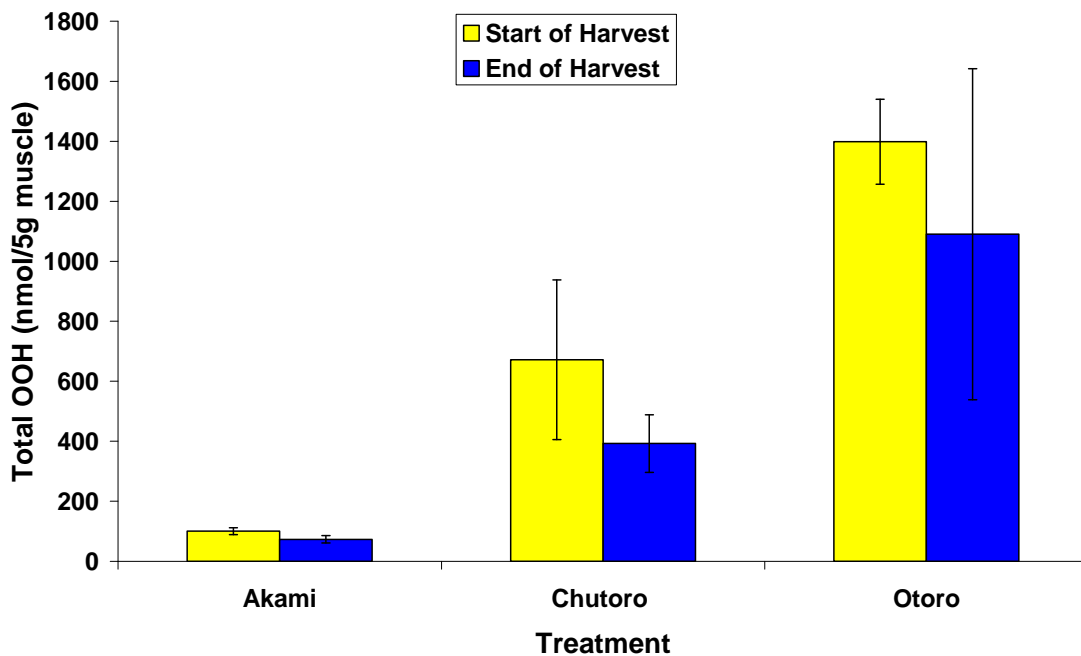


Figure 4.10: Hydroperoxide concentrations (mean \pm SEM) from the muscle samples of the Akami, Chutoro and Otoro cuts of farmed Southern bluefin tuna in Japan taken from the Start and End of a harvest in May of the 2003 farming season in Port Lincoln (Start $n=4$, End $n=4$; day four post-mortem).

No significant differences ($P>0.05$) were found between the Start of Harvest and End of Harvest treatments within and between the cuts of Akami, Chutoro and Otoro for the percentage of the brown coloured pigment metmyoglobin (Fig. 4.11). Further, there were no significant differences ($P>0.05$) found between the Start of Harvest and End of Harvest treatments within the cuts of Akami, Chutoro and Otoro for CIE L, a^* and b^* measures of lightness and colour (Fig. 4.12).

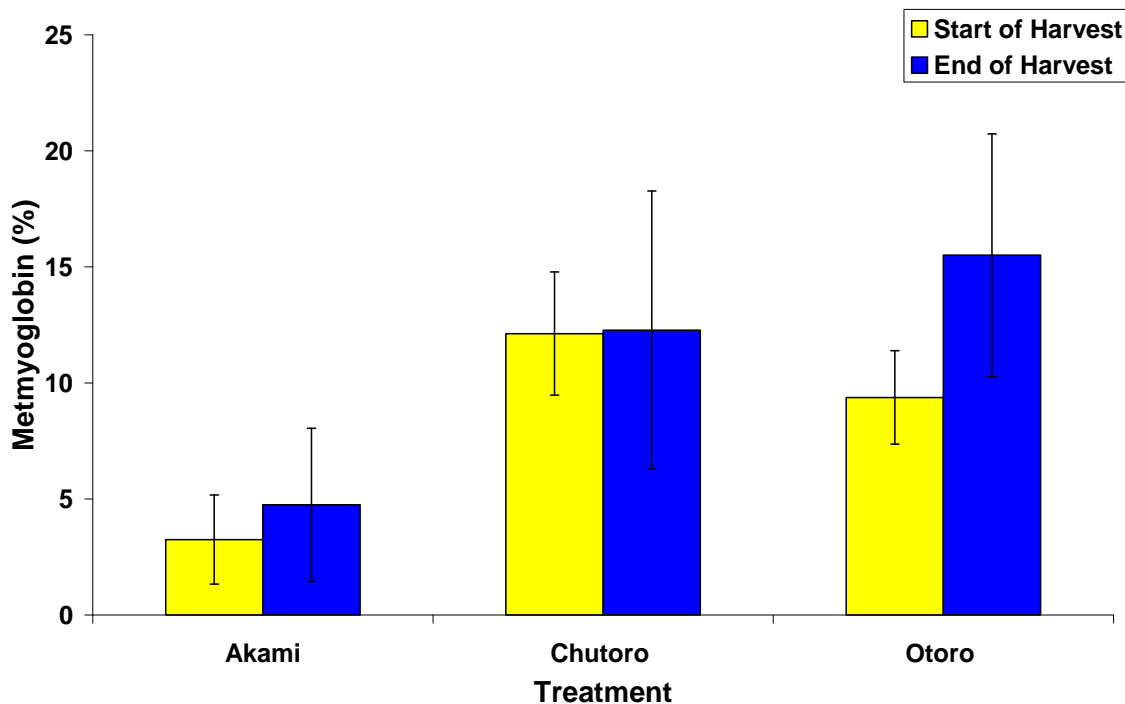


Figure 4.11: Percentage metmyoglobin values (mean \pm SEM) from the muscle samples of the Akami, Chutoro and Otoro cuts of farmed Southern bluefin tuna in Japan taken at the Start and End of a harvest in May of the 2003 farming season in Port Lincoln (Start $n=4$, End $n=4$; day four post-mortem).

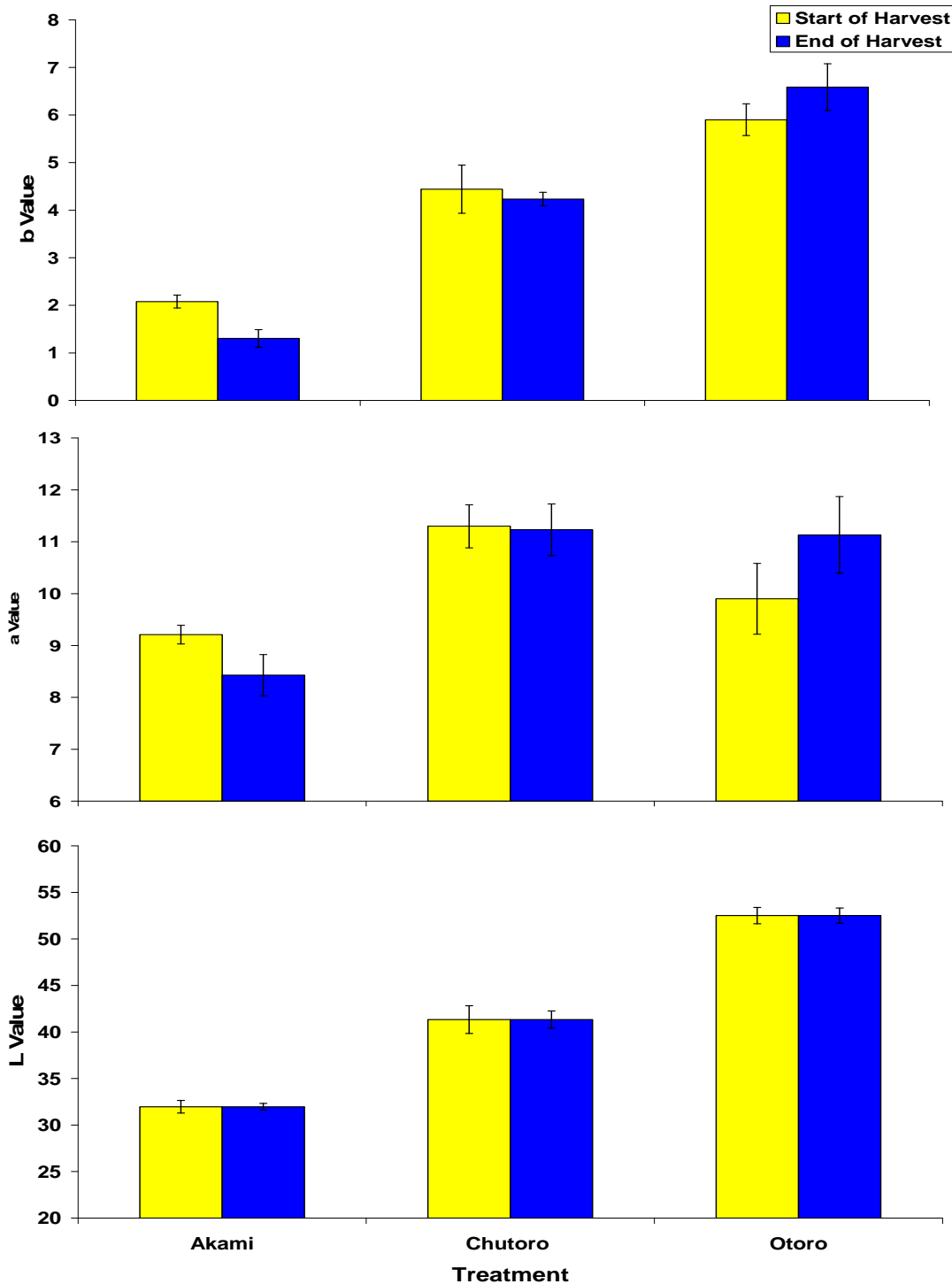


Figure 4.12: CIE Lab values (mean \pm SEM) recorded from the surface of flesh samples of the Akami, Chutoro and Otoro cuts of farmed Southern bluefin tuna in Japan taken from the Start and End of a harvest in May of the 2003 farming season in Port Lincoln (Start n=4, End n=4; day four post-mortem).

In the Akami cut the Start of Harvest treatment had a significantly higher ($P < 0.05$) digital camera RGB ratio than the End of Harvest treatment. No significant differences ($P > 0.05$) for RGB ratio were recorded between the Start of Harvest and End of Harvest treatments for the cuts of Chutoro ($P = 0.115$) and Otoro ($P = 0.854$; Fig. 4.13).

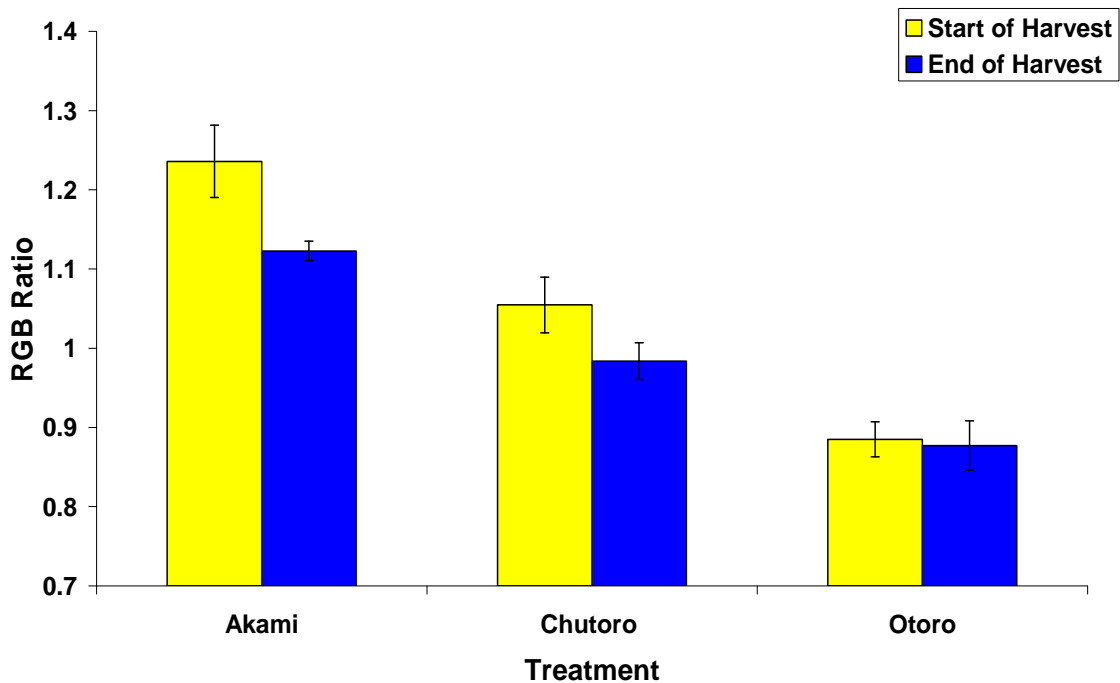


Figure 4.13: Digital camera RGB ratios (mean +/- SEM) recorded from the surface of samples of the Akami, Chutoro and Otoro cuts of farmed Southern bluefin tuna in Japan taken from the Start and End of a harvest in May of the 2003 farming season in Port Lincoln (Start n=4, End n=4; day four post-mortem).

Sensory Analyses

Split plot analysis of variance of the Akami and Chutoro cuts for all eight sensory descriptors returned no statistically significant differences between the Start of Harvest and End of Harvest treatments (Fig. 4.14 and Fig. 4.15). The sensory scores for the Otoro were not significantly different ($P>0.10$) between treatments except for the descriptors of brightness ($P=0.029$) and transparency ($P=0.087$), with the Start of Harvest treatment significantly brighter and more transparent than the End of Harvest treatment (Fig. 4.16).

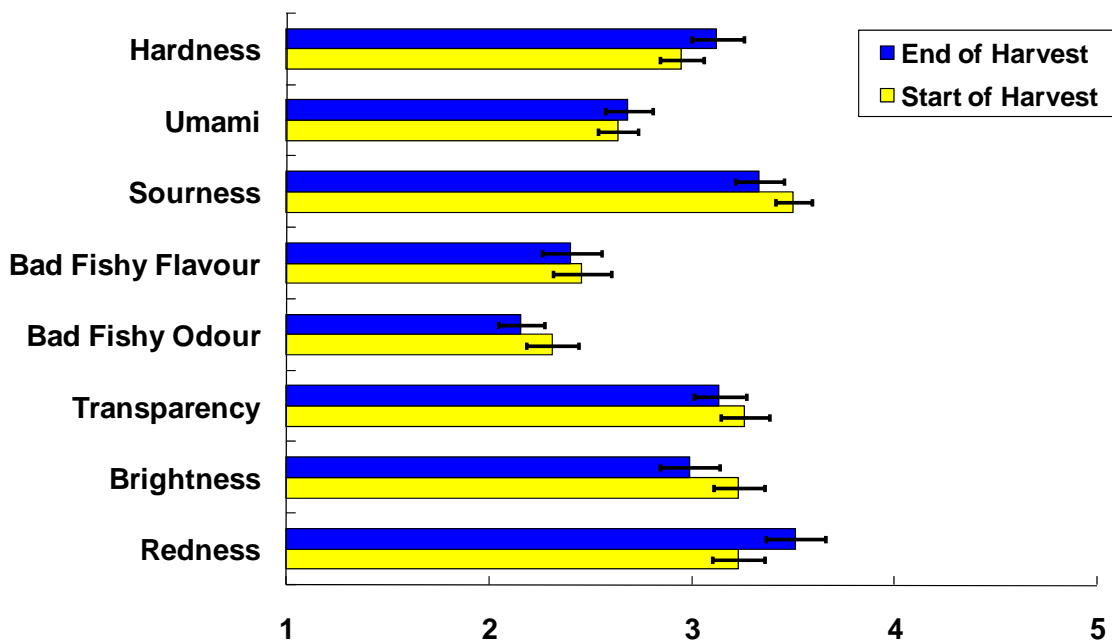


Figure 4.14: Average sensory scores (mean \pm SEM) for sashimi sections ($n=12$) of the Akami cuts of farmed Southern bluefin tuna in Japan taken from the Start and End of a harvest in May of the 2003 farming season in Port Lincoln (Start $n=4$, End $n=4$; day four post-mortem).

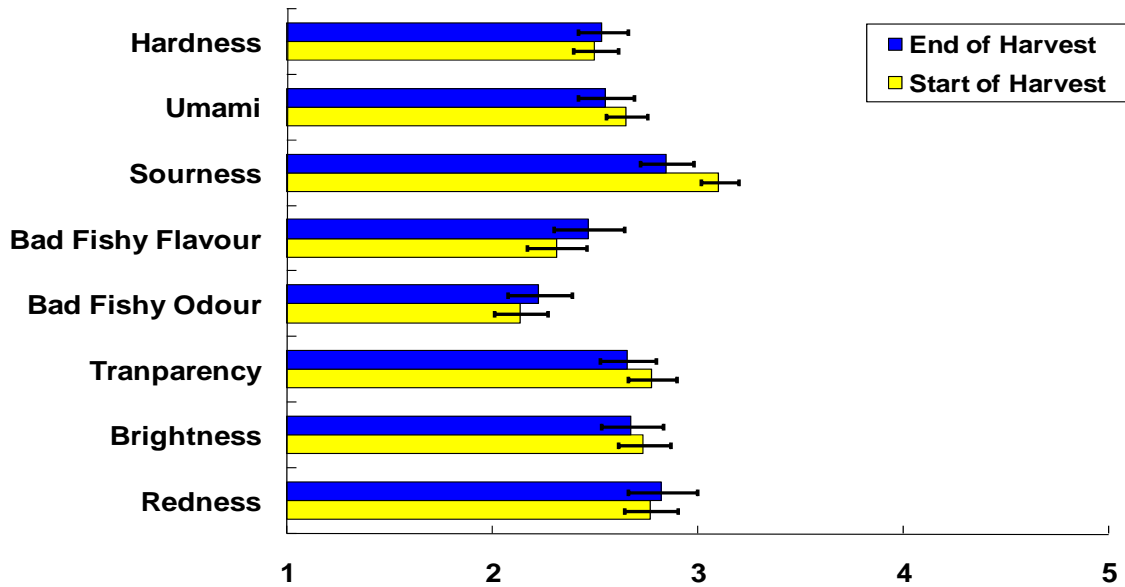


Figure 4.15: Average sensory scores (mean +/- SEM) for sashimi sections (n=10) of the Chutoro cuts of farmed Southern bluefin tuna in Japan taken from the Start and End of a harvest in May, 2003 farming season in Port Lincoln (Start n=4, End n=4; day four post-mortem).

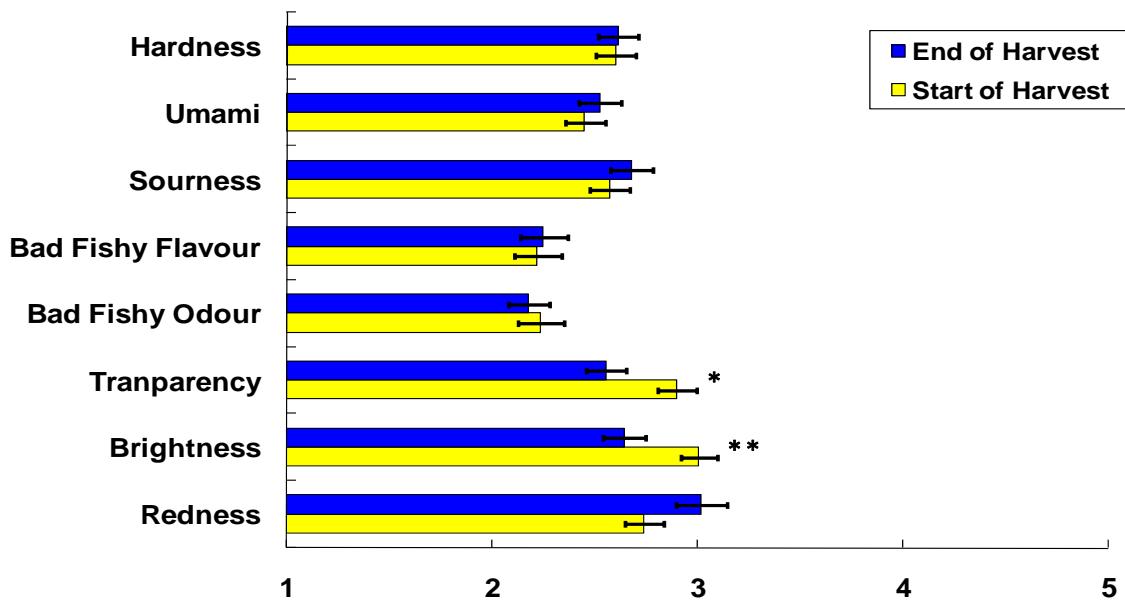


Figure 4.16: Average sensory scores (mean +/- SEM) for sashimi sections (n=18) of the Otoro cuts of farmed Southern bluefin tuna in Japan taken from the Start and End of a harvest in May of the 2003 farming season in Port Lincoln (Start n=4, End n=4; day four post-mortem; * = P<0.10, ** = P<0.05).

Principal components analysis with full cross validation and data weightings of 1.0 were conducted on the sensory data for the cuts of Akami, Chutoro, and Otoro. Two-dimensional scatter plots (bi-plots) of the two main principal components (PCs) with X-loadings for the Akami (Fig. 4.17), the Chutoro (Fig. 4.18), and the Otoro sensory data (Fig. 4.19) explain 73% and 15%, 58% and 19%, and 65% and 21% of the sensory data for each cut respectively.

The plots reveal both sample properties and variable relationships simultaneously. The closer that two samples are in the plot, the more similar they are, and the further away two samples are from each other the more different they are. The plots also show the importance of the different variables for the two components specified. Samples projected far away from the center in the same direction as a given variable have large values for that variable and samples lying in the opposite direction have low values. Samples cannot be discriminated well by variables that are close to the centre.

The two main principal components describing the sensory data for the Akami show that fish 1 and 3 of the Start of Harvest treatment and fish 2 of the End of Harvest treatment were scored highly for the positively correlated variables of sourness, bad fishy odour, and redness. Umami was negatively correlated to these variables. Data for the Chutoro cut revealed the majority of the high fat samples were perceived to be high in umami, whilst fish 3 and fish 4 of the Start and End of Harvest treatments

respectively scored high in the positively correlated variables of bad fishy odour and bad fishy flavour, sourness and hardness. Of the sensory data from the Otoro section three of the four fish from the End of Harvest treatment were located in the lower quadrants with the variables bad fishy flavour and sourness and were negatively correlated to the variables of transparency and brightness.

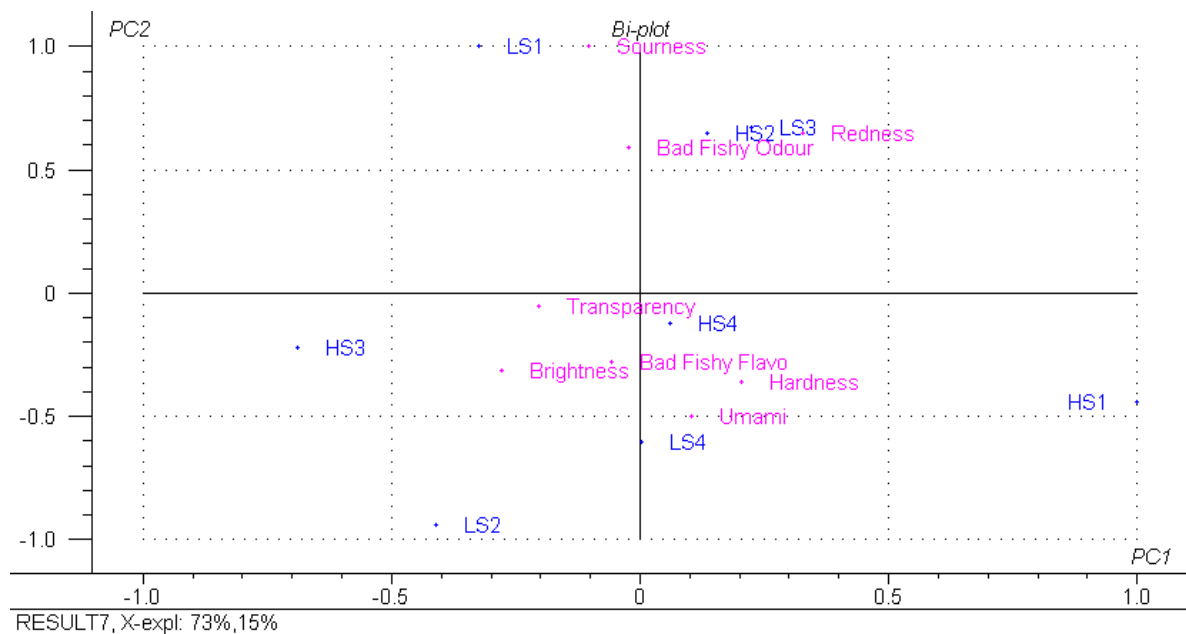


Figure 4.17: Sample scores and variable loadings bi-plot for the sensory data from the Akami sections of farmed Southern bluefin tuna in Japan taken at the Start (LS = potentially low stress) and End (HS = potentially high stress) of a harvest in May of the 2003 farming season in Port Lincoln (Start n=4, End n=4; day four post-mortem).

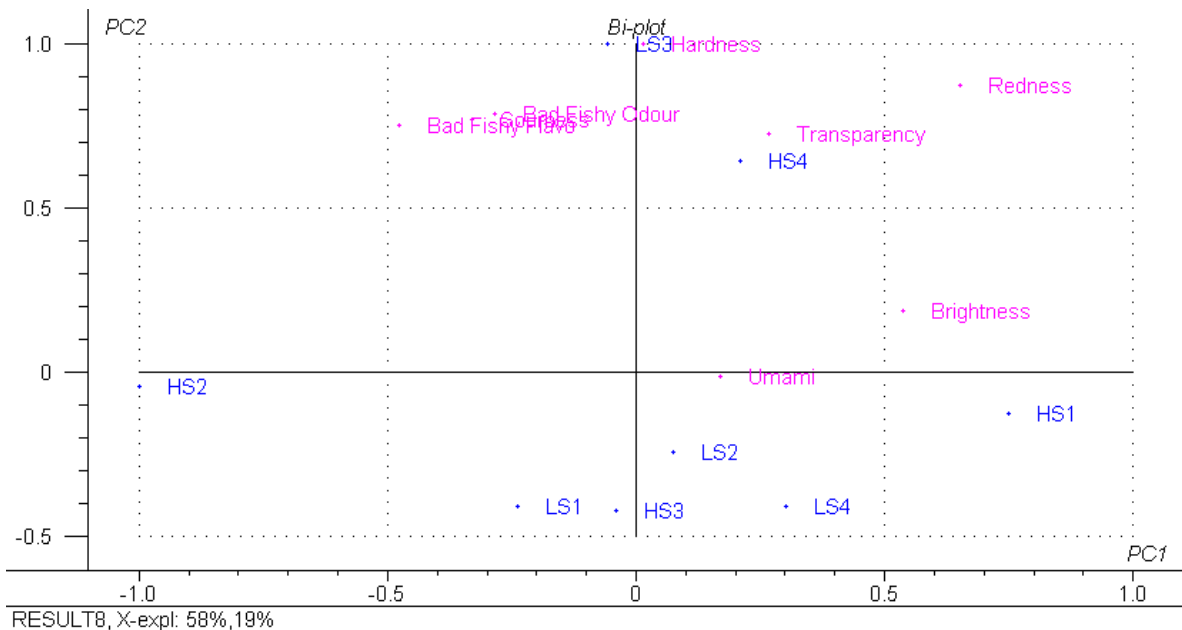


Figure 4.18: Sample scores and variable loadings bi-plot for the sensory data from the Chutoro sections of farmed Southern bluefin tuna in Japan taken at the Start (LS = potentially low stress) and End (HS = potentially high stress) of a harvest in May of the 2003 farming season in Port Lincoln (Start n=4, End n=4; day four post-mortem).

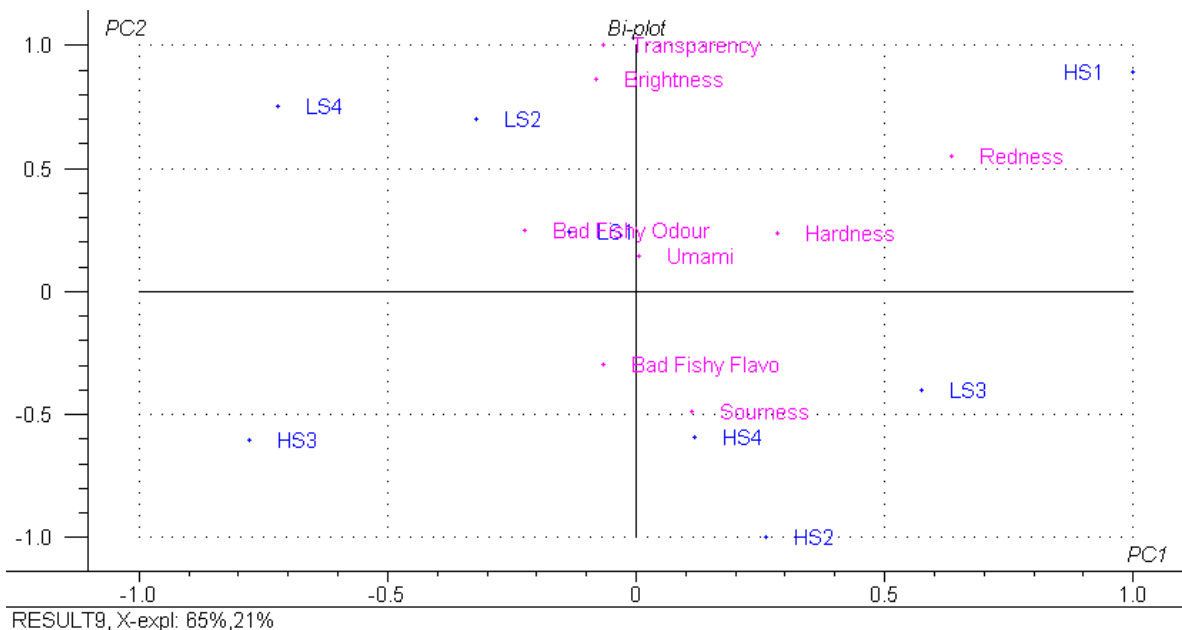


Figure 4.19: Sample scores and variable loadings bi-plot for the sensory data from the Otoro sections of farmed Southern bluefin tuna in Japan taken from the Start (LS = potentially low stress) and End (HS = potentially high stress) of a harvest in May of the 2003 farming season in Port Lincoln (Start n=4, End n=4; day four post-mortem).

4.5 Discussion

Physico-Chemical

Of the physico-chemical parameters examined from the flesh of the three cuts of tuna at day four post-harvest only the concentration of inosine monophosphate ($P=0.024$) and the digital camera RGB ratio data ($P=0.017$) of the Akami cuts recorded significant differences between the Start of Harvest and End of Harvest treatments. The values and the range of the plasma and ultimate pH values were consistent with previously recorded plasma pH and flesh ultimate pH values (5.68 – 5.83) of farmed Southern bluefin tuna (Douglas, unpublished data), were not significantly different between the three cuts of Akami, Chutoro, and Otoro, and between the harvest stress treatments for each cut respectively ($P>0.05$). Plasma pH and ultimate flesh pH could have been tempered by the characteristically high concentrations of bicarbonate in the blood and the very high intracellular non-bicarbonate buffering capacities of tunas, which, when combined with the high activity of lactate dehydrogenase (LDH) of the genus, would have resulted in the rapid buffering of acids *in situ* and minimal intracellular and extracellular pH changes (Dickson and Somero, 1987; Perry *et al.*, 1992; Dickson, 1996). The rate of pH decline and the ultimate values for pH in muscle flesh are said to be determined by the physiological condition of the muscles at the time of euthanasia, and can be related to lactate production and accumulation. However, in the present study, despite significantly higher concentrations of muscle lactate in the working Akami and Chutoro cuts when compared to the comparatively non-working Otoro

cut, this finding was not corroborated by the values of ultimate pH. These lower concentrations of lactate in the Otoro cut may have resulted from its oxidation by lipid hydroxide molecules, which are in high concentration in this high-lipid muscle block, or due to lower levels of glycolytic activity (Driedzic & Hochachka, 1978). This being the case, the animals may be coping 'well', physiologically speaking, with the levels of harvest stress as defined by the traditional parameters of pH and lactate and possibly mobilizing their extensive liver glycogen reserves (D'Antignana, unpublished results). Indeed Henckel *et al.* (2000) reported no significant differences between the ultimate pH of the four major muscles in porcine flesh after pre-slaughter exercise, leading the authors to conclude that the frequently observed differences in ultimate pH of muscle groups are caused by a combination of environmental factors and genotype rather than by differences in the physiological and morphological characteristics of the different muscle groups.

Despite significant differences in the concentrations of lactate between the differing cuts of muscle in a tuna carcass there were no significant differences in lactate concentrations between the harvest stress treatments within these cuts ($P > 0.05$). According to Driedzic & Hochachka (1978), there may be an upper limit to which lactate accumulates as demonstrated with strenuously exercised trout where, after 2 minutes, muscle lactate increased from 7 to 25 $\mu\text{moles/g}$, however, after 3, 7 and 13 minutes the lactate levels appeared to plateau at 28, 32 and 32 $\mu\text{moles/g}$ respectively (Black *et al.*, 1962 in Driedzic & Hochachka, 1978). If this is also the

case with tuna species such as Southern bluefin tuna then the effects of confinement stress alone may raise lactate levels to a plateau even before pursuit and capture stressors have come into the harvesting equation. However this is unlikely as the white muscle of tuna has recorded some of the highest glycolytic enzyme activities in any tissue of any animal (Bushnell & Jones, 1994; Fudge *et al.*, 2001). With most of the research into the effects of stress on fish conducted on salmonids (trout and salmon), which are distantly related to tunas and have a very different ecology (Korsmeyer & Dewar, 2001), extrapolating from model stress responses in salmonids to tuna carries with it a degree of risk. The high aerobic enzyme activities (e.g. citrate synthase and α -glycerophosphate dehydrogenase) of tuna white muscle, combined with an exceptionally high specific activity of lactate dehydrogenase (LDH) when compared to ectothermic scombrid fishes and other teleosts, facilitates rapid lactate clearance and recovery of blood lactate concentrations to normal levels following maximal burst activity in a time-frame that is one-fourth to one-twelfth of that recorded for other teleosts such as rainbow trout (Bushnell & Jones, 1994; Dickson, 1996; Weber & Haman, 1996; Korsmeyer *et al.*, 1996; Mathieu-Costello, 1996). It is therefore possible that any of the pH changes and excess lactate produced during confinement such as encountered by tuna throughout a harvesting event are being buffered, cleared, and/or resynthesised into glucose respectively.

There is a potential temperature difference of up to 10⁰ Celsius between subcutaneous and core white muscle in bluefin tuna in temperate water (Stevens *et al.*, 2000), and the effects of exercise during harvest on carcass temperature, the activity rates of the glycolytic enzymes, and therefore the rates of glycolysis may vary according to the temperature gradients between the various cuts. However, Fudge *et al.* (2001), in an examination of whether the proportions of glycolytic enzymes change along the heterothermic gradient of the white muscle of bluefin tuna, found no adjustments in the concentration or type of glycolytic enzymes along the thermal gradient, as well as no evidence to suggest that the more temperature-sensitive enzymes would exhibit more dramatic compensation. The authors suggest that the near temperature insensitivity of enzymes such as glyceraldehyde-3-phosphate dehydrogenase would help to conserve the glycolytic flux through the heterothermic white muscle of bluefin tuna during seasonal and transient changes in the thermal gradient. Further, the temperature gradient has been said to deteriorate during long bouts of anaerobic activity such as those experienced during a struggle of up to 60 minutes on a rod and reel in cold water (Carey & Teal, 1969 in Fudge *et al.*, 2001) as opposed to the stable gradient witnessed during naturally high levels of activity during the feeding of captive tuna. This suggests there would be a limited impact of the temperature variances witnessed within and between the muscle blocks and between fish of different size as a result of exercise on the glycolytic process at the point of slaughter and as the carcass enters the post mortem state in the first few hours as it cools.

K Value, a ratio of the accumulated concentrations of inosine and hypoxanthine relative to the total pool of ATP and its degraded forms, is a commonly used indicator of fish freshness, with a lower ratio signifying a 'fresher' product. Although pre-mortem stress has been shown to promote the degradation of ATP leading to higher K Values (Izquierdo-Pulido *et al.*, 1992; Lowe *et al.* 1993; Sigholt *et al.* 1997, Erickson *et al.* 1997a; Erikson *et al.* 1999), in the present investigation no significant differences in K Values were recorded between harvest treatments for each cut of muscle indicating that the relative rates of ATP degradation and the accumulation of hypoxanthine and inosine were comparable. Despite this finding the concentrations of the flavour associated nucleotide inosine monophosphate were significantly higher for the Start of Harvest treatment when compared to the End of Harvest treatment for the Akami cut ($P=0.024$). In contrast, the average IMP values for the Chutoro and Otoro revealed trends with higher concentrations of IMP in the End of Harvest fish. This is more consistent with the literature, with white muscle of exercised or exhausted fish having higher IMP levels. Enforced swimming of skipjack tuna (*Katsuwonus pelamis*) for 15 minutes resulted in a significant increase in the concentration of IMP despite minimal overall changes in purine nucleotides (Arthur *et al.*, 1992). In the white muscle of rested and exhausted cod (*Gadus morhua*) and carp (*Cyprinus carpio*) concentrations of inosine monophosphate increased from 1.26 to 4.60 and 1.38 to 4.01 $\mu\text{moles/g}$ respectively (Jones & Murray, 1960; Driedzic & Hochachka, 1976).

Unlike previous investigations, in the present study measures were taken four days post-mortem rather than immediately post-mortem and it is not clear according to Driedzic and Hochachka (1978) when the reactions that result in the depletion of the adenylate pool and increases in the concentrations of IMP start to occur. In the white muscle of tuna the Akami cut has greater aerobic capacity than either the cuts of Chutoro or Otoro, so the start point and the rate of depletion of the adenylate pool in the Akami may precede and be more rapid in this working muscle. Further, the concentrations of IMP rise then fall in the muscle over time, so that both the time of sampling post mortem and the possible degrees of separation due to treatment or varying aerobic capacities of the differing muscle blocks could affect results (Fig. 4.20). Indeed, with the higher average K value in the Akami cut of the End of Harvest treatment when compared to the Start of Harvest treatment, the accumulation and degradation of IMP in the End of Harvest treatment may have preceded that of the Start of Harvest treatment.

As IMP is a ribo-nucleotide associated with flavour perception (de Araujo *et al.*, 2003), a high concentration in the flesh may well be associated with high umami score, and therefore the time frame for a most favourable umami response may vary according to an individual fish's position (i.e. Start, Middle or End) within a harvest and between the differing cuts of white muscle.

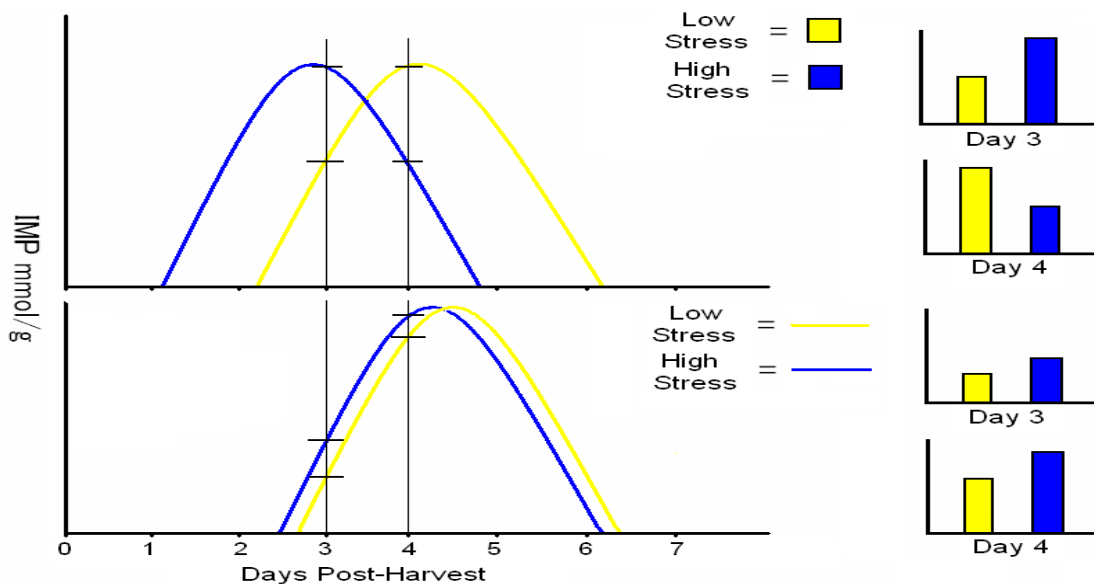


Figure 4.20: Theoretical representation of how the degree of separation and time of sampling could affect the measured concentrations of inosine monophosphate in fish muscle as a result of differing degrees of harvest stress.

There were no statistically significant differences between the concentrations of hydroperoxide between the Start and End of Harvest treatments within the cuts of Akami, Chutoro, and Otoro ($P > 0.05$). The P values were no nearer significance with the scaling of each sample to its percentage fat content. According to Weber and Haman (1996), tuna have a high lipolytic capacities, high maximal rates of circulatory fatty acid transport through modified plasma and cytosolic proteins, increased amounts of intramuscular fat droplets in direct contact with outer mitochondrial membranes in locomotory muscles, and an elevated capacity for lipid oxidation in muscle mitochondria. The comparatively high fat content of farmed tuna and the high aerobic capacity of tuna as a species means it is possible that the levels of stress imposed during a harvest do not result in the significant mobilization

of the fat reserves of the Chutoro and Otoro, or not until the carbohydrate reserves have been expended as the presence of glucose has been shown to spare the release of free fatty acids (Driedzic & Hochachka, 1978). Despite a statistically non-significant result, there were lower average concentrations of hydroperoxide in the Chutoro and Otoro cuts of End of Harvest tuna. If lipid catabolism was not initiated as a result of the harvest treatment it is possible that the lower concentrations of hydroperoxide measured in the cuts of Chutoro and Otoro were a result of the oxidation of lactate instead.

The enzyme metmyoglobin reductase requires nicotinamide adenine dinucleotide (NADH) as a mediator to facilitate metmyoglobin reduction (Chiou *et al.*, 2001). During strenuous exercise, NADH concentration can become depleted to a point where the post-mortem activity of the enzyme is slowed. Despite this, there were no significant differences between the harvest treatments for the percentages of the brown coloured pigment metmyoglobin for the cuts of Akami, Chutoro, and Otoro. The potentially higher stores of glycogen in well fed farmed tuna combined with the animal's naturally high aerobic capacity may buffer against the impacts of stress and exercise as witnessed during their harvest and help maintain high NADH concentrations - thus not inhibiting the activity of metmyoglobin reductase. The oxidation rates of red oxymyoglobin to brown metmyoglobin and the activity of metmyoglobin reductase is also pH dependent. However, the formation and reduction of metmyoglobin could not have been significantly influenced by these

two parameters as there were no statistical differences between the harvest treatments for the concentrations of lactate and the values of pH in the flesh.

According to Chiou *et al.* (2001) tuna meat discolours more rapidly in supermarket storage conditions and has a shorter shelf-life due to lower activity rates and/or the lower stability of tuna metmyoglobin reductase when compared to the metmyoglobin reductase of red meat. Further, the authors found that the immersion of tuna meat in metmyoglobin reductase could extend the colour shelf life of tuna meat – a potential treatment for extending the colour shelf-life of tuna meat should more exhaustive levels of harvest stress prove deleterious.

No significant differences ($P>0.05$) for the CIE L, a, and b measures of lightness, redness and yellowness were found between the harvest stress treatments within the cuts of Akami, Chutoro and Otoro. Activity and crowding associated stress however has been shown to significantly reduce the redness of the flesh of salmon (Robb *et al.*, 2000) and pigs (Rosenvold & Anderson, 2003) as measured by the CIE Lab system. According to Warriss *et al.* (1996) changes in the colour of red meat from animals exposed to pre-slaughter stress stem from a denaturing of the soluble proteins to more insoluble forms due to the rapid drop in pH. This results in water loss from the meat that subsequently changes the reflective properties of light from the surface of meat altering its lightness, hue and chroma. As there were no significant differences in ultimate pH recorded between the harvest treatments,

coupled with the high buffering capacity of tuna to pH change, this suggests that any protein denaturation and insolubility that may have occurred as a consequence of the levels of harvest stress in the present investigation did not affect the reflective properties of the flesh and its colour as measured by the colorimeter. However, the flesh of tuna when compared to that of other meats is much more translucent and potential absorbance and scattering of the incidence light, along with background affects, may significantly decrease the amount of reflected light and the measured values of lightness, hue and chroma respectively (Douglas, unpublished data). Differences in the lightness, hue and chroma would possibly be detected by the colorimeter in severe situations such as occurs when tuna is deemed to be suffering from 'yake' where the flesh is much more pale and opaque.

According to Rosenvold & Anderson (2003), elevated temperatures due to crowding stress on the colour of the porcine *longissimus dorsi* muscle post-mortem resulted in the retention of redness as measured by colorimeter. This effect, the authors hypothesized, may have been due to the partial heat denaturation of oxygen-utilizing enzymes reducing their activity and making more oxygen available for deoxymyoglobin within the surface layer of the muscle. Alternatively, the fading of redness was found to be significantly faster in the *biceps femoris* and *semimembranosus* porcine muscles which, as a result of the crowding stress, had recorded temperatures well above that of the *longissimus dorsi* muscle. Along with heat inactivation of the oxygen-utilizing enzymes, Rosenvold & Anderson (2003)

postulated that the relatively higher temperatures witnessed in the *biceps femoris* and *semimembranosus* porcine muscles may have further resulted in a reduction of the activity of metmyoglobin reductase to a degree where it more than counteracted the positive effects of deactivation of the oxygen-utilizing enzymes. The activity of metmyoglobin reductase of tuna, already shown to be effective in the extension of colour stability in tuna flesh (Chiou *et al.*, 2001), may, along with oxygen-utilizing enzymes, not be as heat sensitive as the enzymes in porcine muscle as tuna are not thermo-regulators but thermo-conformers with body temperatures elevated but variable along with changes in ambient water temperatures and activity rates. Indeed, Fudge *et al.* (2001) found glycolytic enzymes exhibited temperature insensitivity and biochemical symmorphosis throughout the heterothermic white muscle of bluefin tuna. Although there are no known reports of 'tuna burn' (yake) recorded from similar harvesting operations, it is possible that the confinement and capture stress during these harvesting operations raises the body temperatures to a point where flesh properties are affected that are detectable to the human eye but are not sufficient to cause rejection of the meat.

Despite statistically non-significant colorimeter readings between the harvest treatments, digital camera RGB ratios revealed a significant difference between treatments with the start of harvest treatment recording higher values for the cuts of Akami ($P < 0.05$), and, at an alpha of 0.10, a near significance level for the cuts of Chutoro ($P = 0.115$). High RGB ratios have been shown to be related to the higher

subjective quality ranks of wholesalers at the first point of sale (Tsukiji Fish Market, Tokyo), and higher auction floor prices (see Methods Chapter) and this result therefore suggests that tuna subject to shorter periods of confinement and capture stress may receive a higher quality assessment on the market and potentially return higher auction floor prices on any given day.

The physico-chemical data suggest that the harvesting event investigated in the present study did not affect these traditional indicators of flesh quality in the white muscle of fresh, farmed Southern bluefin tuna five days post-harvest in Japan. However, little is known about the recruitment of muscle fibres during high speed and burst swimming of tuna, prompting Altringham and Shadwick (2001) to pose the following questions - does muscle strain increase to power high-speed swimming? At what swimming speeds are fast fibres recruited? What contribution, if any, does the slow muscle make to burst swimming? And what are the energetic costs associated with maximal swimming performance? In previous research Altringham and Block (1997) proposed that the high speed aerobic cruising of tuna may be using more efficient slow twitch red muscle without needing to recruit the less efficient fast twitch white muscle. Tail-beat frequencies of between 8 and 12Hz were recorded by those authors during burst activity associated with feeding. However, at tail-beat frequencies of around 3Hz and a swimming speed of about 2 body lengths per second (BL/s), they calculated that only 50% of the slow twitch red muscle would be required. Yellowfin tuna have been recorded maintaining cruising

speeds of at least 3.5 BL/s for over an hour (Block *et al.*, 1997), possibly not needing to recruit fast-twitch muscle at even these speeds. In order to avoid collision with other tuna, divers, and the nets, farmed Southern bluefin tuna are not able to employ burst swimming for long periods within the confines of the harvesting net, and speeds significantly higher than 3.5 BL/s would not be maintainable for long periods. Therefore, the fast-twitch white muscle block that comprises the Akami, Chutoro and Otoro cuts may not be recruited for any significant periods of time during a harvest.

In other aquaculture industries an examination of the holding densities and confinement times prior to euthanasia reveals densities of 125 kg m⁻³ for 5.5 hours for Atlantic salmon (Erikson *et al.*, 1997b); 69 - 170 kg m⁻³ for up to 11 hours for brook and lake trout (Mc Donald *et al.*, 1993); 167 kg m⁻³ for 10.5 hours for rainbow trout (Ostenfeld *et al.*, 1995); and 12 - 120 kg m⁻³ for 4 – 12 hours for Coho salmon (Specker & Schreck, 1980). The harvesting portion of the seine net used in the current investigation formed approximately a 15m equilateral triangle that was 3m in depth at the divide, increasing gradually to the surface at the opposite edge. A minimum volume for such an area at the end of a harvest would have equated to approximately 150 m³. The 70 tuna in the harvesting divide of the seine net at the maximum harvesting density and confinement period would have approximated to 16 kg m⁻³ over a 30 minute period. In the non-harvesting divide of the net there would have been an average depth no less than 5 metres and therefore fish would

have been confined for 30 minutes at a density no higher than 6 kg m^{-3} prior to transfer into the harvesting divide. Therefore, the times in confinement are considerably shorter and holding densities lower when compared to the other aquaculture industry examples stated, although they may not be considered 'best practice'.

Southern bluefin tuna, as a species, may also be less reactive to stress. Anecdotal evidence suggests that Southern bluefin tuna, when compared to Northern bluefin tuna, are a more sedate species of tuna and frighten less easily on the fishing grounds when the purse of the seine net is being drawn. Further, the revisiting of a single pen to sample fish using a hook and line for repeated sampling did not result in any detectable changes in the blood chemistry parameters of stress (Thomas *et al.*, 2003).

Sensory

Of the sensory descriptors evaluated by panellists only the average values for the appearance descriptors of transparency ($P < 0.10$) and brightness ($P < 0.05$) in the Otoro cut were significantly higher for the Start of Harvest treatment when compared to that of the End of Harvest treatment. In their examination of the slaughter methods of eels, Morzel & Van de Vis (2003) recorded a similar finding in that panellists were only able to distinguish descriptors of appearance, and that the results indicated benefits to minimizing stress. The reduced transparency and

brightness of the Otoro may have been due to increasing protein insolubility similar to that of PSE pork. Although not statistically different ($P = 0.109$) the higher average concentrations of lactate in the End of Harvest Otoro treatment may, through protein denaturation, have been a contributing factor to greater protein insolubility, and therefore greater opacity.

The average values for redness for all three cuts were higher for samples from the End of Harvest treatments when compared to those values recorded by panellists from the Start of Harvest treatments. This finding corroborates with that of Rosenvold & Anderson (2003) in porcine *longissimus dorsi* muscle as measured by a colorimeter where the authors proposed that partial thermal denaturation of the oxygen-utilizing enzymes may have made more oxygen available for deoxymyoglobin within the surface layer of the muscle. A similar finding was recorded by Thomas *et al.* (unpublished data) where the tail cuts of tuna exhausted during harvest were redder in colour than those from control (low stress) fish as assessed by a colour panel at day eight in Japan.

There were no significant differences between the harvest treatments for the sensory descriptors of flavour or texture. Investigations into the effects of differing slaughtering methods on the flavour (Van de Vis *et al.*, 2003) and the texture of salmon (Roth, 2003) have also returned non-significant results. The number of the sensory variables positioned near the centre of the scores and loadings bi-plots

indicates that the panellists may have struggled to differentiate samples – even when there may have been differences (Type II Error). To overcome the likelihood of type two errors occurring, more fish, more panellists, or more training could be undertaken. However, if the problem is more psychophysical than psychometric due to the heterogeneous and labile nature of tuna flesh then more training may not reduce the probability of committing a type two error.

To overcome these difficulties when dealing with heterogeneous flesh samples a new testing protocol could be employed. A single portion of flesh from the same sampling position within the carcass of an individual could be assessed by a number of panellists for appearance traits. Randomizing the order, panellists could rotate from one booth to another doing so repeatedly at set time intervals to account for both the intensity and duration of the flesh bloom and for shelf-life investigations. The remaining portion of the same cut could then be assessed for texture (sensorially or rheologically) and then homogenised to provide a consistent range of samples for the assessment of odour and flavour characteristics. Homogenised (minced) tuna meat known as 'negitoro' is commonly consumed in Japan.

The interactions between the effects of different seasons on the sensory characteristics of the flesh and harvest stress may also be worth consideration. According to Tejada & Huidobro (2002) the flavour scores of fish harvested using comparable slaughter methods but at different times of the season varied –

especially in the early days of storage. Therefore, examination of the sensory characteristics of the product at different points within the tuna farming season, where there are differences in body composition and water temperature, as well as sampling and testing after a longer storage period may return differing findings between harvest treatments.

Consumers and retailers are gradually beginning to determine the ethics of business, and producers need to be concerned not only with the quality and safety of their produce but also with the welfare of the fish and the occupational health and safety of their staff (e.g. the use of chemicals). Therefore the welfare of the animal must be taken into account when alternative harvesting techniques are being considered. According to Van de Vis *et al.* (2003), a slaughter method should render a fish unconscious until death without avoidable excitement, pain or suffering prior to killing. However, when compared to other species of fish, tuna represent a unique challenge because of their capture and slaughter in a cage environment. They are large animals that, as obligatory ram ventilators, are required to swim constantly to avoid asphyxiation. Further, with the *rete mirabile* arrangement of the blood vessels functioning to trap metabolically produced heat, strenuous exercise can elevate body temperatures to a point where muscle proteins denature resulting in *yake* – see Introduction pg11. Tuna therefore present culturists with unique logistical challenges not only to preserve the flesh qualities of the product, but also to ensure the welfare and safety of staff.

The current practice of hand-capture where tuna are grasped by the tail and the isthmus, are turned up-side-down so to disorient them, and then led to a cushioned platform for immediate brain spiking and euthanasia could, at present, be considered 'best-practice' within the farmed Southern bluefin tuna industry considering both the welfare of the animal and the safety of the staff. Alternative harvesting methods for harvesting tuna from cages have been tested; however there are logistical constraints with the use of anaesthetics in the Southern bluefin tuna farming industry as well as potential safety, flesh quality, and market issues that would require investigation. The use of electro-slaughtering in the Atlantic Northern bluefin tuna farming industry is being attempted however there have been reports of spinal damage that complicates the loining process, as well as burns and blood coagula affecting flesh qualities (Mateo *et al.*, 2002).

4.6 Conclusions

In the present investigation there were no significant differences in the majority of sensory and biochemical indicators of quality attributes between fish harvested at the beginning or at the end of a commercial tuna harvest. However, with a sample size of eight, inferential power was at a minimum, and from other studies on crowding stress in fish it is clear that as some fish respond more than others to crowding, the results therefore could have been highly affected by individuals rather than treatment. With the same numbers of preparatory staff and panellists, the

numbers of fish could be increased and only one or two of the different cuts of a tuna carcass could be examined (rather than all three). This would increase inferential power between treatments for a particular cut but would not reveal what is occurring in the whole carcass, and therefore, would carry the risk of missing a quality issue in the cut(s) not examined. The most desirable option would be the repetitive sampling of fish over a number of consecutive days and over the course of a season to build up a robust data set.

With a majority of the biochemical indicators of stress not returning significant results one may conclude that farmed Southern bluefin tuna are not 'stressed' during a harvest of up to one hour in duration. Alternatively, as proposed by Tejada & Huidobro, (2002) from their investigations into gilthead sea bream, the classical physicochemical indicators of stress and/or their analytical methods may not be sensitive enough to detect differences and more research may be needed to determine a more suitable biochemical index for the species. However, from a 'product quality' point of view, the relevance of such a biochemical index should always be calibrated to a trained or expert sensory panel. An extremely sensitive biochemical index capable of detecting differences in the flesh that humans cannot, would yield purely academic results not relevant to the food item and, if not cognizant to the animal, not relevant to its welfare, and subsequently, would be of no concern to the industry. To examine the ability of the tuna to cope with the crowding stressors of a harvest, blood samples from multiple individuals throughout

a harvest and during a season could be collected and plasma pH, lactate, cortisol and other indicators of stress could be investigated. These samples would not cause any commercial damage to the tuna and would reveal any blood chemistry changes within harvests and for the population over the course of a season where the tuna appreciate fat and ambient water temperatures fall significantly.

In the present investigation the samples were examined only at day four post-harvest and day one in Japan, and the data therefore is applicable mostly to the supermarket point of sale and does not take into account the need of the wholesaler or restaurateur who store the product up to ten days (day fifteen post-harvest). If funds and resources had made it possible to conduct biochemical testing for a longer period, treatment effects may have become more evident after more time in storage.

Of the objective flesh quality indicators only the RGB ratio recorded a significant effect regarding the time of harvest on the Akami section – a ratio that is calibrated to auction floor grades of quality and price. Of the subjective flesh quality indicators significant effects of harvest stress were detected in the transparency and brightness of the valuable Otoro section which commonly sells at a price of up to 3.5 times higher than for Akami. These results indicate that extended harvest times negatively affect the appearance and quality grade of fresh, farmed Southern bluefin tuna in Japan, and potentially therefore the market returns for the industry.

The collaborating tuna farming company repeatedly records the highest grades for quality and market price, so the fish used in the current experiment were cultured and harvested in what could be described as the industry's 'best practice'. Higher levels and/or longer periods of stress during harvest could have a far greater deteriorative effect on the qualities of the flesh – both fresh and frozen. Tuna destined for freezing undergo a much longer period of harvest stress with many tuna reaching a state of exhaustion, This situation coupled with the subsequent effects of thawing, may more obviously affect the flesh properties..

Finally, examination of the bio-chemical indicators on a dry weight basis revealed that the three differing cuts that make up tuna white muscle have similar metabolic activities and produce chemicals such as lactate at a similar rate. If a similar trend exists with other quality related biochemicals, such as total myoglobin, it could validate the use of the tail cut as an indirect measure of the quality related compositions of the three valuable cuts of the carcass from this otherwise redundant section.

5 POST-HARVEST HANDLING & FLESH QUALITIES OF FARMED, FRESH SOUTHERN BLUEFIN TUNA FROM PORT LINCOLN, SOUTH AUSTRALIA TO TOKYO, JAPAN

5.1 Introduction

Temperature, from the point of euthanasia, through distribution, and during storage affects not only the microbiological status of food products such as meat but also its bio-chemical and physical structure, and, therefore, the sensory qualities of the final product (Rikert *et al.*, 1957; Bremner *et al.*, 1987; Berry, 1997; Koutsoumanis *et al.*, 2005). Higher temperatures result in the greater scavenging of oxygen by residual respiratory enzymes, elevated oxygen consuming processes, such as fat oxidation, and lower oxygen tensions that promote the auto-oxidation of myoglobin which in turn affect flavour and advance colour change respectively (O'Keefe and Hood, 1980-81). Elevated temperatures also accelerate the degradation of the collagen fibrils of the endomysium and perimysium within and between bundles of muscle fibres (Veland & Torrissen, 1999; Ando, *et al.* 1992; Ando, 1999; Bremner, 1999.), and, via the faster depletion of cellular ATP reserves promotes the onset and resolution of rigor mortis, affecting the texture of meats (Huss, 1995).

Poikilothermic fish have body temperatures only slightly higher than that of the surrounding water temperature. Therefore, the often higher ambient air temperatures following landing can exacerbate the autolytic and bacteriological breakdown of the flesh if not rapidly chilled (Huss, 1995). However, even in chilled

and frozen storage, there can be significant deterioration of the sensory and nutritional qualities of the flesh of fish that lead to reductions in the commercial value (Aubourg *et al.*, 2005; Hamada-Sato *et al.*, 2005; Losada *et al.*, 2005). The rate of degradation initiated by muscle enzymes, and later by microbial enzymes, is not only temperature dependent but also varies according to the species, size, lipid content, condition, and microbial load of the fish (Losada *et al.*, 2005).

Although rapid chilling and lower storage temperatures cannot completely halt degradation it can extend shelf life by reducing the activities of proteolytic and lipolytic micro-organisms and the formation of total volatile bases in the muscle of European hake (Rodriguez *et al.*, 2004), mackerel (Rodriguez *et al.*, 2005) and sardine (Ababouch *et al.*, 1996; Campos *et al.*, 2005); inhibit lipid hydrolysis and oxidation and nucleotide autolysis in sardines (Losada *et al.*, 2004a) and farmed turbot (Pineiro *et al.*, 2005; Rodriguez *et al.*, 2006); slow nucleotide degradation and extend the shelf life of striped bass (Boyd *et al.*, 1992), horse mackerel (Mochizuki & Sato, 1994; Losada *et al.*, 2005), Nile perch (Karungi *et al.*, 2004), European hake (Losada *et al.*, 2004b), yellowfin tuna (Chiou & Ding, 2005), and European eel (Özogul *et al.*, 2006); and inhibit the production of biogenic amines and total volatile bases in Mediterranean hake (Baixas-Nogueras, 2002). These specific reports concur with the general principle that the major deteriorative changes in post-mortem fish flesh are very temperature sensitive in the chill range, with a doubling of rates occurring by even such a small temperature change as from 0°C

to 4°C as indicated in the relationship $r = (1 + 0.1t)^2$ where r is the rate and t is temperature in degrees Celsius (Bremner *et al.*, 1987). Temperature affects food safety and the sensory related qualities of fish muscle and therefore the most important aspect in the distribution and storage of fresh fish is the effective control of the temperature conditions from the time of harvest to the time of consumption (Giannakourou *et al.*, 2005).

Tuna, unlike thermo-regulators or thermo-conformers, have temperature profiles that are neither constant nor homothermic - either within or across muscle blocks. A *rete mirabile* arrangement of their circulatory system provides counter currents between the warm venous blood and the cold arterial blood enabling the exchange and conservation of metabolically produced heat energy for enhanced physiological performance. Known as *regional endothermy*, the result is a heterothermic temperature profile with heat concentrated in the core of an individual's body gradually lessening towards the extremities. The specific values and the range of temperatures are a function of an individual's physical and metabolic activity as well as its size, condition, and the ambient water temperature (Carey & Teal, 1969).

High levels of activity, such as during a feeding event, can cause the body of a tuna to 'overheat'. To counter this, wild tuna can dive to the cooler depths of the ocean in order to cool down and return their body temperatures to more favourable zones (Holland *et al.*, 1992). In wild fisheries the struggling of a tuna on a hook, in a net, or

on the deck of a boat can rapidly elevate temperatures to a point where, combined with lactic acid build-up, muscle proteins can denature. Termed *yake* in the sashimi markets of Japan, the resulting flesh is opaque, pale in colour, rough in texture, and has limited to no value as sashimi (Ohta *et al.*, 2004). To counter this tuna carcasses are chilled immediately post-capture in iced or refrigerated sea water for the fresh sashimi market, or blast frozen as soon as possible to cool their core temperature.

This rapid chilling of tuna immediately after capture has been shown to not only slow microbial growth but also the detrimental biochemical reactions affecting the production and supply of fresh, sashimi grade tuna with acceptable shelf life (Price, 1991). Tuna have high levels of free histidine in the muscle tissue that can be transformed by histidine-decarboxylating bacteria to histamine, and its accumulation, along with biogenic amines, has been used as an index of tuna quality deterioration (Perez-Villarreal & Pozo, 1990). However, the use of specific levels of histamine as an indicator of quality has not been considered as a reliable index of spoilage (Guizani *et al.*, 2005), and for sashimi grade fish such as tuna, the freshness indicator K value, a ratio of the different breakdown products of adenosine triphosphate (Saito *et al.* 1959), is preferred over histamine (Hamada-Sato *et al.*, 2005).

According to Perez-Alonso *et al.* (2003) research on the quality changes in most fish species during storage has focussed mainly on changes in the sensory attributes, the formation of volatile amines and hypoxanthine, and/or the changes in proteins and physical properties of the muscle. However, the lipid fraction of fatty species is now the subject of much attention since the healthy polyunsaturated fatty acids, such as the omega 3's, are prone to oxidation and are linked to off odours (Undeland *et al.*, 1999; Perez-Alonso *et al.*, 2003).

With the advent of tuna farming, where the fish are fattened in cages prior to harvest for the sashimi market, comes greater control over the post-mortem time-temperature management of the fresh product. In the Australian industry a number of options are available to farm managers regarding the chilling method, the duration of carcass chilling, the packaging materials, how the tuna are packed, the transport environment, the type of aircraft unit load device (ULD), and the shipping route and time in transit to market . The most common post-mortem chilling method for fresh tuna is submersion of the carcass into a mixture of flake ice and sea water following on-board gilling and gutting. Several hours later landed tuna are cleaned and prepared for shipment at processing facilities where they are either wrapped singly (fish weight >35kg) or with other tuna (two or three fish up to 35kg) in polyethylene plastic with ice packs and/or ice gels placed in the visceral and gill cavities and around the carcass(es). The wrapped tuna are placed into fibreboard boxes termed 'coffins' (~1420mm x ~410mm x ~310mm), and then loaded onto

wooden pallets and hauled in refrigerated trucks to storage facilities near the major airports of Adelaide or Melbourne. At the airport freight handling facilities the coffins are loaded into or onto the unit load devices (ULD) of aircraft for air-freight to Japan via one or more domestic and/or international flights. Typically, tuna coffins are loaded into either AV or AK type containers (Rate Class 8) or on pallets with netting (Rate Class 5) that carry up to 20 or 55 tuna coffins respectively (Fig. 5.1).

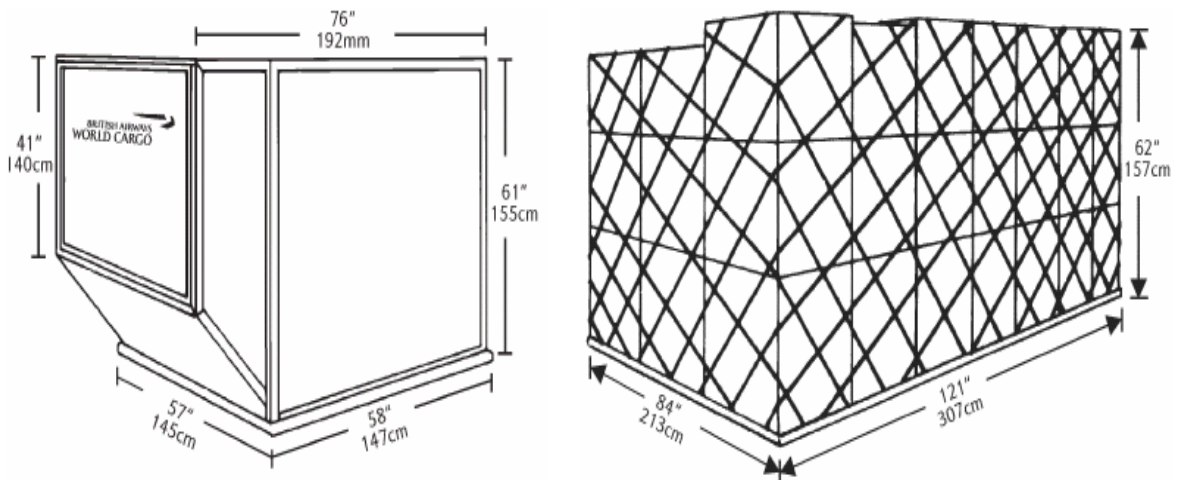


Figure 5.1: (Left) Rate Class 8 (LD3/AKE/AVA), and (Right) Rate Class 5 (PAP/PIP/PAG) aircraft unit load devices (ULD).

Depending on whether the tuna are freighted by truck to Adelaide or Melbourne, and on the contracting airline company, the shipment can take a number of differing routes to Japan (Fig. 5.2).

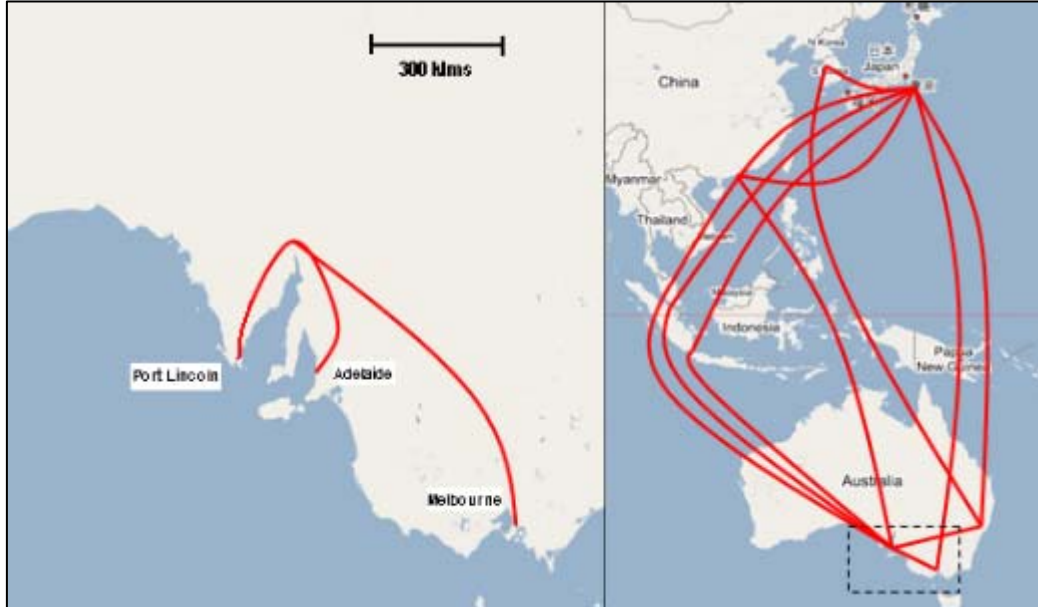


Figure 5.2: Potential trucking (left) and airline shipping routes (right) of farmed Southern bluefin tuna from Port Lincoln to Narita, Japan.

The most direct route to Japan with the least number of transfers is haulage of the tuna by freezer truck to Melbourne for loading onto a direct flight to Narita International Airport. Other routes include shipment via Singapore, Kuala Lumpur, Jakarta, Incheon, and Hong Kong through a variety of regional carriers, and where a number of transfers can be involved. One of the more indirect routes used to freight tuna to Japan is haulage by freezer truck to Adelaide, a domestic flight to Sydney, an international flight to Incheon, South Korea, and finally, an international flight to Narita, Japan. After arrival and following customs clearance at Narita the tuna are either hauled by refrigerated trucks to the central and outer markets of Tokyo, or further air-freighted on to other markets in Japan. From the point of departure in Port Lincoln to the point of arrival at Narita in Japan the time in transit can vary from 40 to 50 hours.

In 1999 a majority of companies in the Southern bluefin tuna farming industry switched from processing tuna approximately 24 hours post-harvest to processing the tuna on the same day of harvest – termed ‘next day’ and ‘same day’ processing by the industry respectively. According to a phone poll of farm managers in 2002 the reason for the switch was unclear but it was mostly thought to be at the request of the auctioneers at the tuna markets in Japan that had stated that fish processed on the same day had shown ‘better’ colour at the time of auction. As a consequence, the majority of fresh tuna is processed and dispatched on the same day as harvest.

Farmed, fresh Southern bluefin tuna air-freighted from Australia to Japan are one of the most valuable fish species in the world and therefore the cold chain is managed to a level where there have been no known bacteriological incidents since the industry’s birth in the early 1990s. However, as premiums are paid for fish of the ‘highest quality’, it is warranted to investigate the affects of current cold chain management practices on the flesh quality attributes of this temperature-sensitive product.

5.2 Aims & Objectives

The aims and objectives of this investigation were to firstly examine the range of carcass and ambient temperatures from the point of harvest in Port Lincoln to Tokyo, Japan at differing times of the year and of tuna processed either on the same day or the day after harvest. Secondly, to compare some of the sensory and bio-chemical attributes of the flesh in Japan from a single shipment of tuna processed on the same day as harvesting to tuna processed the day after harvesting in Port Lincoln, Australia.

5.3 Methods

Time-Temperature Logging: Disk-shaped data loggers (17mm by 6mm; KOOLTRAK) programmed to record temperature values every 15 minutes were sealed in protective plastic bags, and inserted into the post-pectoral bleed-cut space of tuna following an on-board gilling and gutting operation. Harvested tuna were placed in ice slurry immediately after this procedure as per industry practice. To measure ambient temperatures data loggers were attached to laminated sheets for placement inside the tuna coffins, or attached to the outside of the tuna coffins at the processing facilities (Fig. 5.3).



Figure 5.3: Insertion of a data logger into the post-pectoral bleed cut (top left), attachment to a laminated card for placement inside a tuna coffin (top right), and attachment and taping of a data logger to the outside of a tuna coffin in Port Lincoln, Australia (bottom two photos).

Objective One – Time-Temperature Profiling

March Harvest – Same Day: Tuna were harvested on the morning of the 15th of March 2002 and processed in the afternoon. Loggers were inserted into the bleed cuts of thirty fish and on the inside and outside of ten tuna coffins (n=50). The loggers placed on the inside of the boxes were placed between the cardboard and the plastic-wrapped tuna. The tuna were then freighted to Adelaide in a freezer truck, unloaded the following morning, and placed on board flight QF738 to arrive in Sydney before noon. The tuna were then placed into chilled storage before being loaded onto flight QF021 to Narita International Airport. Following their arrival and unloading on the morning of the 17th of March the tuna were transported to a distribution centre located in the vicinity of the airport and all temperature loggers were removed from the carcasses and from inside and outside the coffins.

August Harvest – Next Day: Tuna were harvested on Tuesday the 6th of August 2002 and processed the following morning. Data loggers were inserted into eight* fish and on the inside and outside of eight tuna coffins. The loggers placed on the inside of the boxes were plastic-wrapped together with the tuna. The tuna were then shipped to Melbourne by freezer truck arriving the morning of the 8th and placed on board flight QF434 that departed Melbourne at 13:00 and arrived in Sydney at approximately 1400 hrs. The tuna were then placed into chilled storage before being loaded onto flight KE812 to Incheon International Airport in South Korea, arriving at approximately 04:30 hours on the 9th of August. After loading onto flight

KE701 which departed Incheon at approximately 09:00 hours they arrived at Narita, Japan at approximately 12:00 hours that afternoon. After release from customs, the shipment was then transported to a distribution centre located in the vicinity of the airport, and then trucked to the Tsukiji Wholesale Fish Market. All temperature loggers were removed at approximately 00:30 hours on the 10th of August.

* Lower numbers of tuna and cartons were examined in the August harvest when compared to the March harvest due to the tracking of the tuna through to Tsukiji Wholesale Fish Markets in Tokyo rather than just the distribution centre at Narita International Airport.

Objective Two – Same Day Vs Next Day Processing

On Friday the 29th of August, 2003 five tuna from a commercial harvest were equipped with internal temperature loggers (via the bleed cut) and placed in ice slurry as per normal practice. These five fish were left in ice slurry in a processing shed overnight. The following day another harvest of fish was taken from the same pontoon and a further five fish were also equipped with temperature loggers. To minimize the possible effects of harvest stress on either the temperature and/or biochemistry of the flesh, tuna of equal size and position within the harvest were selected for each treatment.

The five fish harvested on Friday 29th ('next day') were processed (cleaned and boxed with gel ice) and shipped together with the five fish harvested on Saturday the 30th ('same day'). Following processing, and the insertion and attachment of data loggers to the inside and outside of the coffins respectively, the tuna were trucked to Adelaide and loaded on to flight SQ230 departing Adelaide at 13:05 on

Sunday the 31st of August to Singapore. Later that evening the tuna were loaded on to flight SQ998 from Singapore to Narita International Airport arriving at 7:05 on Monday the 1st of September. After customs clearance the tuna were trucked to the Tsukiji Wholesale Markets in Tokyo on the morning of the 2nd of September.

The tuna used for the experiment by-passed the auction floor and were loined at collaborating wholesalers at the Tsukiji market. The front, left portion (*kami*) that encompasses both Akami and Otoro cuts of each carcass were retrieved from the wholesalers for use in the experiment with the remainder of the carcasses sold for cost recovery (Fig. 5.4). The *kami* sections were transported to the Tokyo University of Fisheries and assessed by trained Japanese panellists (n=13) on Day 1 (Tuesday 2nd of September) and Day 4 (Friday 5th of September); Days 4 & 5, and Days 8 & 9 post-harvest for same day and next day processed fish respectively.

Subjective descriptors of quality assessed by panellists on Day 1 and Day 4 were redness, brightness, and degree of colour change – the latter being a direct translation from a Japanese term and refers to the amount of browning. Bad fishy odour was added as a quality descriptor on Day 4 only. All descriptors were assessed using sliding scales (Fig. 5.5). A hedonic descriptor of quality was also asked of the panellists using a scale of one to ten with one being the 'highest quality' and ten being the 'lowest quality'.

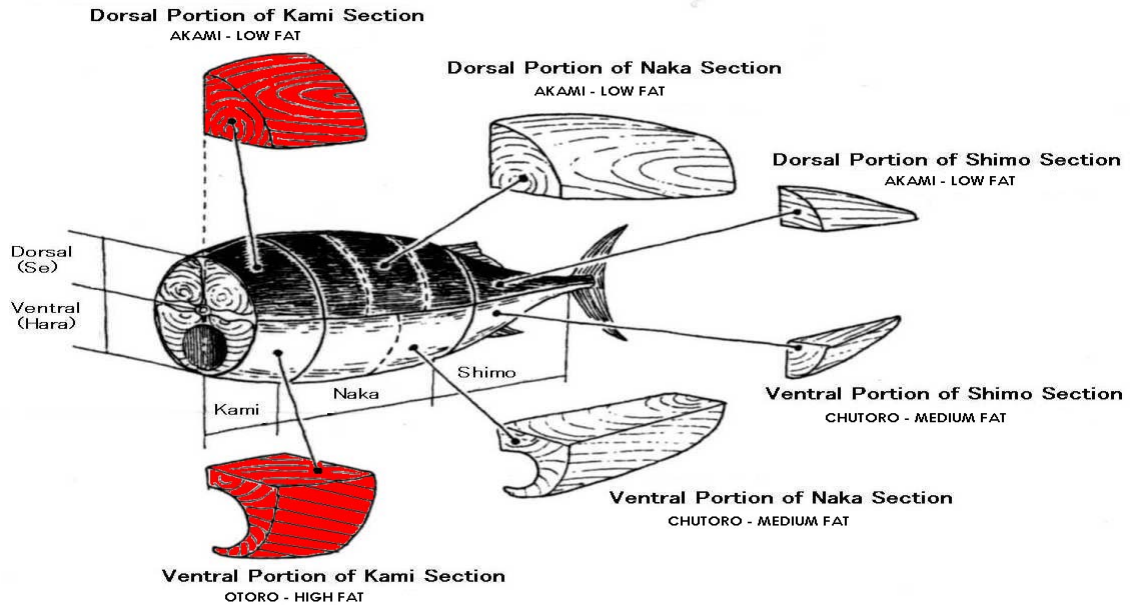


Figure 5.4: Otoro (ventral) and Akami (dorsal) portions of the *kami* section of a tuna carcass.

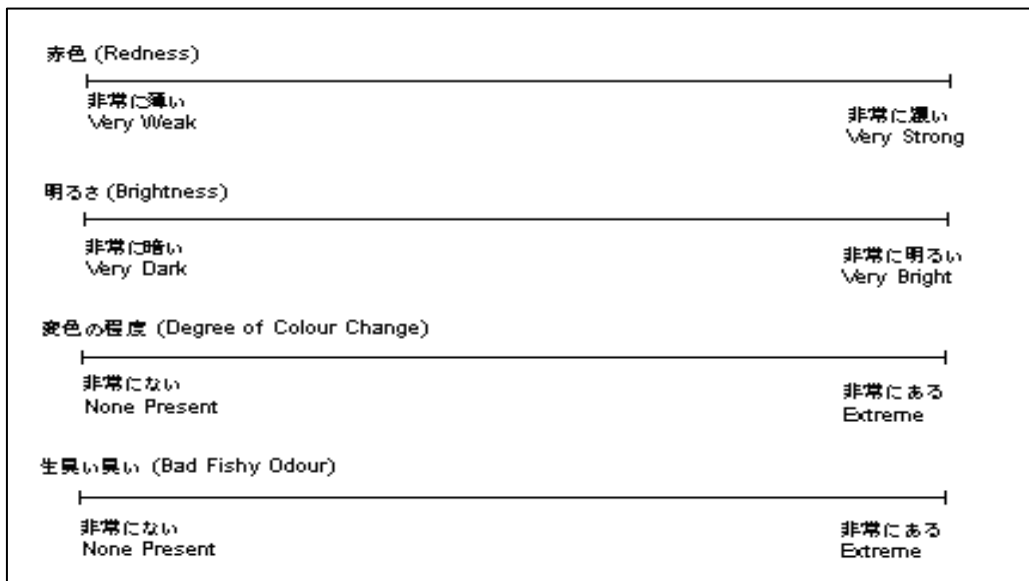


Figure 5.5: Examples of the anchored, sliding scales used in the assessment of the Akami and Otoro sections.

Samples of muscle tissue were removed immediately following panel assessment for the objective analyses of Hydroperoxide (Otoro) and K Value (Akami) on both

days (see Methods Chapter 2.2). Sensory and biochemical data was analysed using one-way ANOVA (SPSS).

5.4 Results

Objective One: *Time-Temperature Profiling*

March Harvest - Ambient temperatures rose considerably in both Adelaide and Sydney prior to loading and following off-loading of aircraft (Fig. 5.6). Temperatures were extremely variable between coffins with loggers recording values as high as 38⁰C and as low as 7⁰C in Adelaide during the loading of QF738 (Fig. 5.7). In Sydney, ambient temperatures as high as 47⁰C and as low as 8⁰C were recorded immediately after the tuna were off-loaded from flight QF738. The average box internal temperatures mirrored the trend of the average box external temperatures but were buffered by the insulating properties of the cardboard and the thermal mass of the fish (Fig. 5.8). Average fish internal temperatures fell below 5⁰C just before 12am on the night of the 15th, with the highest and the lowest recordings measuring at 8⁰C and 2⁰C respectively at this point in time (Fig. 5.9). The lowest average fish internal temperature of 3.86⁰C was recorded at 15:05 on the 16th. From this point on the average internal fish temperatures began to slowly increase to a final temperature of 5.28⁰C at 15:03 in the afternoon of the 17th where the loggers were removed at the distribution centre in Narita, Japan. The rise in internal temperatures followed the heating event of loading onto flight QF021 and may have resulted from partial thawing of the ice packs.

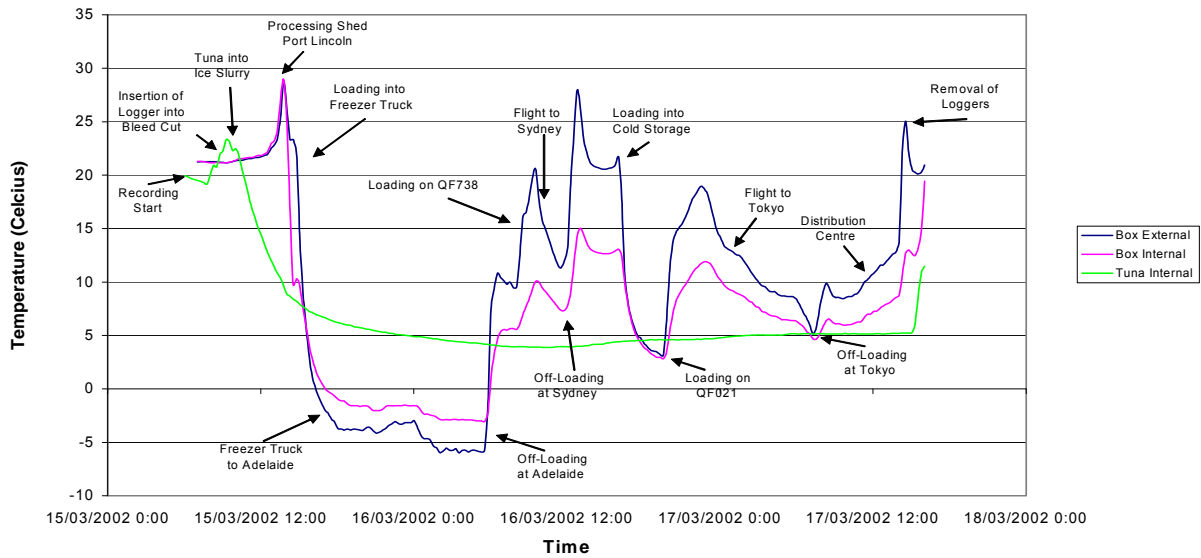


Figure 5.6: Average box external, box internal, and tuna internal logged temperatures from Port Lincoln to Narita International Airport via Adelaide and Sydney for a shipment processed on the same day as harvest in March 2002.

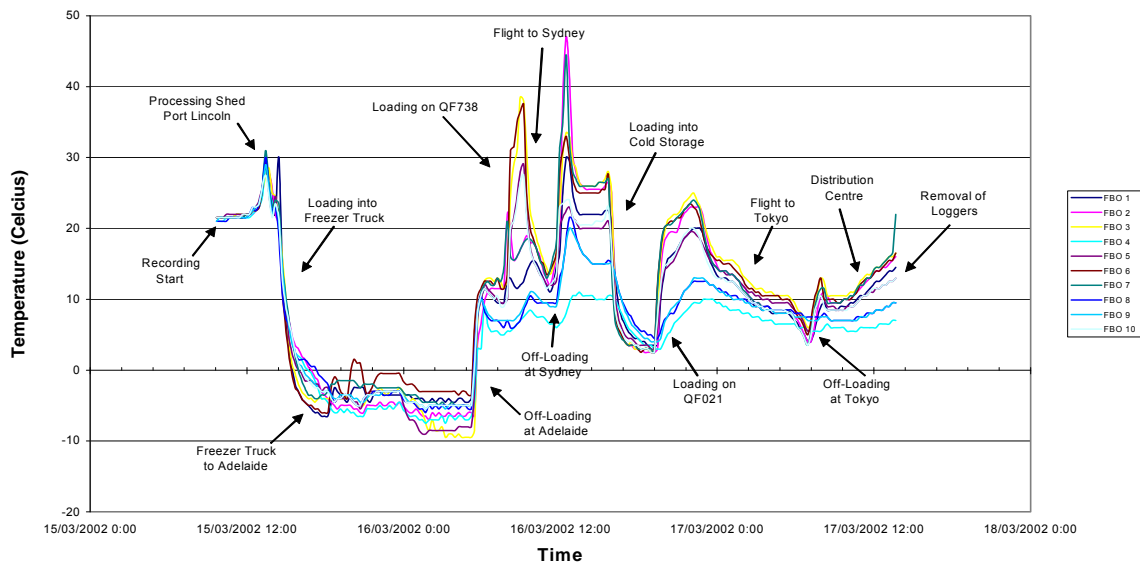


Figure 5.7: Individual box external logged temperatures from Port Lincoln to Narita International Airport via Adelaide and Sydney for a shipment processed on the same day as harvest in March, 2002.

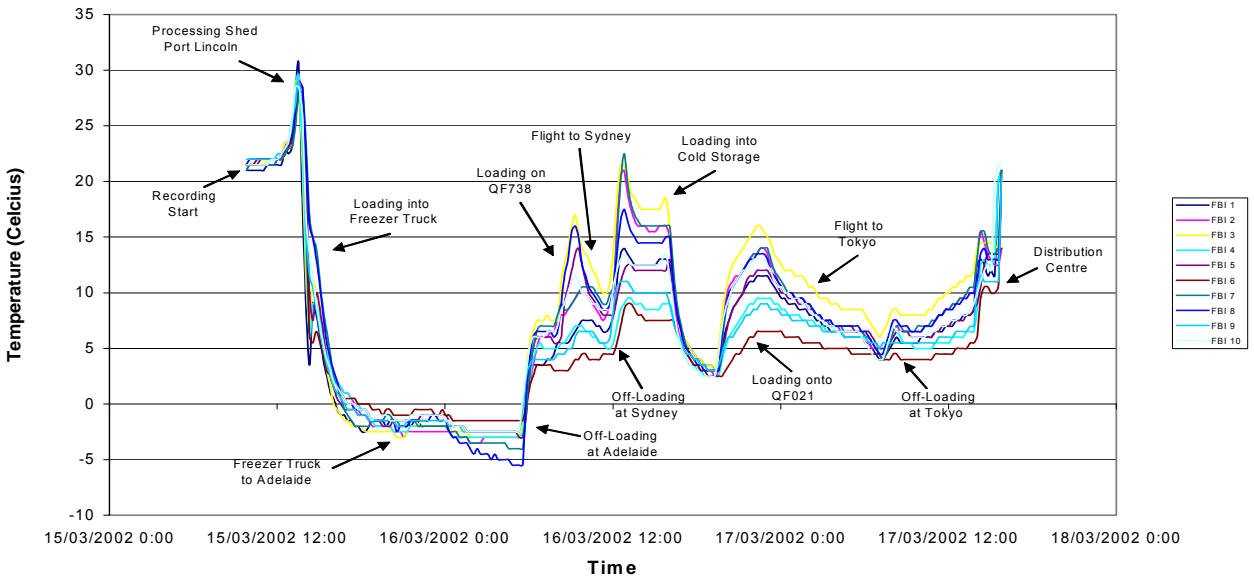


Figure 5.8: Individual box internal logged temperatures from Port Lincoln to Narita International Airport via Adelaide and Sydney for a shipment processed on the same day as harvest in March, 2002.

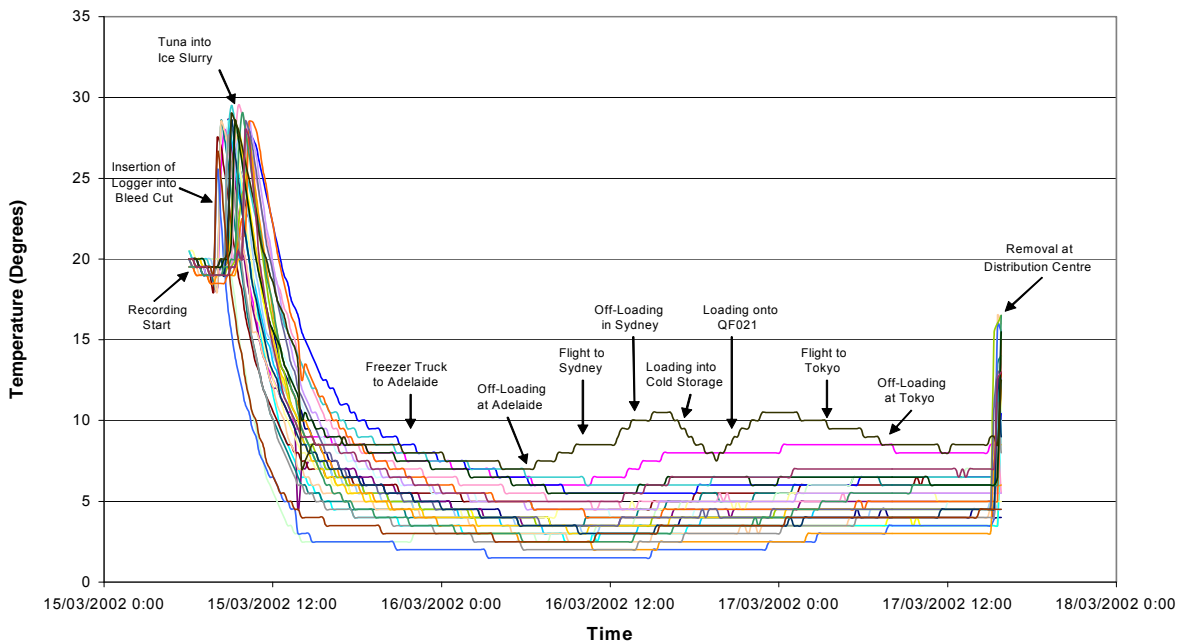


Figure 5.9: Individual tuna internal logged temperatures from Port Lincoln to Narita International Airport via Adelaide and Sydney for a shipment processed on the same day as harvest in March, 2002.

August Harvest - Average box external temperatures rose considerably at Melbourne, Sydney, Incheon and Tokyo prior to loading and following off-loading of aircraft (Fig 5.10). Alarming high temperatures were observed in Tokyo with box external loggers recording temperatures ranging between 30.5⁰C and 40.5⁰C at 14:01 hours following off-loading (Fig. 5.11). The average box internal temperature also mirrored the trend exhibited by the external loggers, although being buffered by the insulating properties of the cardboard it was naturally lower. There were two box internal loggers that recorded sub-zero temperatures during transit, FBI 5 (light blue line) and FBI 4 (yellow line) (Fig. 5.12). FBI 5 consistently recorded temperatures well below other loggers indicating possible logger malfunction, and FBI 4 more closely mirrored the trends seen in externally attached loggers. This may have been caused by a damaged box that was not effectively insulating the internal temperature. Average fish internal temperatures fell below 5⁰C at approximately 20:00 hours on the night of the 6th, with the highest and lowest temperatures measuring at 8.5⁰C and 1.5⁰C respectively at that time (Fig. 5.13). The lowest average fish internal temperature of 1.08⁰C was recorded at 12:00 hours on the 8th of August. From this point the average internal fish temperatures increased to 3.83⁰C at 16:31, following the heating event that occurred after the off-loading of flight KE701 in Tokyo. Loggers C5 (aqua line), C10 (brown line), and D1-9 (light blue) recorded sub-zero temperatures indicating possible contact with the external environment (e.g. ice packs in the gill cavity) and/or possible logger malfunction.

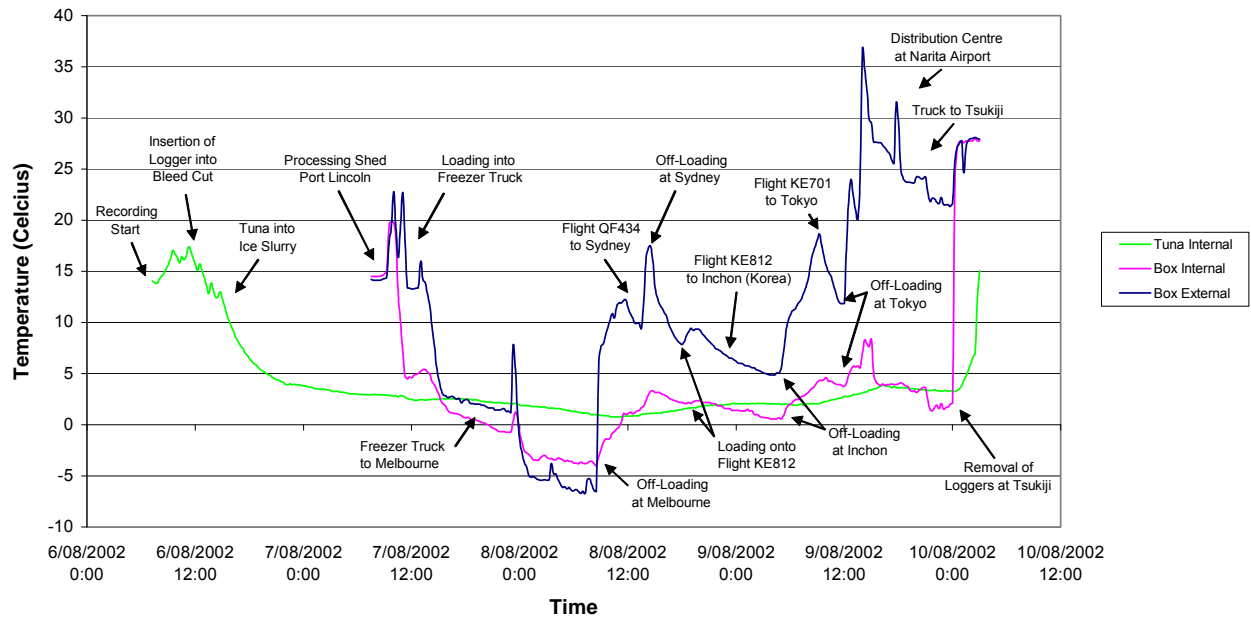


Figure 5.10: Average box external, box internal, and tuna internal logged temperatures from Port Lincoln to Tsukiji Wholesale Market via Melbourne, Sydney, and Incheon (South Korea) for a shipment processed on the day after harvest in August 2002.

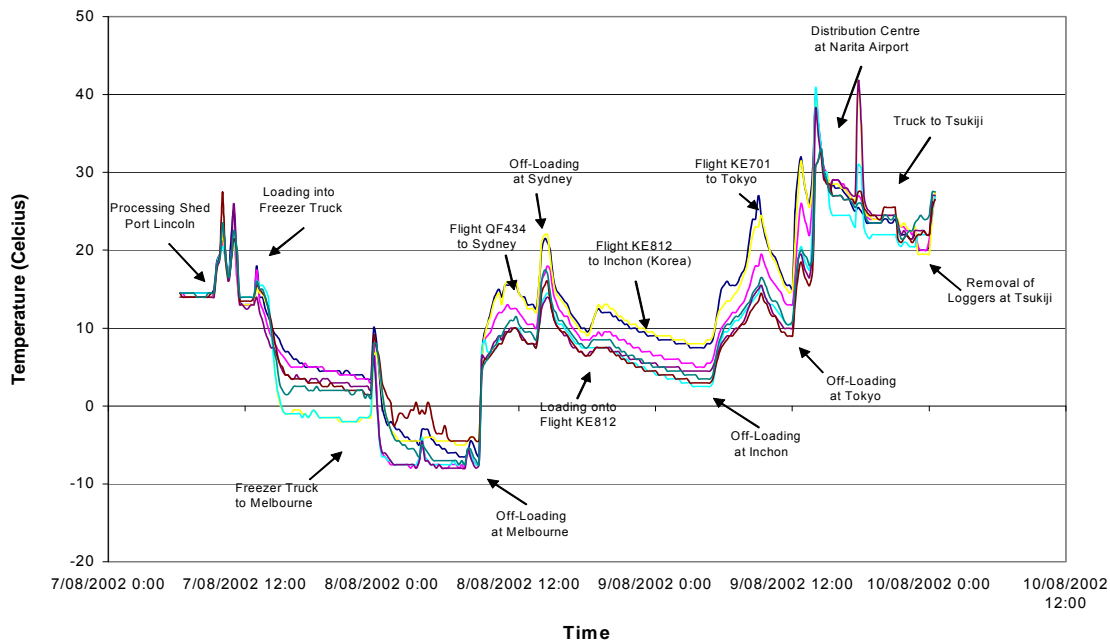


Figure 5.11: Individual box external logged temperatures from Port Lincoln to Tsukiji Wholesale Market via Melbourne, Sydney, and Incheon (South Korea) for a shipment processed on the day after harvest in August 2002.

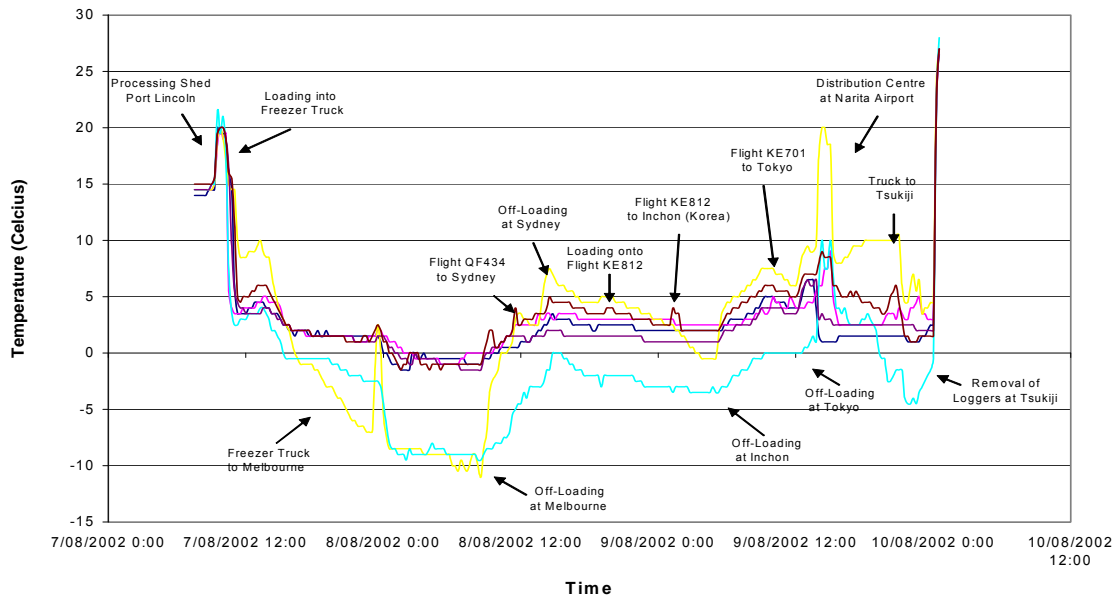


Figure 5.12: Individual box internal logged temperatures from Port Lincoln to Tsukiji Wholesale Market via Melbourne, Sydney, and Incheon (South Korea) for a shipment processed on the day after harvest in August 2002

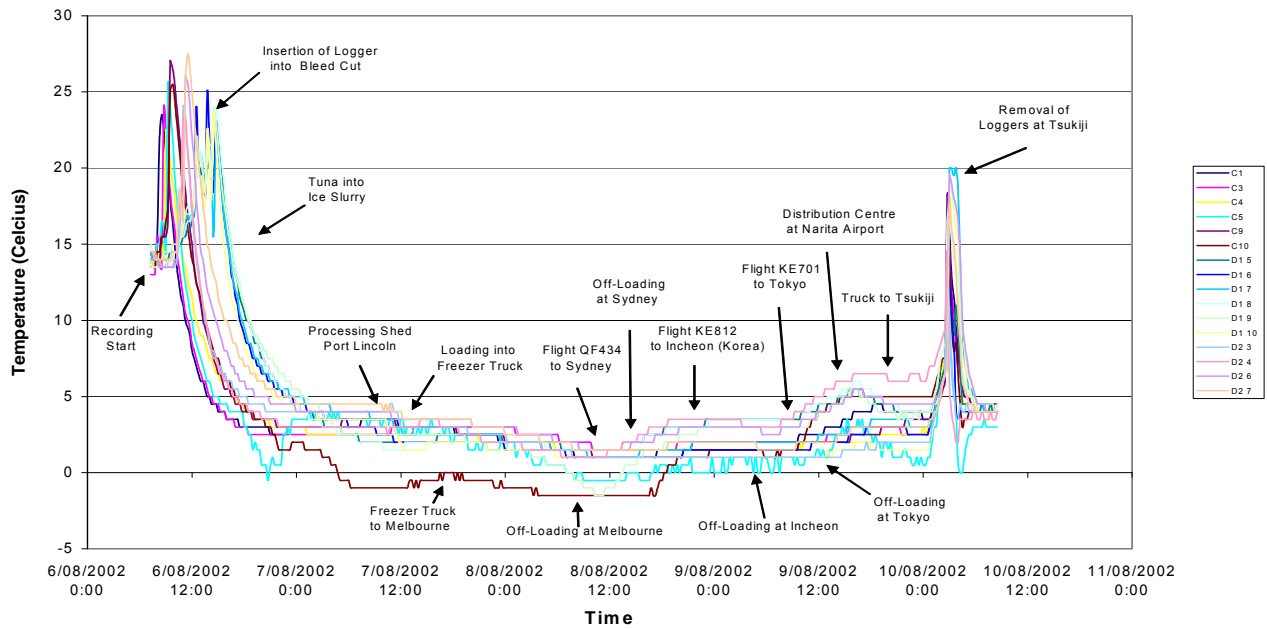


Figure 5.13: Individual tuna internal logged temperatures from Port Lincoln to Tsukiji Wholesale Market via Melbourne, Sydney, Incheon (Korea) for a shipment processed on the day after harvest in August, 2002.

Objective Two: *Same Day Vs Next Day Processing*

Time-Temperature: No time-temperature data was retrieved for this experiment due to a technical failure of the loggers which failed deactivation commands and the data was overwritten.

Sensory: There were no significant differences ($p>0.05$) between the Akami cuts from fish processed either on the same day as harvest or the next day after harvest for the average appearance scores of redness, brightness, and degree of colour change on day one and day four in Japan (Fig. 5.14). There were also no significant differences between the treatments for the degree of colour change in the cuts of Otoro (Fig. 5.15).

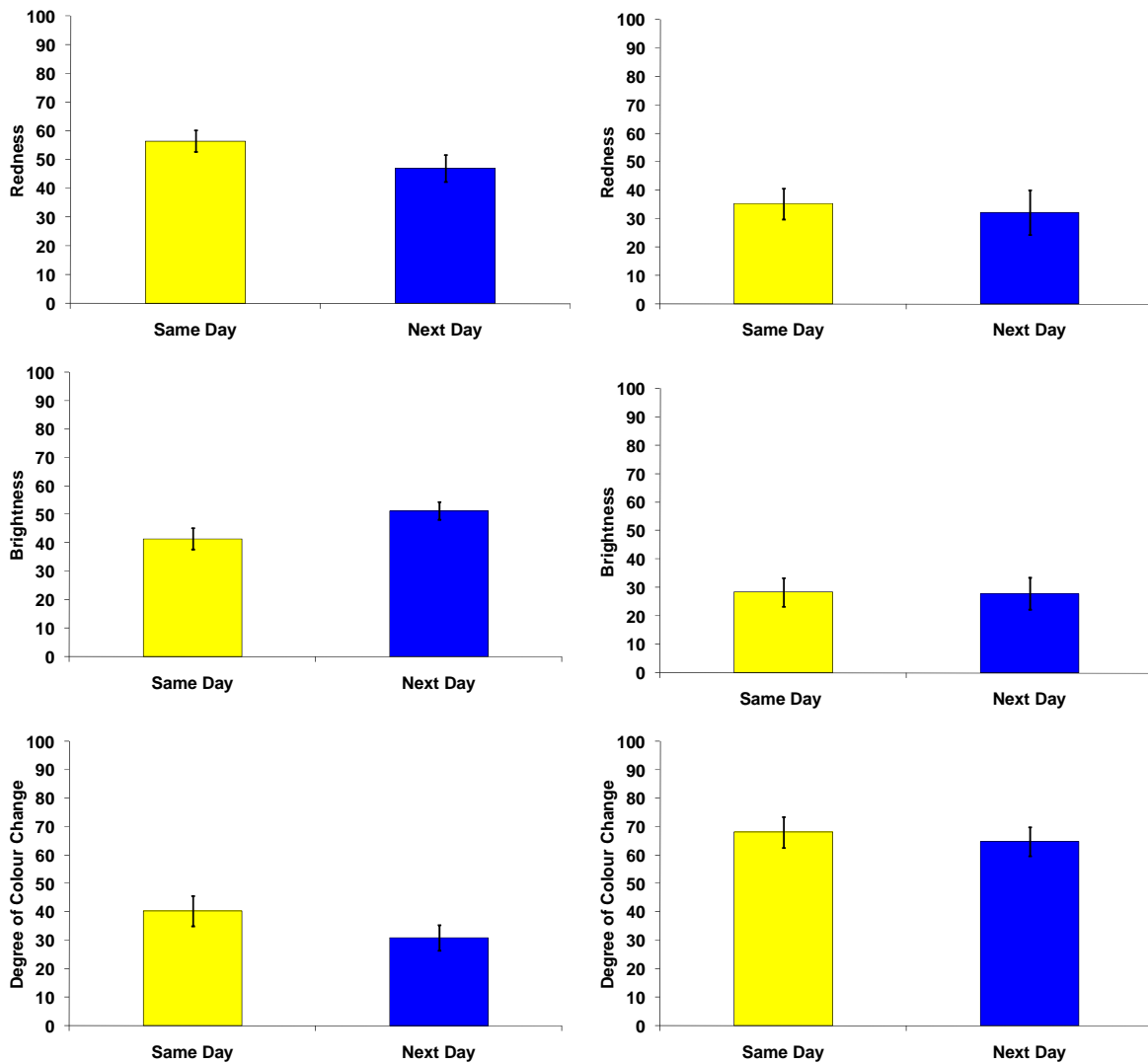


Figure 5.14: Average scores for redness, brightness and degree of colour change from the Akami cuts of farmed, fresh Southern bluefin tuna on day one (left column) and day four (right column) in Japan that were processed on the same day as harvest or the next day after harvest in Port Lincoln, Australia.

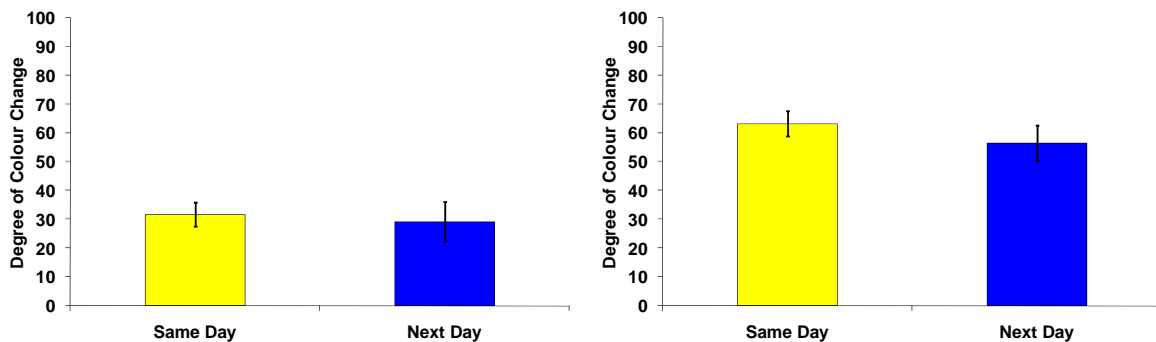


Figure 5.15: Average scores for the degree of colour change from the Otoro cuts of farmed, fresh Southern bluefin tuna on day one (left) and day four (right) in Japan that were processed on the same day as harvest or the next day after harvest in Port Lincoln, Australia.

There were no significant differences ($p > 0.05$) between the Akami and Otoro cuts from fish processed either on the same day as harvest or the next day after harvest for the average sensory scores of bad fishy odour on day four in Japan (Fig. 5.16).

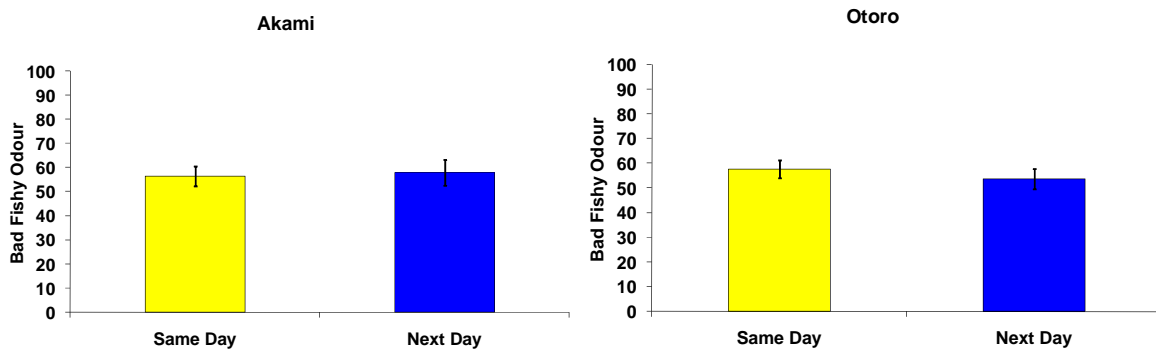


Figure 5.16: Average scores for the bad fishy odour from the Akami (left) and Otoro cuts (right) of farmed, fresh Southern bluefin tuna on day four in Japan that were processed on the same day as harvest or the next day after harvest in Port Lincoln, Australia.

There were no significant differences ($p>0.05$) between the Akami and Otoro cuts from fish processed either on the same day as harvest or the next day after harvest for the average hedonic scores of 'quality grade' on day one and day four in Japan (Fig. 5.17).

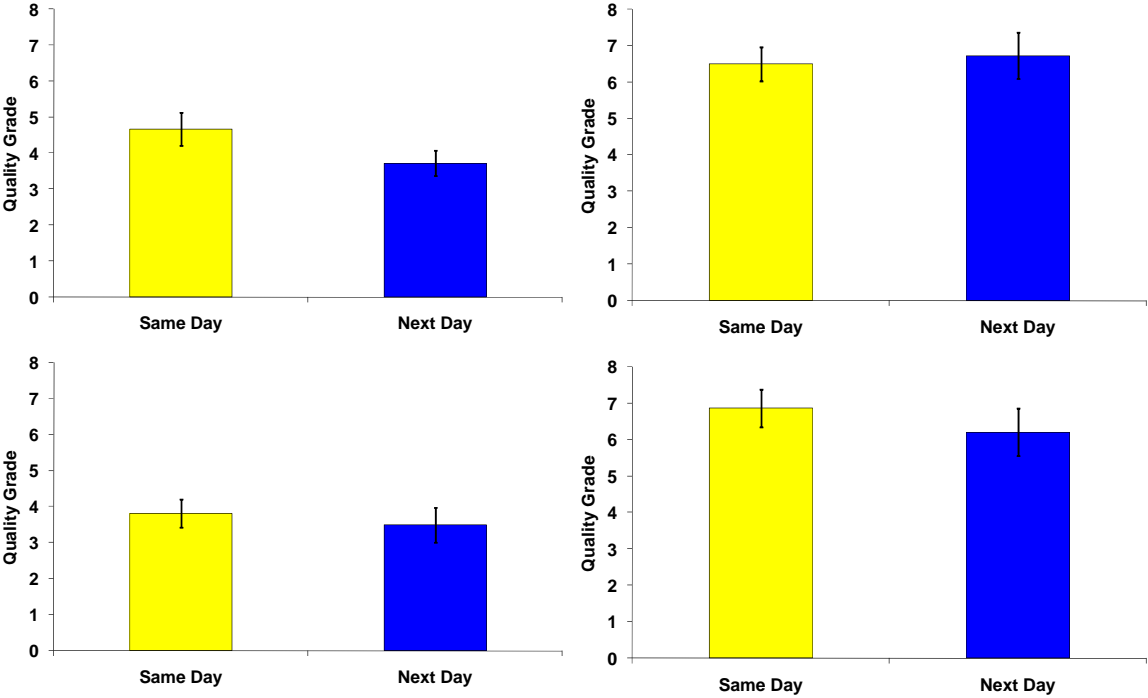


Figure 5.17: Average scores for the quality grade from the Akami (upper figures) and Otoro cuts (lower figures) of farmed, fresh Southern bluefin tuna on day one (left column) and day four (right column) in Japan that were processed on the same day as harvest or the next day after harvest in Port Lincoln, Australia.

Biochemical: There were no significant differences ($p>0.05$) between the Otoro cuts from fish processed either on the same day as harvest or the next day after harvest for concentrations of hydroperoxide on day one and day four in Japan (Fig. 5.18).

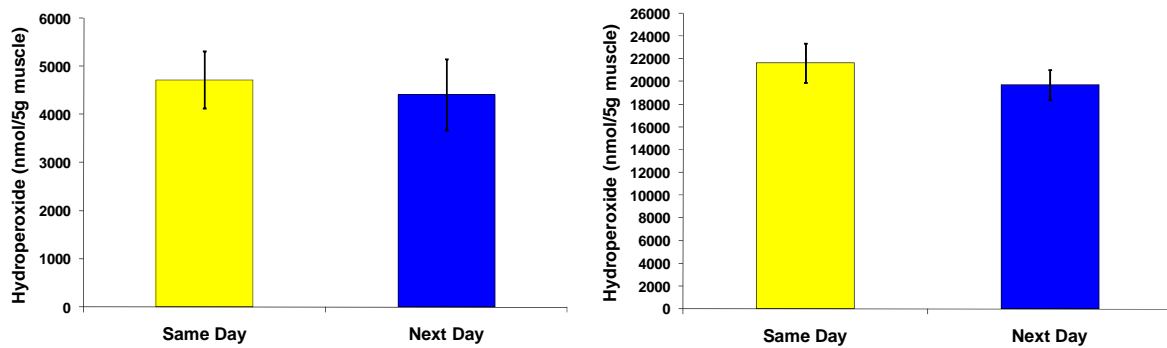


Figure 5.18: Average concentrations of hydroperoxide (nmol/5g muscle) in the Otoro cuts of farmed, fresh Southern bluefin tuna on day one (left) and day four (right) in Japan that were processed on the same day as harvest or the next day after harvest in Port Lincoln, Australia (n=5).

Unfortunately due to technical difficulties at the Tokyo University of Fisheries, samples were not analysed for nucleotides.

5.5 Discussion

Time-temperature Profiling

The first objective of these trials was to examine the changes in the ambient and carcass temperatures of fresh, farmed Southern bluefin tuna during shipment from Port Lincoln, Australia, to Tokyo, Japan. In order to examine variations between season and shipping strategy, temperatures were logged in both March and August, and for fish processed on the same day and the following day of harvest.

As with all cold-chains the weakest links were found at the transfer points (Giannakourou *et al.*, 2005). In March the external temperatures during freezer truck haulage from Port Lincoln to Adelaide were all sub-zero and in the range of -5°C to 0°C (Fig. 5.7). However, in August, the time spent in haulage was marked by two distinct temperature regimes (Fig. 5.11). Within the first leg of the journey from Port Lincoln to a destination unknown (possibly a shunting yard between Port Lincoln and Melbourne) all loggers recorded temperatures above zero indicating that the freezer fans were not set at the requested level of -10°C . During the second leg of the journey to Melbourne however all loggers recorded sub-zero temperatures. Whether this was deliberate or accidental is unknown although there were concerns raised by tuna farm managers that drivers were switching off their freezer fans after leaving Port Lincoln to reduce costs. The data was shown to some of the freight forwarders operating out of Port Lincoln and in subsequent trials where the time-temperature profiles were logged the problem did not re-occur.

As it is summer in March in Australia and summer in August in Korea and Japan, the highest average external temperatures of 20.55⁰C and 27.9⁰C (Adelaide and Sydney; Fig. 5.7), and 18.6⁰C (Korea) and 36.7⁰C (Japan; Fig. 5.11) were recorded in these months and locations respectively. Maximum recordings of 38.0⁰C and 47.0⁰C in Adelaide and Sydney, and 27.0⁰C and 40.5⁰C in Korea and Tokyo highlight the dangers of exposure to direct sunlight and high air temperatures during the loading and offloading of the tuna into and from the aircraft during these months. This is especially the case in Japan, as the ice blocks have melted by up to 50% of their original volume during transit (verified at the distribution centre during re-icing). The variability in external temperatures between coffins within a shipment in some instances may be a result of the random distribution of loggers throughout an AV and the exposure of some loggers but not others to direct sunlight. In transit, the proximity of some loggers to cooling fans may also result in large discrepancies between recorded temperatures. The implications of these factors are known to freight and airline companies as upon consultation they said practices were being altered to minimize the exposure to direct sunlight and high temperatures. Indeed, at Adelaide Airport, *Australian Air Express* were in the process (2003) of building a cover to shield perishable goods from direct sunlight prior to their movement to the terminals.

The logged temperatures inside the boxes during both shipments generally mirrored the trends of the temperatures logged on the outside of the boxes. Due to the fact the loggers used in the March shipment (Fig. 5.8) were outside of the plastic wrap around the tuna carcasses the logged temperatures more closely mirrored the externally logged temperatures when compared to the logged temperatures of the August shipment when the loggers were inside the wrap (Fig. 5.12). In both March and August, during freezer truck haulage, the lower temperatures outside and inside the boxes appeared to further reduce carcass temperatures – even the carcasses that had been left in ice slurry over night, suggesting that circulation of the slurry may further help to lower carcass temperatures pre-shipment . Internal temperatures were more sensitive to changes in the external temperature following the offloading in Korea and Japan and most probably due to the partial melting of the ice packs.

In August, with all data included, the lowest average temperature recorded inside the box and plastic wrapping was during trucking (-4.0°C), with a range of -1.0°C to -11.0°C (Fig. 5.12). The highest average value of 8.3°C , with a range of 1.0°C to 20.0°C , was recorded in Tokyo. However, as with temperatures recorded on the outside of the boxes, there was a great deal of variation in internal temperatures which may have resulted from differing position of the box and logger in an AV, the position of the logger in the box, the number of tuna packed in a single box, and possible damage to the box resulting in a reduced insulating capacity. For example,

two of the loggers recorded extremely low temperatures both during trucking and offloading. However, as these loggers were recording almost identical temperatures to the other loggers at both the beginning and end of trial it is difficult to dispel the data and put it down to logger malfunction.

With these two loggers removed however there is a tighter range and the lowest and highest average internal temperatures move from -4°C to -1°C and 8.3°C to 6°C respectively. Regardless of whether the possible outliers are in or out of the calculation, both the latter averages are too high for the preservation of fresh fish (Huss, 1995). On the other hand, temperature can also be too low with the result being partial freezing of the flesh. Whether a particular fish freezes is a function of its size, composition, and the starting temperature of the carcass as well as time. To determine the optimal cooling conditions it would be necessary to determine the thermal properties of tuna carcasses of different size and condition and generate a model. If such a model were available post-harvest handling techniques, such as minimum time in slurry and on-boat sorting and processing according to size rather than the harvesting order may be defined or implemented respectively. Further, with real-time, wireless thermostatic control it may be possible to maintain ambient and carcass temperatures at optimum levels during shipping (McAteer *et al.*, 2001).

The March 'same day processed as harvested' shipment recorded an average carcass temperature at processing of approximately 11.5°C , with a range extending

from 3.5⁰C up to 18.5⁰C (the latter taken toward the end of a harvest; Fig. 5.9). In August, the tuna processed the day after harvest resulted in an average temperature at processing of 2.9⁰C, with a range extending from 2.0⁰C to 4.5⁰C (outliers excluded; Fig. 5.13). Both the average and range of carcass temperatures show that, at the time of processing, carcasses left in ice slurry overnight enter the shipping stage at much lower and less variable temperatures.

Of the carcass temperatures recorded during shipment in March, the lowest average temperature recorded was 3.9⁰C, with a range of 1.5⁰C to 10.5⁰C during off-loading at Sydney Airport (Fig. 5.9). In August, at the same point during shipment, the lowest average temperature recorded was 1.1⁰C, with a range of 1.0⁰C to 1.5⁰C (excluding outliers; Fig.5.13).

If a true reflection of the tuna carcass temperatures (and not due to logger malfunction or dislocation) the upper range in the March shipment should be of great concern. All other factors being equal, a tuna at 8⁰C would be deteriorating at a rate nearly twice as fast as tuna at 3⁰C (using $r = (1 + 0.1t)^2$; Bremner, *et al.*, 1987). Although possibly not recognisable in the short term, undesirable qualities would show up in the stored meat. The implication is that the quality characteristics of tuna can vary both between and also within shipments and may result in a poor reputation for the product. Control of all harvest and post-harvest factors leads to brand protection.

At processing and during shipment the critical time of the year in Australia is therefore at the start of the season (March) due to the higher ambient water and air temperatures – especially for fish processed on the same day as harvesting. Fish of similar size need to be processed in the order they were harvested to ensure maximum time in ice slurry. When fish are of variable size it may be warranted to place carcasses into cooling bins according to size and process the smaller fish first and the larger, warmer fish last giving them longer in slurry conditions.

Naturally, at all times of the year it is necessary to process the tuna as rapidly as possible and have the tuna coffins loaded on to freezer trucks immediately following packaging, with the boxes stacked with spaces between them to allow the cold air to ventilate. According to the carcass temperature data, thermostats in the trucks could be set to lower levels than recorded on this trial, however, it must be noted that the position of the loggers was close to the core and not the most thermally sensitive part of the carcass – the tail region – which is the most susceptible to freezing damage.

The average and range of carcass temperatures recorded in March in Japan were 5.2⁰C, and 3.0⁰C to 8.5⁰C at the distribution centre near Narita Airport (Fig. 5.9). At the same handling stage in August the average and range of temperatures recorded were 3.5⁰C, and 1.5⁰C to 6.5⁰C respectively (Fig. 5.13). As the shipments differ in

the time of year, supply route used, and method, it is not possible to compare the two beyond the fact that packing the day after harvest resulted in less temperature variation between individuals along the supply chain. However, with some carcasses registering temperatures above 5⁰C at both times of the year it is evident from these trials that improvements could be made in the cold-chain management of the product regardless of the processing strategy – especially during the summer months in Australia and Japan at either end of the season.

In this experiment the data loggers were placed into the post-pectoral bleed-cut position after a core sample had been removed. This region is in close proximity to the buccal cavity which is well exposed during ice slurry, and is packed with ice at processing. The extremely low temperatures recorded by a number of the loggers would indicate possible contact or close proximity to the ice packs. Further, as tuna exhibit variable temperature profiles both throughout the carcass of an individual and between similarly proportioned individuals there is a need to examine more points within the carcass to get an understanding of these temperature profiles and how they may relate to ambient water temperatures, harvest stress, and post-harvest manipulations and environments. It may be possible in the future, with enough data, to model and predict carcass temperature profiles using a single sampling point.

This study has highlighted the temperature profiles of tuna carcasses and the shipping environments for two of the supply routes currently used by the industry, at two different periods within a single season, and has been effective in pin-pointing areas of concern along the supply chain at these periods. Of the remaining possible supply routes, and any new routes that become available, it is worth investigating the affects of the time-temperature profiles on the quality characteristics of the end product.

Same Day Vs Next Day Processing

The second objective of these trials was to examine the affect of processing the tuna on the same day as harvest as opposed to processing the tuna the day after harvest in Port Lincoln on a range sensory and biochemical parameters in Tokyo. For all the sensory and the single biochemical indicators of quality there were no statistically significant differences in mean scores between same day and next day treatments ($P>0.05$). Thus, it would appear that any benefits of shipping the tuna at colder and more constant temperatures are possibly cancelled out by getting the fish to market 24 hours earlier. However, the average values of all indicators except 'redness' on day one for the Akami section suggested that processing the day after harvest resulted in flesh quality attributes that were more favourable to the wholesaler and the consumer (i.e. next day processed fish tended to have less colour change, were brighter, were higher in quality grade, and lower in hydroperoxide value) than from fish processed on the same day as harvest (see

Table 5.1). This is in keeping with general principles (Huss 1995; Bremner *et al.*, 1987).

Parameter	Day in Japan	Same Day		Next Day	
		Akami	Otoro	Akami	Otoro
Hydroperoxide	Day 1	-	X	-	✓
	Day 4	-	X	-	✓
Redness	Day 1	✓	-	X	-
	Day 4	✓	-	X	-
Brightness	Day 1	X	-	✓	-
	Day 4	✓	-	X	-
Colour Change	Day 1	X	X	✓	✓
	Day 4	X	X	✓	✓
Quality Grade	Day 1	X	X	✓	✓
	Day 4	✓	X	X	✓
Fishy Odour	Day 4	✓	X	X	✓
Frequency Table		Same Day		Next Day	
		Akami	Otoro	Akami	Otoro
Total Day 1		1	0	3	3
Total Day 4		4	0	1	4
Grand Total		5	0	4	7

Table 5.1: Hedonic comparison of the average objective and subjective quality parameters of the Akami and Otoro cuts from fresh, farmed Southern bluefin tuna on day one and day four in Japan processed on the same day or the next day of harvest in Port Lincoln, Australia (✓ = preferable; X = not preferable; - = not measured).

However, for the Akami, this trend had reversed by day four, with fish processed on the same day as harvest revealing more preferable flesh quality characteristics for this cut than tuna processed the day after harvest. In contrast, the average values for the Otoro on day four were consistent with those recorded on day one with all quality related parameters favouring next day processed tuna to that of processing tuna on the same day as harvest. These trends may stem from a greater sensitivity

to temperature of the lipid fraction compared to the non-lipid fraction (lipid oxidation), and a greater sensitivity to time of the non-lipid fraction compared to the lipid fraction (myoglobin oxidation).

Employing strategies that keep the carcasses of fish at colder and at more constant temperatures has been shown to inhibit the hydrolysis and oxidation of the lipid fraction, and according to Losada *et al.*, (2005), is recommended for relatively fat fish species in order to obtain safer and higher quality fish products. In colder water temperatures there is an increased degree of un-saturation of the lipid fraction with the conversion of saturated fatty acids of the biological membrane phospholipids typical of the warm season into poly-unsaturated fatty acids typical of the cold season (Calabretti *et al.*, 2003). It is the instability of these unsaturated fatty acids, especially at higher temperatures, that would make the winter product from Australia susceptible to oxidation following off-loading in summer Japan.

With the industry's *raison d'être* being the production of fatty tuna the average results for seven quality related parameters for the Otoro not favouring the common practice of processing tuna the same day as harvest should be of concern to the industry. However, with 'colour' said to be the quality trait of concern for farmed tuna by the market the more favourable score for redness of the Akami in this experiment supports the notion that same day processing meets the request of the market. However, the 'market' in this sense may only represent the auctioneers who, with only a cut tail on display, present the non-lipid Akami to wholesalers for grading prior

to the auction. This 'redder' Akami of the tail of same day processed tuna may help to generate higher auction prices but lead to a slightly compromised Akami portion at day one and the Otoro portion for the life of the product.

Current industry practice is to use an ice slurry to chill the carcass immediately post-harvest, it would be worth examining the use of biphasic fluid ice (flow-ice) that consists of small spherical ice crystals suspended in iced water at a temperature slightly above the freezing temperature of fish ($\sim -1.5^{\circ}\text{C}$). This system of fluid ice has a faster chilling rate than flake ice or refrigerated ice water due to a greater heat exchange capacity and limits physical damage to the carcass owing to the spherical geometry of its microscopic ice crystals (Rodriguez *et al.*, 2005), and this greater chilling ability of this system could overcome some of the time constraints to same-day processing.

5.6 Conclusions

Within the cold chain for fresh, farmed air-freighted Southern bluefin tuna the most hazardous periods are during the loading and off-loading of the tuna coffins at the airports – especially during the summer periods in either Australia or Japan. As there are no known cases of spoilage or food poisoning directly associated with the poor cold chain handling of the product it has not been highlighted as an area of concern. However, from a non-bacteriological quality standpoint, the industry could benefit from paying more attention to the cold chain to assure maximum

preservation of desirable quality attributes and shelf-life.

As tuna exhibit variable temperature profiles both throughout the carcass of an individual and between individuals of the same proportions there is a need to examine more points within the carcass to get an understanding of these temperature profiles and how they may relate to ambient water temperatures, harvest stress, and post-harvest manipulations. It would be possible to model and predict carcass temperature profiles using a single sampling location if enough data were available.

In order to ensure both the physical and microbiological qualities of the product each stakeholder within the supply chain should be party to a '*Chain of Responsibility*' agreement which binds them to a duty of care for the handling of the product. Incorporating temperature monitoring tracing technologies will enable the identification of where problem points occurred within the chain and who is liable for any claim (Mc Ateer *et al.*, 2001).

Although there were no statistically significant differences between the sensory and biochemical parameters from fish processed on the same day as harvest compared to fish processed a day after harvest, averages appear to favour the latter. Due to logistical and budgetary restraints sample numbers were at the lowest possible level ($n=5$) to detect a difference (at $\alpha=0.05$) and therefore a more rigorous test or

cumulative tests may produce statistically significant results. Further, as the water and air temperatures in Port Lincoln during the end of the season are much lower than at the start of the season, resulting in lower carcass temperatures at the commencement of shipping, investigations examining the effects of processing on the day or the day after harvest at differing times of the year should be undertaken to account for these seasonal variations in temperature.

The longer that fresh fish, especially fatty species, are kept at the lowest possible temperatures without freezing, lipid hydrolysis and oxidation is limited - even following disturbances in the time-temperature profiles (Pineiro *et al.*, 2005; Losada *et al.*, 2005). Therefore, investigations into combining the time benefits of same day processing with lower and constant temperature shipping could be considered. This might involve the loading of fluid ice bins direct from the boats on to trucks leaving Port Lincoln for Adelaide or Melbourne on the day of harvest for processing at facilities near those airports.

6. SUMMARY & CONCLUSIONS

With the advent of tuna farming a new product was created for the Japanese sashimi market differing from its wild caught counter-part in terms of its biochemical and physical characteristics, its seasonality, its volume, and its consistency. Although farming raises practical issues that need to be dealt with, it also offers for the first time the possibility of looking at the affects of husbandry, harvest and post-harvest factors on product quality using an R&D approach. Furthermore, it allows us to use what we have learned about the importance of husbandry, harvest and post-harvest handling on the product qualities of other farmed animals. The following summarises the findings of the first market-based research into the qualities of farmed Southern bluefin tuna, how they are impacted upon by husbandry, harvest, and post-harvest applications within the South Australian industry, and possible future directions for research .

In Japan a tuna carcass is classified and sectioned into three portions – Akami (inner portions of the dorsal and ventral loins), Chutoro (outer portions of the dorsal and ventral loins), and Otoro (belly portions of the ventral loins). However, unlike the cuts of traditional meats (i.e. beef, lamb, chicken etc) that are sectioned, named, and sold according to their physical location within a particular muscle or the skeletal frame, the three cuts of tuna are from the single ‘white’ muscle block and have less precise boundaries. At the shop front or sushi counter a tuna that is high in fat will be sectioned along different cut lines to one that is less fat, with the fatter

tuna yielding a greater amount of the pink Chutoro and Otoro relative to the red Akami. This is evidenced by the fact that some sushi restaurants advertise and sell 'toro', preferring to link the higher priced fattier portions of Chutoro and Otoro together. As a result, a fatter tuna will not only produce a higher yield overall but will yield a greater amount of the more valuable cuts. This greater yield of more expensive flesh may more than compensate for the higher costs of feeding fattier baitfish. This should be of particular interest to the industry that has, on a whole for the past three years, been feeding a higher proportion of low-fat baitfish that have lower feed conversion ratios which result in production of a tuna with lower overall fat levels than in previous years. This lower cost practice may result in poorer overall return, when the lower conversion ratios and lower yields of the more highly valued Chutoro and Otoro cuts are taken into account than if fattier baitfish are fed to the tuna. The feeding of low fat baitfish may be a false economic strategy and the cost and benefits of feeding less quantities of fattier baitfish is worthy of investigation. Results from trials with fattier baitfish in conjunction with compositional data on proportion of the various cuts and the price ratios between them could be used to derive a model that estimates returns. This would provide industry with a decision making tool to choose sources of baitfish and feed practices as prices, availability and composition of baitfish fluctuate. The tool would be an aid in projecting returns in conjunction with other factors such as supply, demand and currency fluctuations.

Proximate analysis of the three cuts of farmed Southern bluefin tuna reveal significant differences in fat and moisture levels. The three cuts also show variations in the concentrations of a suite of biochemical indicators related to quality attributes. However, analysis of these biochemical indicators of quality on a solids basis, revealed that the structural and functioning parts of the three differing cuts that make up tuna white muscle have similar metabolic activities. Although more investigations are required, this finding potentially validates the use of muscle samples from any of the three cuts as an indirect measure of carcass-level quality and may justify using the redundant tail cut as the test muscle for all three cuts. This is significant for tuna in that grading is performed on gilled and gutted fish, head- and skin-on complete with scales, and in contrast to the flesh quality examinations of other meat industries such as beef in which appraisals are performed on the skinned, dehorned, deheaded and trimmed carcasses providing direct access to the flesh. Tuna flesh is mainly assessed on a tail cut so that flesh sampling and analysis could be performed in both Australia and Japan. This means that the results would be relevant to the end product in a way that would not affect the value of the carcass.

Development and application of the world's first sensory testing procedure for tuna revealed statistically significant differences between the Akami and the Chutoro and Otoro cuts for a number of sensory descriptors. Principal Components Analysis of the sensory data coupled with instrumental data further revealed clustering of the

samples into the cuts of Akami, Chutoro, and Otoro. The predictive capacity of the suite of instrumental variables examined on the sensory response variables was low (E-explained=58%; Y-explained=22%) and potentially a result of the low sample numbers examined for each cut. To improve the predictive capacities of instrumental data of the sensory characteristics more sample numbers would be required for each cut of Akami, Chutoro, and Otoro. Using the same experimental design developed in the present investigation, it would be possible to raise the sample number from eight to twenty-four fish and examine only one of the three cuts in a carcass. Alternatively, multiple trials examining all three cuts could be conducted over time and the data pooled and the cuts modelled separately. Both these options have design flaws as there is potential for missing a quality-related issue (e.g. tuna burn etc.) in an unexamined cut or of introducing unintentional procedural variation. Furthermore, tuna farming industries contrast greatly with many other food industries that apply a stepwise approach to building predictive models, such as the processed foods industries, in that the costs and variability of the end product is high – tuna are very costly experimental units. However, to avoid these costs, physiologically similar surrogates could be identified and farmed alongside the tuna. Results on these surrogates could then be used to help build predictive models in conjunction with the target species.

Regardless of the economic barriers to creating predictive models, the development, however, of the statistically balanced experimental design and standard procedures

for the sensory analysis of Southern bluefin tuna, mean that it is now possible to examine the affects of any on-farm or in-chain manipulation on the sensory properties of the flesh. This in turn could be used by farmers to identify inputs and/or practices that produce desirable traits in the product from the aspect of quality improvement, or undesirable traits that may arise in any attempt to reduce costs. Using both approaches, the sensory analytical tool is one of the most powerful means a farmer has to produce the highest quality product for the lowest cost.

Flesh grading of the desirable and/or undesirable traits in meat is common in industries such as beef, pork, and poultry, and can be used by wholesalers and retailers and consumers to guide their pricing and purchasing decisions respectively. In these industries a range of subjective and objective measures of quality are used in a variety of countries, yet for farmed tuna there are no grading standards in any country where an industry is established, and none in its major market - Japan. This is despite large differences in 'quality' between individual fish placed on this market, and the average yield and 'quality' between particular producers within a particular industry.

With a view to quantify these quality variations at the market, investigations were tested to see if digital camera technology could be calibrated to the 'eye' of a tuna quality expert on the Tsukiji auction floor of Tokyo. Significant differences between a ratio of the average red, green, and blue values ($R/(G+B)$) extracted from digital

images of the flesh of the tail sections of tuna graded into quality ranks A, B, and C by an expert technician showed potential for use of the technology as a rapid, non-destructive, objective method for tuna quality grading. Furthermore these RGB ratios and quality ranks were also correlated to the auction floor prices received for the tuna.

A premium is paid for fatter fish in the market and the higher quality ranks are ascribed to 'rounder' individuals. Digital colour analysis could be combined with rapid analytical techniques of body composition and carcass conformation. Additionally, to account for changes in quality characteristics of the flesh over time, an indicator of freshness such as K value regularly calibrated to an expert grader, could form the basis of a quality ranking system. Alternatively, developing a quantitative approach to classifying the sensory characteristics that equate to a particular rank could lead to a technician based grading system. Incorporating a Quality Indexing Method (QIM) into such a grading system could also account for quality changes over time.

A standardised grading system within a particular farming industry could assist producers, intermediaries, and retailers in maximizing their economies of scale, as well as aid consumers with purchasing decisions. At present, there is limited transparency within the tuna industry as a whole and it operates mostly on trust relationships between the various parties within a particular supply chain. A quality

grading system linked to pricing would allow producers to more effectively predict market returns and/or negotiate fixed prices that are better balanced with the costs of producing a target level of quality. This would allow retailers to understand their consumers buying habits for a given quality grade and adjust policy and procurement accordingly.

The important role of the micro-nutrients vitamin C, vitamin E, and selenium for *in vivo* cellular function and structure, and *ex vivo* in muscle foods have been well documented within a number of industries. Their properties as anti-oxidants (vitamin C and E) and role in enzyme function and cellular integrity (selenium) in helping to maintain the colour of muscle foods are well known. In the Australian industry vitamin E and C have been supplementally fed to farmed tuna which, when compared to wild tuna, have come under criticism in Japan for having a shorter colour shelf-life. The industry's common method of scattering a pre-mix powder containing vitamin E and C on baitfish prior to feeding did not significantly raise the flesh concentrations of these micro-nutrients. In fact, Vitamin C and selenium concentrations were shown to decline over the course of the season, whereas vitamin E levels remained constant. Whether or not the decreases were due to a dietary shortfall of vitamin C and selenium or were associated with other aspects of the farming process needs to be determined. Research into the nutritional profiles, the storage temperatures and times of the baitfish species that are currently fed to farmed tuna has since been carried out in order to improve the quality and supply of

not only the macronutrient but also the micronutrient fraction of the tuna's diet.

Despite these findings, tuna fed higher concentrations of vitamin E and C in the pre-mix powder significantly held their colour for longer than those fed lower concentrations, warranting the cost of the practice. Research to develop a moist pellet as an improved delivery mechanism for these essential nutrients is also under way, and proposed for investigation at the time of writing is the potential use of micro-injection techniques to add vitamins to both baitfish and the tuna muscle post-mortem.

The stress of capture and slaughter has been shown to greatly affect the qualities of beef, pork, poultry, and fish. Assessment of the qualities of tuna taken at the beginning and the end of a commonly used batch harvesting procedure lasting approximately one hour revealed no significant differences in the majority of sensory and biochemical indicators when the fish were examined at day one in Japan. Southern bluefin tuna may therefore be coping 'well' with the stressors of this type of harvest. Alternatively, the physicochemical indicators of stress and/or the analytical methods applied may not have been sensitive enough to detect differences. However, it should be noted that test sensitivity is relevant to the industry only if detectable differences relate either to problems with animal welfare or to issues of product quality as assessed by trained and experienced panellists.

The only flesh quality indicators that showed a statistically significant effect of harvest stress were the digital camera RGB ratios of the Akami section, and the sensory descriptors of transparency and brightness of the Otoro section. As the RGB ratios were calibrated to the grades of an expert technician and correlated to auction floor price, the result indicates that higher levels of stress during harvest could negatively affect the appearance and quality grade of the product in Japan, and potentially therefore the market returns for the industry. Further, with the sensory attributes of transparency and brightness of the valuable Otoro portion negatively affected by harvest stress, reputational damage may be caused by a shortening of the shelf life of the product in the storage units of wholesalers and sushi restaurants.

It is important to mention that the harvesting procedure examined in this study is considered 'best practice' at the time of writing. Therefore it is necessary to investigate the quality outcomes of the harvesting procedures that more intensively crowd and stress the fish for longer periods – such as those procedures used to harvest fish destined for the frozen market. However, as tuna are large, endothermic, ram ventilators they present aquaculturists with unique logistical challenges at harvest time to not only preserve the flesh qualities of the product, but also to ensure the welfare and safety of staff. In other aquaculture industries a variety of tools and techniques such as fish pumps, cages with raiseable floors, gases and chemical anaesthetics have been used to minimize stress levels and/or

confinement times prior to euthanasia. In tuna, anaesthetics and electric harpoons have been trialled with only the latter being adopted by a few farms in the Mediterranean industry – although still undergoing refinement due to safety and flesh quality concerns. With the development of new harvesting techniques costly to research and refine it is desirable within current practices to minimize stress as much as possible.

Within the cold chain for fresh air-freighted, farmed Southern bluefin tuna the critical control points are during the loading and off-loading of the tuna coffins at the airports in Australia, at hubs along the way, and upon arrival to Japan – especially during the summer periods at either location. As there are no known cases of insurance claims against the product that have been directly associated with poor cold chain handling it has not been highlighted as a major area of concern. However, from a non-bacteriological, quality standpoint, the industry should pay more attention to the cold chain to assure maximum preservation of desirable quality attributes and shelf-life.

Of the two industry practices of either shipping tuna on the same day as harvest or the day after harvest, no statistically significant differences were detected between a selection of sensory and biochemical parameters measured in samples from tuna of either treatment sent in a single shipment in Japan. Although averages did appear to favour shipping the day after a harvest, due to logistical and budgetary

restraints sample numbers were at the lowest possible level to detect a difference and therefore a more rigorous test and/or cumulative tests at differing times of the season may produce significant results.

The longer time that fatty fish such as farmed, Southern bluefin tuna are kept at the lowest possible temperatures, just avoiding freezing damage, lipid hydrolysis and oxidation is inhibited. Therefore, investigations into combining the time benefits of shipping fish on the same day as harvest with low and constant temperature preservation techniques such as biphasic fluid ice could be investigated. Further, as tuna exhibit variable temperature profiles both throughout the carcass of an individual and between individuals of the same proportions there is a need to examine more points along and within the carcass to get a greater understanding and potentially model temperature profiles and how they may relate to ambient water temperatures, harvest stress, and post-harvest manipulations.

In order to ensure both the sensory, physicochemical and microbiological qualities of the product, each stakeholder within the supply chain should be party to a '*Chain of Responsibility*' agreement which binds them to a duty of care for the handling of the product. Incorporating temperature monitoring tracing technologies will enable the identification of where problem points occur within the chain and who is liable for any claim should they occur.

Market-based research enabled the quality attributes of the product to be defined and assessed according to variations in the farming process, harvesting techniques, and in shipping methods and routes to Japan. These tools and techniques can be used and/or developed to help the industry better understand the fish, the culture system, the end product, and the market. It is important to note that the notions of 'quality' for all products though are culturally and economically framed, and are not static. Subsequently, the relevance of quality definitions and assessment techniques require regular review and calibration in order for producers to be able to produce the quality characteristics that maximise consumer satisfaction and profitability over time.

Scientific Outcomes Summary

- Identified a rapid, inexpensive, non-invasive technique for the quality assessment of sashimi grade tuna using digital camera technology.
- Formulated the first statistically balanced, whole carcass, sensory analytical method for bluefin tuna.
- First sensory profiling of the different cuts within the carcass of a bluefin tuna.
- Detailed a strategy to build a model and predict the sensory characteristics of the flesh based on instrumental techniques.
- Confirmed that vitamin supplementation extended the colour shelf of sashimi grade tuna.
- Revealed that the appearance of both the low fat portion according to digital

camera RGB values, and the high fat portion according to the sensory descriptors of brightness and transparency, were negatively affected by exposure to the exercise/stressors during harvest.

- Identified the critical control points within the cold-chain for air-freighted fresh tuna from Port Lincoln to Japan.
- Revealed that although the low fat, low value portion of tuna processed and shipped the same day of harvest had brighter red colour day one in Japan, the fattier, high value portion of tuna had less desirable quality traits both on day one and day four in Japan.

Industry Implications Summary

- The rapid, non-destructive instrumental technique and the sensory method provide the Southern bluefin tuna farming industry have been provided with two approaches to identify the various husbandry, harvest, and post-harvest practices that negatively, neutrally, or positively affect the market relevant qualities of the end product. Research results can now be fed back into these operations to in order to minimise costs and improve production efficiencies or maximise quality and potential market returns.
- This project was the first to confirm that the industry technique of vitamin supplementation extended colour shelf life in the market and was not a production cost without benefit. This research led to further research into the delivery method to improve vitamin uptake and extend the colour shelf life –

which is still the greatest quality issue with farmed Southern bluefin in the tuna markets of Japan and elsewhere.

- The project discovered that even with industry's best harvest practice there were quality related issues in tuna exposed to greater amounts of exercise/stress. Developing harvesting methods that minimise the amounts of exercise/stress at harvest should yield quality benefits.
- Through detection of the critical control points within the cold-chain for air-freighted fresh tuna, and the greater temperature sensitivity of the high value, fattier portion of the carcasses, it was shown that the flesh qualities and shelf life of the end product would benefit from a more temperature stable cold-chain. To ensure this, improve qualities, and potentially obtain higher market returns, greater in-chain collaboration is required.
- The potential for more effective chilling pre-processing, either before or during transportation to airports, should be investigated.

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Figure 2.27: Percentages of metmyoglobin (+/- Std Err) in the three main cuts from the carcasses of farmed Southern bluefin tuna in Japan air-freighted fresh from Australia (n=8; day four post-mortem).

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Figure 2.29: Lactate concentration (+/- Std Err) in the three main cuts from the carcasses of farmed Southern bluefin tuna in Japan air-freighted fresh from Australia (n=8; day four post-mortem).

Figure 2.30: Average sensory scores (+/- Std Err) of redness, brightness, transparency and bad fishy odour for the three main cuts from the carcasses of farmed Southern bluefin tuna in Japan air-freighted fresh from Australia (n=8; day four post-mortem).

Figure 2.31: Average sensory scores (+/- Std Err) of bad fishy flavour, sourness, umami, fattiness, and hardness for the three main cuts from the carcasses of farmed Southern bluefin tuna in Japan air-freighted fresh from Australia (n=8; day four post-mortem).

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Figure 2.33: Two-Dimensional scatter bi-plot of the loadings of instrumental variables and the scores of Akami, Chutoro, and Otoro flesh samples taken from fresh, farmed Southern bluefin from Port Lincoln, Australia in Tokyo at day five post-harvest (n=8; day four post-mortem).

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Table 2.1: Yield data from the dorsal loin of three tuna.

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Table 2.3: Combination numbers were ascribed to each of the 36 possible combinations and then further divided into 6 blocks of 6 numbers each (left table). Within the 6 blocks random numbers were ascribed, and the rows numbered 1 and 2 were deleted for each (yellow rows, middle table). New random numbers were ascribed for randomization of subjects within the panel (right table).

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Table 2.6: Inosine monophosphate, total nucleotides, and lactate concentrations expressed on a wet weight basis and fat-free dry weight basis for the three cuts of Akami, Chutoro, and Otoro (n=8).

Table 2.7: Normality statistics for untransformed, square root transformed and log 10 transformed hydroperoxide data.

3. Vitamin Supplementation and the Flesh Qualities of Farmed, Fresh Southern Bluefin Tuna in Port Lincoln and Japan

Figures

Figure 3.1: Vitamin E concentrations (mean +/- SEM) in flesh samples from wild Southern bluefin tuna caught in January (Wild; n=15), and in flesh samples from farmed Southern bluefin tuna fed low and high levels of vitamin supplements that were harvested in March (Harvest 1; High n=15, Low n=16) and in May (Harvest 2; High n=10, Low n=10) of the 2002 farming season in Port Lincoln (different letters indicate statistical significance $p < 0.5$).

Figure 3.2: Vitamin C concentrations (mean +/- SEM) in flesh samples from wild Southern bluefin tuna caught in January (Wild; n=15), and in flesh samples from farmed Southern bluefin tuna fed low and high levels of vitamin supplements that were harvested in March (Harvest 1; High n=15, Low n=16) and in May (Harvest 2; High n=10, Low n=10) of the 2002 farming season in Port Lincoln (different letters indicate statistical significance $p < 0.5$).

Figure 3.3: Selenium concentrations (mean +/- SEM) in flesh samples from wild Southern bluefin tuna caught in January (Wild; n=15), and in flesh samples from farmed Southern bluefin tuna fed low and high levels of vitamin supplements that were harvested in March (Harvest 1; High n=15, Low n=16) and in April (Harvest 2; High n=10, Low n=10) of the 2002 farming season in Port Lincoln, Australia (different letters indicate statistical significance $p < 0.5$).

Figure 3.4: Surface pH values (mean +/- SEM) of core flesh samples of tuna fed high and low vitamin supplementation from January until harvest in March (a) and April (b) 2002 in Port Lincoln. Samples were stored on white tiles under cellophane wrap in constant temperature conditions (4°C) for an eight day period.

Figure 3.5: Chromameter L*a*b* values (mean +/- SEM) recorded from the core flesh samples of Southern bluefin tuna fed high and low vitamin supplementation from January until harvest in March 2002. Samples were stored on white tiles under cellophane wrap in constant temperature conditions (4°C) for an eight day period.

Figure 3.6: Chromameter L*a*b* values (mean +/- SEM) recorded from the core flesh samples of Southern bluefin tuna fed high and low vitamin supplementation from January until harvest in April 2002. Samples were stored on white tiles under cellophane wrap in constant temperature conditions (4°C) for an eight day period.

Figure 3.7: Colour rank frequencies from an eight-day evaluation by a panel of nine people of the colour of core flesh samples taken from farmed Southern bluefin tuna fed low and high levels of vitamin supplements and harvested in March 2002 (High n=15, Low n=16) in Port Lincoln.

Figure 3.8: Colour rank frequencies from a three-day evaluation by a panel of seven people of the colour of tail cut samples taken from farmed Southern bluefin tuna fed low and high levels of vitamin supplements and harvested in March 2002 (High n=15, Low n=16) in Tokyo, Japan.

Figure 3.9: Colour rank frequencies from an eight-day evaluation by a panel of six people of the colour of core flesh samples taken from farmed Southern bluefin tuna fed low and high levels of vitamin supplements and harvested in April 2002 (High n=15, Low n=16) in Port Lincoln.

Figure 3.10: Colour rank frequencies from a four-day evaluation by a panel of six people of the colour of tail cut samples taken from farmed Southern bluefin tuna fed low and high levels of vitamin supplements and harvested in April 2002 (High n=7, Low n=8) in Tokyo, Japan.

Tables

Table 3.1: Colour ranking criteria of core samples of the flesh of fresh, farmed Southern bluefin tuna.

4. Commercial Level Harvest Stress and the Physico-Chemical and Sensory Flesh Qualities of Farmed Southern Bluefin Tuna in Japan

Figures

Figure 4.1: A typical harvesting method of farmed Southern bluefin tuna using a seine net to capture a desired number of tuna (A), the attachment of a dividing line (B), the raising of the dividing line (C), resulting in a holding area (deeper left side) and a harvesting area (shallower right side) with approximately equal numbers of tuna in each (D).

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Figure 4.4: Carcass time-temperature profiles of farmed Southern bluefin tuna subject to low and high levels of harvest stress in May of the 2003 farming season in Port Lincoln (Low n=4, High n=4).

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Figure 4.6: Ultimate pH values (mean +/- SEM) recorded from the surface of flesh samples of the Akami, Chutoro and Otoro cuts of farmed Southern bluefin tuna in Japan that were subject to low and high levels of harvest stress in May of the 2003 farming season in Port Lincoln (Low n=4, High n=4; day four post-mortem).

Figure 4.7: Lactate concentrations (mean +/- SEM) from the muscle samples of the Akami, Chutoro and Otoro cuts of farmed Southern bluefin tuna in Japan that were subject to low and high levels of harvest stress in May of the 2003 farming season in Port Lincoln (Low n=4, High n=4; day four post-mortem).

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Figure 4.9: Inosine monophosphate concentrations (mean +/- SEM) from the muscle samples of the Akami, Chutoro and Otoro cuts of farmed Southern bluefin tuna in Japan that were subject to low and high levels of harvest stress in May of the 2003 farming season in Port Lincoln (Low n=4, High n=4; day four post-mortem).

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Figure 4.11: Percentage metmyoglobin values (mean +/- SEM) from the muscle samples of the Akami, Chutoro and Otoro cuts of farmed Southern bluefin tuna in Japan that were subject to low and high levels of harvest stress in May of the 2003 farming season in Port Lincoln (Low n=4, High n=4; day four post-mortem).

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Figure 4.13: Digital camera RGB ratios (mean +/- SEM) recorded from the surface of samples of the Akami, Chutoro and Otoro cuts of farmed Southern bluefin tuna in Japan that were subject to low and high levels of harvest stress in May of the 2003 farming season in Port Lincoln (Low n=4, High n=4; day four post-mortem).

Figure 4.14: Average sensory scores (mean +/- SEM) for sashimi sections (n=12) of the Akami cuts of farmed Southern bluefin tuna in Japan that were subject to low and high levels of harvest stress in May of the 2003 farming season in Port Lincoln (Low n=4, High n=4; day four post-mortem).

Figure 4.15: Average sensory scores (mean +/- SEM) for sashimi sections (n=10) of the Chutoro cuts of farmed Southern bluefin tuna in Japan that were subject to low and high levels of harvest stress in May, 2003 farming season in Port Lincoln (Low n=4, High n=4; day four post-mortem).

Figure 4.16: Average sensory scores (mean +/- SEM) for sashimi sections (n=18) of the Otoro cuts of farmed Southern bluefin tuna in Japan that were subject to low and high levels of harvest stress in May of the 2003 farming season in Port Lincoln (Low n=4, High n=4; day four post-mortem; * = P<0.10, ** = P<0.05).

Figure 4.17: Sample scores and variable loadings bi-plot for the sensory data from the Akami sections of farmed Southern bluefin tuna in Japan that were subject to low (LS) and high (HS) levels of harvest stress in May of the 2003 farming season in Port Lincoln (Low n=4, High n=4; day four post-mortem).

Figure 4.18: Sample scores and variable loadings bi-plot for the sensory data from the Chutoro sections of farmed Southern bluefin tuna in Japan that were subject to low (LS) and high (HS) levels of harvest stress in May of the 2003 farming season in Port Lincoln (Low n=4, High n=4; day four post-mortem).

Figure 4.19: Sample scores and variable loadings bi-plot for the sensory data from the Otoro sections of farmed Southern bluefin tuna in Japan that were subject to low (LS) and high (HS) levels of harvest stress in May of the 2003 farming season in Port Lincoln (Low n=4, High n=4; day four post-mortem).

Figure 4.20: Theoretical representation of how the degree of separation and time of sampling could effect the measured concentrations of inosine monophosphate in fish muscle as a result of differing degrees of harvest stress.

5. Post-Harvest Handling & Flesh Qualities of Farmed, Fresh Southern Bluefin Tuna from Port Lincoln, South Australia to Tokyo, Japan

Figures

Figure 5.1: (Left) Rate Class 8 (LD3/AKE/AVA), and (Right) Rate Class 5 (PAP/PIP/PAG) aircraft unit load devices (ULD).

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Figure 5.3: Insertion of a veneer encased data logger into the post-pectoral bleed cut (top left), attachment to a laminated card for placement inside a tuna coffin (top right), and attachment and taping of a data logger to the outside of a tuna coffin in Port Lincoln, Australia.

Figure 5.4: Otoro (ventral) and Akami (dorsal) portions of the *kami* section of a tuna carcass.

Figure 5.5: Examples of the anchored, sliding scales used in the assessment of the Akami and Otoro sections.

Figure 5.6: Average box external, box internal, and tuna internal logged temperatures from Port Lincoln to Narita International Airport via Adelaide and Sydney for a shipment processed on the same day as harvest in March 2002.

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Figure 5.8: Individual box internal logged temperatures from Port Lincoln to Narita International Airport via Adelaide and Sydney for a shipment processed on the same day as harvest in March, 2002.

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Figure 5.10: Average box external, box internal, and tuna internal logged temperatures from Port Lincoln to Tsukiji Wholesale Market via Melbourne, Sydney, and Incheon (South Korea) for a shipment processed on the day after harvest in August 2002.

Figure 5.11: Individual box external logged temperatures from Port Lincoln to Tsukiji Wholesale Market via Melbourne, Sydney, and Incheon (South Korea) for a shipment processed on the day after harvest in August 2002.

Figure 5.12: Individual box internal logged temperatures from Port Lincoln to Tsukiji Wholesale Market via Melbourne, Sydney, and Incheon (South Korea) for a shipment processed on the day after harvest in August 2002

Figure 5.13: Individual tuna internal logged temperatures from Port Lincoln to Tsukiji Wholesale Market via Melbourne, Sydney, Incheon (Korea) for a shipment processed on the day after harvest in August, 2002.

Figure 5.14: Average scores for redness, brightness and degree of colour change from the Akami cuts of farmed, fresh Southern bluefin tuna on day one (left column) and day four (right column) in Japan that were processed on the same day as harvest or the next day after harvest in Port Lincoln, Australia.

Figure 5.15: Average scores for the degree of colour change from the Otoro cuts of farmed, fresh Southern bluefin tuna on day one (left) and day four (right) in Japan that were processed on the same day as harvest or the next day after harvest in Port Lincoln, Australia.

Figure 5.16: Average scores for the bad fishy odour from the Akami (left) and Otoro cuts (right) of farmed, fresh Southern bluefin tuna on day four in Japan that were processed on the same day as harvest or the next day after harvest in Port Lincoln, Australia.

Figure 5.17: Average scores for the quality grade from the Akami (upper figures) and Otoro cuts (lower figures) of farmed, fresh Southern bluefin tuna on day one (left column) and day four (right column) in Japan that were processed on the same day as harvest or the next day after harvest in Port Lincoln, Australia.

Figure 5.18: Average concentrations of hydroperoxide (nmol/5g muscle) in the Otoro cuts of farmed, fresh Southern bluefin tuna on day one (left) and day four (right) in Japan that were processed on the same day as harvest or the next day after harvest in Port Lincoln, Australia (n=5).

Tables

Table 5.1: Hedonic comparison of the average objective and subjective quality parameters of the Akami and Otoro cuts from fresh, farmed Southern bluefin tuna on day one and day four in Japan processed on the same day or the next day of harvest in Port Lincoln, Australia (✓ = preferable; ✗ = not preferable; - = not measured).

9. STATISTICAL SUMMARY TABLES

RGB Ratio	A Grade	B Grade	C Grade
Average	1.25	1.16	1.12
Std. Dev.	0.14	0.14	0.12
Std. Err.	0.02	0.01	0.02
Price	A Grade	B Grade	C Grade
Average	3936	3517	3366
Std. Dev.	561	459	326
Std. Err.	71	42	52

Table 9.1: RGB ratio (R/(G+B)) and auction floor price averages, standard deviations, and standard errors as a function of quality grade (A = High Quality, n=64; B = Medium Quality, n=146; C = Low Quality, n=51) as ascribed to air-freighted, farmed Southern bluefin tuna from Port Lincoln, Australia at the Tsukji Wholesale Markets, Tokyo by an expert grader in the 2002 season. (Section 2.3.1)

Akami												
	pH	L Value	a Value	b Value	IMP(mmol/g)	K value(%)	OOH Value	% MetMyo	RGB Ratio	Lactate ug/g	Fat %	Moisture %
Average	5.74750	32.15688	8.82000	1.69125	5.80000	19.30000	86.89125	4.00000	1.17936	12.22162	1.37817	70.49974
Std. Dev.	0.07450	0.95337	0.65933	0.47888	0.89722	2.33506	24.86950	4.73682	0.08092	1.87067	0.79374	1.09737
Std err.	0.02634	0.33707	0.23311	0.16931	0.31721	0.82557	8.79270	1.67472	0.02861	0.66138	0.28063	0.38798
Chutoro												
Average	5.77563	41.47563	11.26500	4.33625	5.02500	22.11250	532.00104	12.20000	1.01925	11.31845	8.80171	65.02287
Std. Dev.	0.05641	2.29803	0.85010	0.69495	0.53918	4.36984	399.44986	8.58604	0.06697	1.78507	3.18943	2.19459
Std err.	0.01994	0.81248	0.30056	0.24570	0.19063	1.54497	141.22685	3.03562	0.02368	0.63112	1.12763	0.77590
Otoro												
Average	5.75063	53.20063	10.51563	6.24375	3.68750	18.98750	1244.32575	12.43750	0.88105	7.01048	31.22012	50.27379
Std. Dev.	0.04403	1.73130	1.47318	0.85983	0.49982	3.29564	764.02591	8.03758	0.05043	1.72910	5.02067	3.21069
Std err.	0.01557	0.61211	0.52085	0.30400	0.17671	1.16519	270.12395	2.84171	0.01783	0.61133	1.77508	1.13515

Table 9.2: Averages, standard deviations, and standard errors for the physicochemical parameters of the cuts of Akami, Chutoro, and Otoro from the carcasses of farmed Southern bluefin tuna in Japan air-freighted fresh from Australia (n=8; day four post-mortem) (Section 2.3.2).

Akami	Redness	Brightness	Transparency	Bad Fishy Odour	Bad Fishy Flavour	Sourness	Umami	Fatty	Hardness
Average	3.3697917	3.1093750	3.1979167	2.2343750	2.4322917	3.4166667	2.6614583	2.7031250	3.0364583
Std. Dev.	0.9631221	0.9520148	0.8629178	0.8396526	0.9989990	0.7492686	0.7486647	0.9221774	0.8311210
Std. Err.	0.0982982	0.0971646	0.0880712	0.0856967	0.1019599	0.0764719	0.0764103	0.0941193	0.0848259
Chutoro	Redness	Brightness	Transparency	Bad Fishy Odour	Bad Fishy Flavour	Sourness	Umami	Fatty	Hardness
Average	2.7965909	2.7068182	2.7147727	2.1818182	2.3886364	2.9738636	2.6000000	2.7772727	2.5170455
Std. Dev.	0.9200761	0.8564556	0.8343956	0.9134431	1.0496230	0.9955642	0.8180436	0.7806168	0.7483655
Std. Err.	0.0980804	0.0912985	0.0889469	0.0973734	0.1118902	0.1061275	0.0872037	0.0832140	0.0797760
Otoro	Redness	Brightness	Transparency	Bad Fishy Odour	Bad Fishy Flavour	Sourness	Umami	Fatty	Hardness
Average	2.8779762	2.8244048	2.7261905	2.2083333	2.2351190	2.6250000	2.4880952	3.5267857	2.6071429
Std. Dev.	1.0096792	0.8993342	0.9004482	0.9763930	1.0637264	0.9346138	0.9155221	0.9481449	0.8842312
Std. Err.	0.0763246	0.0679833	0.0680675	0.0738084	0.0804102	0.0706502	0.0692070	0.0716730	0.0668416

Table 9.3: Averages, standard deviations, and standard errors for the sensory parameters of the cuts of Akami, Chutoro, and Otoro from the carcasses of farmed Southern bluefin tuna in Japan air-freighted fresh from Australia (n=8; day four post-mortem) (Section 2.3.2).

Selenium (wet wt.) mg/kg					
	Wild Fish	Harvest One Low	Harvest One High	Harvest Two Low	Harvest Two High
Average	1.06100	0.81500	0.82692	0.75800	0.75900
Std. Dev.	0.23723	0.14405	0.12750	0.09175	0.08900
Std. Err.	0.07502	0.03850	0.03536	0.02901	0.02814
Vitamin C (ug/g)					
	Wild Fish	Harvest One Low	Harvest One High	Harvest Two Low	Harvest Two High
Average	11.48952	7.88088	9.65855	6.49711	7.18415
Std. Dev.	3.66166	3.30413	2.68029	1.81844	2.15718
Std. Err.	0.97862	0.82603	0.69205	0.57504	0.68216
Vitamin E (ug/g)					
	Wild Fish	Harvest One Low	Harvest One High	Harvest Two Low	Harvest Two High
Average	8.23846	8.87500	9.34667	8.98889	8.31000
Std. Dev.	2.79837	2.61827	1.78040	2.96287	2.66310
Std. Err.	0.77613	0.65457	0.45970	0.98762	0.84215

Table 9.4: Averages, standard deviations, and standard errors for the concentrations of selenium, vitamin C, and vitamin E in flesh samples from wild Southern bluefin tuna caught in January (Wild; n=15), and in flesh samples from farmed Southern bluefin tuna fed low and high levels of vitamin supplements that were harvested in March (Harvest 1; High n=15, Low n=16) and in April (Harvest 2; High n=10, Low n=10) of the 2002 farming season in Port Lincoln, Australia (Section 3.4).

Harvest One -Australia									
pH Low Vitamin									
Day	0.00000	1.00000	2.00000	3.00000	4.00000	5.00000	6.00000	7.00000	8.00000
Average	5.77125	5.76063	5.75438	5.77063	5.77125	5.76563	5.76938	5.75875	5.77063
Std. Dev.	0.06043	0.07962	0.08406	0.07655	0.08625	0.07685	0.07057	0.06407	0.07160
Std. Err.	0.01511	0.01991	0.02102	0.01914	0.02156	0.01921	0.01764	0.01602	0.01790
pH High Vitamin									
Average	5.82067	5.76267	5.76133	5.76267	5.76400	5.75600	5.76533	5.76067	5.75733
Std. Dev.	0.13019	0.06041	0.06323	0.06670	0.05755	0.05501	0.05083	0.04920	0.04773
Std. Err.	0.03362	0.01560	0.01633	0.01722	0.01486	0.01420	0.01312	0.01270	0.01232
L Value Low Vitamin									
Day	0.00000	1.00000	2.00000	3.00000	4.00000	5.00000	6.00000	7.00000	8.00000
Average	30.45813	33.57063	33.94688	34.96000	35.20188	35.20813	37.23875	38.20375	38.78813
Std. Dev.	3.77662	2.78826	2.53256	2.08906	2.86378	3.88262	3.55250	3.22495	3.51061
Std. Err.	0.94415	0.69706	0.63314	0.52226	0.71595	0.97066	0.88813	0.80624	0.87765
L Value High Vitamin									
Average	29.19067	32.45067	32.84667	33.78667	34.06333	34.62800	35.12867	35.56000	37.43133
Std. Dev.	2.79440	1.94056	2.23857	2.75774	3.00924	3.18605	3.80854	6.76169	2.74164
Std. Err.	0.72151	0.50105	0.57800	0.71205	0.77698	0.82263	0.98336	1.74586	0.70789
a* Value Low Vitamin									
Day	0.00000	1.00000	2.00000	3.00000	4.00000	5.00000	6.00000	7.00000	8.00000
Average	13.38938	17.61688	15.50750	13.25688	9.98375	7.35625	5.96875	5.15813	4.75500
Std. Dev.	2.50792	2.22565	3.31364	4.27097	4.13593	3.59143	2.78617	2.06559	1.77695
Std. Err.	0.62698	0.55641	0.82841	1.06774	1.03398	0.89786	0.69654	0.51640	0.44424
a* Value High Vitamin									
Average	12.78000	16.82267	16.26400	13.50667	10.12933	9.00933	7.34133	6.10600	5.45133
Std. Dev.	2.36136	3.49059	1.59534	2.69765	3.79017	3.71279	3.59653	2.96295	1.85374
Std. Err.	0.60970	0.90127	0.41192	0.69653	0.97862	0.95864	0.92862	0.76503	0.47863
b* Value Low Vitamin									
Day	0.00000	1.00000	2.00000	3.00000	4.00000	5.00000	6.00000	7.00000	8.00000
Average	3.66188	8.36313	8.24713	7.62625	7.87438	8.71375	9.92125	10.58688	11.58688
Std. Dev.	1.25196	1.29199	1.10798	1.12159	1.84435	1.93198	2.13847	2.15026	2.26575
Std. Err.	0.31299	0.32300	0.27699	0.28040	0.46109	0.48299	0.53462	0.53757	0.56644
b* Value High Vitamin									
Average	3.24000	7.73600	7.98067	7.44733	7.97533	8.49000	9.59667	10.46267	11.21933
Std. Dev.	1.13952	1.75420	0.89949	1.05309	2.00181	2.11346	2.76808	2.52230	2.48907
Std. Err.	0.29422	0.45293	0.23225	0.27191	0.51687	0.54569	0.71471	0.65126	0.64268

Table 9.5: Averages, standard deviations, and standard errors for the pH, L values, a* values, and b* values in flesh samples from farmed Southern bluefin tuna fed low and high levels of vitamin supplements and harvested in March (Harvest 1; High n=15, Low n=16) of the 2002 farming season in Port Lincoln, Australia (Section 3.4 – Harvest One, Australia)

Harvest Two - Australia									
pH Low Vitamin									
Day	0.00000	1.00000	2.00000	3.00000	4.00000	5.00000	6.00000	7.00000	8.00000
Average	5.79800	5.75200	5.76300	5.70400	5.66500	5.70700	5.68800	5.73600	5.70100
Std. Dev.	0.03190	0.05181	0.02791	0.02011	0.01958	0.03831	0.01932	0.04858	0.03348
Std. Err.	0.01009	0.01638	0.00883	0.00636	0.00619	0.01212	0.00611	0.01536	0.01059
pH High Vitamin									
Average	5.82900	5.82000	5.81100	5.76400	5.72500	5.76500	5.75100	5.78100	5.76500
Std. Dev.	0.03315	0.05164	0.04067	0.03748	0.04223	0.05083	0.05152	0.03414	0.04007
Std. Err.	0.01048	0.01633	0.01286	0.01185	0.01335	0.01607	0.01629	0.01080	0.01267
L Value Low Vitamin									
Day	0.00000	1.00000	2.00000	3.00000	4.00000	5.00000	6.00000	7.00000	8.00000
Average	29.30100	32.30100	33.04000	33.93000	34.41300	35.01500	35.81600	37.07800	37.35600
Std. Dev.	2.11659	2.05380	2.30141	2.73528	3.13812	3.54424	3.29729	3.52177	2.91094
Std. Err.	0.66932	0.64947	0.72777	0.86497	0.99236	1.12079	1.04270	1.11368	0.92052
L Value High Vitamin									
Average	28.49300	31.87800	32.98700	33.62600	34.42000	35.08400	36.02900	36.77100	37.20700
Std. Dev.	1.33437	0.90884	2.07235	1.92853	1.77782	2.76420	3.19120	3.06948	3.29833
Std. Err.	0.42196	0.28740	0.65533	0.60985	0.56220	0.87412	1.00915	0.97065	1.04302
a* Value Low Vitamin									
Day	0.00000	1.00000	2.00000	3.00000	4.00000	5.00000	6.00000	7.00000	8.00000
Average	12.19100	18.71400	17.18200	15.79800	14.04800	10.81800	8.95500	6.61100	5.66800
Std. Dev.	1.31684	1.52436	1.08702	1.10911	1.13263	2.52259	2.86718	2.28240	1.91211
Std. Err.	0.41642	0.48204	0.34375	0.35073	0.35817	0.79771	0.90668	0.72176	0.60466
a* Value High Vitamin									
Average	13.10000	18.55600	17.44400	15.98000	14.75800	12.26900	11.27100	8.26200	6.40500
Std. Dev.	1.37878	1.99532	1.65337	1.73538	2.00748	3.31259	3.76024	3.44542	2.84512
Std. Err.	0.43601	0.63098	0.52284	0.54878	0.63482	1.04753	1.18909	1.08954	0.89971
b* Value Low Vitamin									
Day	0.00000	1.00000	2.00000	3.00000	4.00000	5.00000	6.00000	7.00000	8.00000
Average	3.26900	8.96200	8.34400	8.21400	7.87900	8.59200	8.57600	9.71100	10.59200
Std. Dev.	0.56027	0.80811	0.72540	0.80122	1.24121	1.79640	1.86369	2.40677	2.46114
Std. Err.	0.17717	0.25555	0.22939	0.25337	0.39251	0.56807	0.58935	0.76109	0.77828
b* Value High Vitamin									
Average	3.49000	8.50900	8.24000	8.26300	8.24700	9.10800	9.25000	9.53900	10.21700
Std. Dev.	0.74318	1.34024	1.00803	1.20720	1.11907	2.62629	2.00437	2.20231	2.72092
Std. Err.	0.23501	0.42382	0.31877	0.38175	0.35388	0.83051	0.63384	0.69643	0.86043

Table 9.6: Averages, standard deviations, and standard errors for the pH, L values, a* values, and b* values in flesh samples from farmed Southern bluefin tuna fed low and high levels of vitamin supplements that were harvested in April (Harvest 2; High n=10, Low n=10) of the 2002 farming season in Port Lincoln, Australia (Section 3.4).

	Harvest One			Harvest Two			
Low Vitamin	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 4
Average	2.7767857	3.9910714	5.1696429	3.1632653	3.7142857	4.7142857	5.4693878
Std. Dev.	1.0941636	1.2718498	0.9607519	0.568017	0.5216405	0.6116777	0.4342034
Std. Err.	0.2825118	0.3283902	0.2480651	0.2146903	0.1971616	0.2311925	0.1641134
High Vitamin	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 4
Average	2.5047619	3.2095238	4.8095238	2.8928571	3.4107143	4.1785714	4.9464286
Std. Dev.	0.7690566	0.9939565	0.717679	0.3393528	0.7058171	0.9250325	0.6521438
Std. Err.	0.1985696	0.2566385	0.1853039	0.1199793	0.249544	0.3270484	0.2305677

Table 9.7: Averages, standard deviations, and standard errors of the colour ranking data in Japan measured from the tail cuts taken from farmed Southern bluefin tuna fed low and high levels of vitamin supplements and harvested in March (Harvest 1; High n=15, Low n=16) and in April (Harvest 2; High n=10, Low n=10) of the 2002 farming season in Port Lincoln, Australia (Section 3.4)

Harvest One								
Low Vitamin	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
Average	1.00694	2.10417	2.66667	3.48438	4.20313	4.88393	5.33594	5.79464
Std. Dev.	0.11088	0.95965	1.06959	1.13273	1.21439	1.01716	0.75515	0.44864
Std. Err.	0.02772	0.23991	0.26740	0.28318	0.30360	0.25429	0.18879	0.11216
High Vitamin	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
Average	1.02963	1.91111	2.42222	3.21667	3.86667	4.48571	5.10833	5.53333
Std. Dev.	0.12921	0.29457	0.66295	1.08198	1.27078	1.15276	0.93047	0.64674
Std. Err.	0.03336	0.07606	0.17117	0.27937	0.32811	0.29764	0.24025	0.16699

Harvest Two								
Low Vitamin	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
Average	1.20000	1.88333	2.10000	2.30000	3.10000	3.83333	4.60000	5.13333
Std. Dev.	0.40406	0.41545	0.39915	0.56148	1.25819	1.20966	1.13794	0.94719
Std. Err.	0.05216	0.05363	0.05153	0.07249	0.16243	0.15617	0.14691	0.12228
High Vitamin	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
Average	1.20000	1.78333	1.98333	2.26667	2.80000	3.30000	3.76667	4.48333
Std. Dev.	0.40406	0.41545	0.12910	0.54824	0.97076	1.12446	1.35755	1.11221
Std. Err.	0.05216	0.05363	0.01667	0.07078	0.12532	0.14517	0.17526	0.14359

Table 9.8: Averages, standard deviations, and standard errors for of the colour ranking data in Australia measured from cores taken from farmed Southern bluefin tuna fed low and high levels of vitamin supplements and harvested in March (Harvest 1; High n=15, Low n=16) and in April (Harvest 2; High n=10, Low n=10) of the 2002 farming season in Port Lincoln, Australia (Section 3.4)

Akami									
Start of Harvest	Avg pH	Avg L	Avg a	Avg b	IMP(mmol/g)	K value(%)	OOH Value	% MetMyo	RGB Ratio
Average	5.73750	32.34125	9.21000	2.07875	6.20000	18.20000	100.56743	3.25000	1.23586
Std. Dev.	0.11529	1.34476	0.36159	0.27363	0.90554	0.64807	23.15828	3.84057	0.09131
Std. Err.	0.05764	0.67238	0.18080	0.13681	0.45277	0.32404	11.57914	1.92029	0.04566
End of Harvest	Avg pH	Avg L	Avg a	Avg b	IMP(mmol/g)	K value(%)	OOH Value	% MetMyo	RGB Ratio
Average	5.75750	31.97250	8.43000	1.30375	5.27500	20.40000	73.21508	4.75000	1.12287
Std. Dev.	0.03524	0.72439	0.78923	0.36913	0.51235	3.30857	24.78384	6.60177	0.02473
Std. Err.	0.01762	0.36220	0.39461	0.18456	0.25617	1.65429	12.39192	3.30088	0.01236
Chutoro									
Start of Harvest	Avg pH	Avg L	Avg a	Avg b	IMP(mmol/g)	K value(%)	OOH Value	% MetMyo	RGB Ratio
Average	5.78375	41.61500	11.29875	4.44000	4.80000	21.35000	671.67773	12.12500	1.05470
Std. Dev.	0.08097	2.98086	0.82980	1.00834	0.70711	2.42006	532.23957	5.31311	0.07030
Std. Err.	0.04049	1.49043	0.41490	0.50417	0.35355	1.21003	266.11979	2.65656	0.03515
End of Harvest	Avg pH	Avg L	Avg a	Avg b	IMP(mmol/g)	K value(%)	OOH Value	% MetMyo	RGB Ratio
Average	5.76750	41.33625	11.23125	4.23250	5.25000	22.87500	392.32435	12.27500	0.98381
Std. Dev.	0.02630	1.83980	0.99731	0.28538	0.20817	6.09501	192.36061	11.99038	0.04662
Std. Err.	0.01315	0.91990	0.49865	0.14269	0.10408	3.04751	96.18031	5.99519	0.02331
Otoro									
Start of Harvest	Avg pH	Avg L	Avg a	Avg b	IMP(mmol/g)	K value(%)	OOH Value	% MetMyo	RGB Ratio
Average	5.74500	53.88625	9.90000	5.90125	3.35000	20.40000	1398.43803	9.37500	0.88505
Std. Dev.	0.04143	1.75451	1.36596	0.66718	0.42032	3.56183	282.96927	4.02854	0.04429
Std. Err.	0.02072	0.87726	0.68298	0.33359	0.21016	1.78092	141.48463	2.01427	0.02214
End of Harvest	Avg pH	Avg L	Avg a	Avg b	IMP(mmol/g)	K value(%)	OOH Value	% MetMyo	RGB Ratio
Average	5.75625	52.51500	11.13125	6.58625	4.05000	17.90000	1090.21348	15.50000	0.87705
Std. Dev.	0.05218	1.63158	1.47899	0.98342	0.20817	2.73130	1103.92173	10.46422	0.06269
Std. Err.	0.02609	0.81579	0.73949	0.49171	0.10408	1.36565	551.96087	5.23211	0.03135

Table 9.9: Averages, standard deviations, and standard errors for the physicochemical parameters recorded from muscle samples of the Akami, Chutoro and Otoro cuts of farmed Southern bluefin tuna in Japan taken from the Start and End of a harvest in May of the 2003 farming season in Port Lincoln (Start n=4, End n=4; day four post-mortem) (Section 4.4).

Akami								
Start of Harvest	Redness	Brightness	Transparency	Bad fishy odour	Bad fishy flavor	Sourness	Umami	Hardness
Average	3.22917	3.22917	3.26042	2.31250	2.45833	3.50000	2.63542	2.94792
Std. Dev	0.89893	0.88100	0.83786	0.89695	1.00442	0.63581	0.69756	0.75962
Std. Err.	0.12975	0.12716	0.12094	0.12946	0.14498	0.09177	0.10068	0.10964
End of Harvest	Redness	Brightness	Transparency	Bad fishy odour	Bad fishy flavor	Sourness	Umami	Hardness
Average	2.82500	2.67727	2.65455	2.22727	2.46591	2.84545	2.55000	2.53409
Std. Dev	1.01316	1.01316	0.89168	0.77977	1.00348	0.84635	0.80309	0.89621
Std. Err.	0.14624	0.14624	0.12870	0.11255	0.14484	0.12216	0.11592	0.12936
Chutoro								
Start of Harvest	Redness	Brightness	Tranparency	Bad fishy odour	Bad fishy flavor	Sourness	Umami	Hardness
Average	2.76818	2.73636	2.77500	2.13636	2.31136	3.10227	2.65000	2.50000
Std. Dev	0.77798	0.74339	0.79623	0.82978	1.03094	1.15242	0.78292	0.73589
Std. Err.	0.12975	0.12716	0.12094	0.12946	0.14498	0.09177	0.10068	0.10964
End of Harvest	Redness	Brightness	Tranparency	Bad fishy odour	Bad fishy flavor	Sourness	Umami	Hardness
Average	2.82500	2.67727	2.65455	2.22727	2.46591	2.84545	2.55000	2.53409
Std. Dev	1.05767	0.96998	0.86386	0.99682	1.08047	0.82108	0.86441	0.77308
Std. Err.	0.16723	0.15337	0.13659	0.15761	0.17084	0.12982	0.13668	0.12223
Otoro								
Start of Harvest	Redness	Brightness	Tranparency	Bad fishy odour	Bad fishy flavor	Sourness	Umami	Hardness
Average	2.73810	3.00595	2.89881	2.23810	2.22024	2.57143	2.45238	2.60119
Std. Dev	0.85896	0.80096	0.85651	1.02227	1.06802	0.91554	0.89382	0.88760
Std. Err.	0.09372	0.08739	0.09345	0.11154	0.11653	0.09989	0.09752	0.09684
End of Harvest	Redness	Brightness	Tranparency	Bad fishy odour	Bad fishy flavor	Sourness	Umami	Hardness
Average	3.01786	2.64286	2.55357	2.17857	2.25000	2.67857	2.52381	2.61310
Std. Dev	1.12862	0.95870	0.91513	0.93346	1.06562	0.95578	0.94073	0.88614
Std. Err.	0.12314	0.10460	0.09985	0.10185	0.11627	0.10428	0.10264	0.09669

Table 9.10: Averages, standard deviations, and standard errors for the sensory parameters recorded from muscle samples of the Akami, Chutoro and Otoro cuts of farmed Southern bluefin tuna in Japan taken from the Start and End of a harvest in May of the 2003 farming season in Port Lincoln (Start n=4, End n=4; day four post-mortem) (Section 4.4).

	Akami				Otoro	
Next Day Processing	Redness	Brightness	Colour Change	Quality Grade	Colour Change	Quality Grade
Average	47.09231	51.30769	30.93846	3.72308	29.20000	3.49231
Std. Dev.	10.44263	6.95548	10.01735	0.77421	15.36158	1.06948
Std. Err.	4.67009	3.11059	4.47990	0.34624	6.86991	0.47829
Same Day Processing	Redness	Brightness	Colour Change	Quality Grade	Colour Change	Quality Grade
Average	56.56923	41.50769	40.46154	4.66154	31.69231	3.81538
Std. Dev.	8.24273	8.38507	11.94093	1.03002	9.37231	0.88812
Std. Err.	3.68626	3.74992	5.34015	0.46064	4.19143	0.39718

Table 9.11: Averages, standard deviations, and standard errors for the sensory parameters measured from cuts of Akami and Otoro of farmed, fresh Southern bluefin tuna from Port Lincoln, Australia, on day one in Japan and that were processed on the same day (day four post-mortem in Japan) as harvest or the next day after harvest (day five post-mortem in Japan) (Section 5.4).

	Akami					Otoro		
Next Day Processing	Redness	Brightness	Colour Change	Bad Fishy Odour	Quality Grade	Colour Change	Bad Fishy Odour	Quality Grade
Average	32.23333	27.93333	64.80000	57.96667	6.72727	56.45455	53.78636	6.20909
Std. Dev.	17.67034	12.66028	11.48997	12.11857	1.41129	13.65489	9.20261	1.45014
Std. Err.	7.90242	5.66185	5.13847	5.41959	0.63115	6.10665	4.11553	0.64852
Same Day Processing	Redness	Brightness	Colour Change	Bad Fishy Odour	Quality Grade	Colour Change	Bad Fishy Odour	Quality Grade
Average	35.35000	28.41667	68.10000	56.48333	6.49818	63.20455	57.73636	6.86909
Std. Dev.	12.15802	11.30035	12.10449	9.27688	1.03753	9.75027	8.17397	1.15482
Std. Err.	5.43723	5.05367	5.41329	4.14874	0.46400	4.36045	3.65551	0.51645

Table 9.12: Averages, standard deviations, and standard errors for the sensory parameters measured from cuts of Akami and Otoro of farmed, fresh Southern bluefin tuna from Port Lincoln, Australia, on day four in Japan and that were processed on the same day (day seven post-mortem in Japan) as harvest or the next day after harvest (day eight post-mortem in Japan) (Section 5.4).