Waterford Institute *of* Technology

An Investigation of cold chain management system breaches during exportation on the quality and shelf life of fresh fish

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DECLARATION

No element of the work described in this thesis has been previously submitted for a degree at this or any other institution. The work in this thesis has been performed entirely by the author.

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ABSTRACT

Chilling fish involves establishing and maintaining consistent chilled storage (cold chain) of between 0 and 2 °C using a combination of ice and refrigeration. This process offers logistical advantages to fish producers, maintaining a high quality for longer during distribution. Megrim (*Lepidorhombus whiffiagonis*) and pollack (*Pollachius pollachius*) are typically caught in Irish waters, with the majority of each catch (approximately 90%) exported to Europe. As part of this research and to ensure high quality exports from Ireland to continental Europe, temperatures were assessed and breaches during the distribution of whole chilled fish were identified. To better understand the effect of temperature on product quality these breaches were replicated under controlled laboratory conditions, where both species were evaluated to determine the resultant changes in fish quality and microbial growth. Samples were analysed using proximate composition, microbial analysis, colour, texture, total volatile basic nitrogen (TVB-N), and sensory analysis. Both species had low microbial counts on day 1 of storage, this gradually increased over time, as spoilage occurred. It was observed that when fish samples were stored outside of optimum conditions (0-2 °C), the rate of spoilage increased, with unacceptable microbial level thresholds exceeded in within two days. Similar results were encountered for both species across all other quality testing parameters (colour, texture, TVB-N and sensory), however proximate composition was not significantly affected. In conclusion, the main findings of this study suggest that temperature breaches can easily occur throughout the cold chain, drastically reducing the shelf life and quality of fish. Once fish was exposed to temperatures outside of the recommended (0-2 °C) for chilled fish, quality was severely degraded.

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ABBREVIATIONS

ANOVA:	Analysis of variance
ATP:	Adenosine triphosphate
CCMS:	Cold chain management systems
ICMSF:	The International Commission on microbiological specification for foods
MAP:	Modified atmosphere packaging
NMR:	Nuclear magnetic resonance
PCA:	Plate count agar
RFID:	Radio-frequency identification
SIH:	Seafood Innovation Hub
SSOs:	Specific spoilage organisms
TMA:	Trimethylamine
TMAO:	Trimethylamine-N-oxide
TPA:	Texture profile analysis
TTI:	Time temperature indicator
TVB-N:	Total volatile base nitrogen
TVC:	Total viable count

NOMENCLATURE

WI:	Whiteness index
ΔE:	Total colour difference
L*:	CIE lightness coordinate
a*:	CIE red (+)/green (−) colour attribute
b*:	CIE yellow (+)/blue (−) colour attribute

1 LITERATURE REVIEW

1.1 Introduction

With an ever-increasing population comes the increased demand and requirement for the safe preservation, transportation and storage of food. Adequate food preservation practices are essential to prevent the microbial spoilage of food without altering its texture, taste or nutritional composition.

It has previously been reported that 30% of the world's landed fish are discarded annually due to preventable microbial activity (Amos, 2007). Fish is universally regarded as being a highly perishable commodity, with a naturally short shelf life. The general spoilage of fish is directly linked to that of chemical, enzymatic or microbial activity. Fresh fish spoilage can be very rapid after it is caught and is accelerated due to contamination and improper storage at higher temperatures (Unklesbay, 1992). As the fish begins to spoil, the result is a breakdown of a variety of components, and the production of new compounds. These new compounds are the catalyst for the degradation of the fish appearance, flavour, odour and muscle texture. In order to develop and maintain optimal preservation practices, understanding the factors responsible for their degradation is paramount.

In 2017 Ireland's seafood export market was valued at € 666 million, accounting for 60% of the overall seafood market value in Ireland. Most Irish seafood is exported to foreign markets such as the UK, the EU and Asia (BIM, 2017). Two of the most commonly exported species are megrim (*Lepidorhombus whiffiagonis*) and pollack (*Pollachius pollachius*). The growth of the Irish seafood industry is predominately driven by exports. Currently, 90% of both species are exported to European countries. Spain accounts for 99% of all Irish exports of megrim while France accounts for 89% of all Irish pollack exports (Bord Bia, 2017). France is the most substantial market for these exports, accounting for over one quarter of the overall seafood market value in 2018. Spain accounts for 10% of the export market value. The remaining 55% is exported to the UK, other EU countries, Asia, Africa and the Middle East. By 2020, Ireland aims to position itself as an international leader in the global seafood industry (BIM, 2018).

In order to grow the export sector, to create sustainable Irish seafood throughout the supply chain, from catch to consumer, and to build on Ireland's positive image, the seafood export sector must be assisted in its journey. Factors such as currency, the UK market, supply chain problems and increased competition make it essential to dilute risk by increasing product preservation techniques.

Food business operators in Ireland are required by law to ensure that all frozen and chilled foods are kept at the correct temperature during transport, storage, delivery and display (European Parliament and Council, 2002). This can be achieved by implementing a cold chain management system (CCMS). A CCMS is crucial for the preservation and safety of perishable foodstuffs (Zoller S. , Wachtel, Knapp, & Steinmetz, 2013). A CCMS is a sequence of interlinked operations from the point of origin to the point of consumption in the intake, production, storage, distribution and retail of perishable temperature-sensitive products. Seafood safety, quality and hygiene will be compromised if the cold chain is breached at any point. Therefore, from the Irish exports market perspective, ensuring the integrity of the CCMS is crucial. From a consumer perspective, traceability and sustainability are becoming increasingly more important (Verbeke, Sioen, Brunsø, Henauw, & Camp, 2007). Freshness is one of the main considerations for the fish industry and maintaining a constant temperature during transportation is vital for product freshness. To ensure optimal freshness a CCMS is essential for the industry to adhere to. On the occasion that temperature failure occurs during the distribution of perishable foodstuffs, the result of quality decline and product loss can be as high as 35% (BIM, 2017). Typically, in vehicles and warehouses, humidity and thermometer sensors are installed to monitor fractures within the CCMS. However, these technologies are not 100% fail-safe, and there are no current on-board temperature monitoring studies when transporting these species of fish. Therefore, further research on the entire CCMS with particular focus during exportation is needed. Poor CCMS practices have a ripple effect not only limited to processing and storage conditions of seafood, but can also affect the economic and nutritional values, along with the shelf life of the final product (Blaha & Gonçalves, 2010).

Literature studies indicate that the temperature along the cold chain often rises above the desired limit, consequently producing food waste and endangering food safety. Weak

links within the cold chain regarding temperature control can be found everywhere, whether it is at the beginning of the chain during pre-cooling or throughout logistical phases, where food may be indirectly subject to environmental factors (such as pH, sunlight and ambient temperatures). At the end of the chain, during processing, transport and retail display where temperature control is out of the hands of the manufacturer and understanding of issues related to temperature misuse may not be as competent. However, such temperature increases are not uniformly spread along the cold chain. There are many areas, particularly distribution, that are prone to a cold chain breach.

The purpose of this study was to identify factors within the cold chain that, when exported, may adversely affect the proximate composition, value and shelf life of whole megrim (*Lepidorhombus whiffiagonis*) and pollack (*Pollachius pollachius*). Therefore, determining the source and impact of temperature fluctuations during chilled distribution, with an emphasis on the quality of fish when storage exceeds ≥ 5 °C is paramount.

1.2 Megrim and Pollack

1.2.1 Characteristics

Megrim is a left-eyed flat fish and is a member of the *Scophthalmidae* family (Figure 1.1); other family members include turbot, brill and topknot. Megrim reach sexual maturity at 2.5 to 4 years and can live up to 15 years (Seafish Industry Authority, 2014). Megrim have been reported to grow up to 60 cm, but commonly grow between 35 and 45 cm; the minimum EU landing size when caught is 20 cm. It can be found at depths between 100 to 600 m off the western coast of Ireland. Spawning occurs between January and April; once the eggs are laid they will hatch within 4 to 5 days. Young megrim swim in waters until they are about 2 cm in length, then migrate to the muddy and sandy sea bottom.

Pollack is a round fish with a distinctly crooked lateral line, a strong under bite and is a member of the *Gadidae* family (Figure 1.2); other family members include cod, haddock and whiting. Pollack reaches maturity at 3 years and migrates to deeper waters as it ages. It has been reported to live to a maximum age of 15 years. The minimum EU landing size when caught is 30 cm but has been reported to reach lengths of 120 to 130 cm (European Commission, 2018). It can be found at depths up to 200 m in the northeast Atlantic but is

more commonly found amongst rocky shores and wrecks. Spawning occurs between February and April; once spawned, eggs and larvae drift along the water column en route to shallower coastal regions. Juveniles can reach up to 17 cm in length within the first year. Juveniles dwell among algae and rocks, feeding primarily on crustaceans in shallow waters, before venturing into depths between 40 and 100 m when they are approximately 40 cm of length and around three years of age (Wheeler, 1969).

Both species are carnivorous demersal fish that occupy the sea floor, this can be further broken down into two sub-sets of benthic and benthopelagic fish. Megrim is classified as a benthic fish; they have a denser composition compared to water with negative buoyancy which enables them to lay on the sea floor without effort. Pollack is classified as a benthopelagic species. Pollack have neutral buoyancy, which enables them to float with little effort in the water column just above the sea floor.

1.2.2 Composition

In order to create and maintain fish muscle, a combination of water, protein and fat is required to form 95% of the overall composition, with the remaining 5% comprising minerals (Ambily & Nandan, 2018). Generally, the chemical composition of megrim comprises 80% moisture, 17.8% protein and between 1.0 to 3.9% fat, similar to other members of the *Scophthalmidae* family such as turbot, brill and topknot (FAO, 2001). Pollack has a slightly different composition consisting of 79% moisture, 16.4 to 20.3% protein and 0.6 to 0.8% fat, similar to other *Gadidae* family members like cod, haddock and whiting (FAO, 2001).

The body composition of fish species can significantly alter depending on the phase of sexual maturity once caught, particularly with female fish pre-, during and post-spawning. Prior to spawning the female fish will not eat, therefore once the carbohydrates are depleted the fish's body will then start to break down fats and proteins for energy resources. During this time, the fish is surviving at the muscle proteins' expense. Therefore, it is possible to conclude that the proximate composition can significantly vary across the spawning period. Similarly, the water content of spent fish can rise as high as 91%. With protein levels substantially depleted, the meat becomes relatively soft resulting in a loss of potential yield and quality compared to pre-spawning caught fish

(Ingolfsdottir, Stefánsson, & Kristbergsson, 1998). Studies on other species during spawning such as hake (*Merluccius merluccius*) (Domínguez-Petit, Saborido-Rey, & Medina, 2010), brown trout (*Salmo trutta*) (Pickering, 1986) and winter flounder (*Pseudopleuronectes americanus*) (Sims, Wearmouth, Ge, Southward, & Hawkins, 2004) have suggested that this is a similar trait across a multitude of species.

Both species have a predicted shelf life of between 12 and 14 days which is dependent on size, sexual maturity and other environmental factors (FAO, 2001).

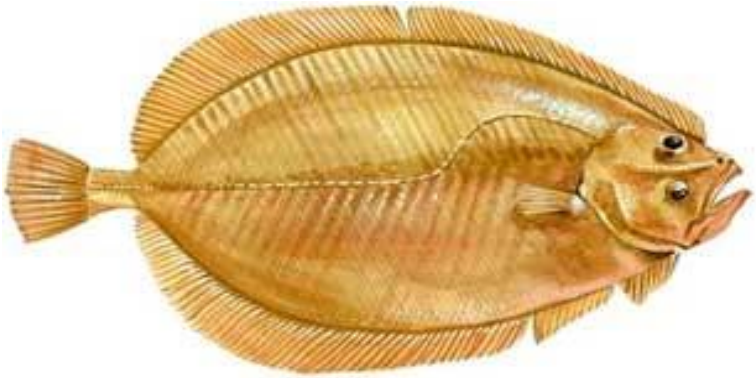

Figure 1.1: Megrim (*Lepidoehombus whiffigonis*) (Skaarhoj, 2019)

Figure 1.2: Pollack (*Pollachius pollachius*) (The Norwegian Seafood Council, 2019).

1.3 Cold Chain

A cold chain or cool chain is a temperature-controlled, uninterrupted supply chain, denoted by a series of actions and equipment used to keep a product within a specified low-temperature range. Chilled and frozen products typically go through a variety of storage and transport phases before reaching the end consumer. The phases along the route must consist of a full 'cold chain', from the point of origin to the point of

consumption covering production, storage, distribution and retail without any significant temperature increase. Cold chains are used for preserving, extending and maintaining the shelf life of products such as chemicals, fresh agricultural products, frozen food and seafood (Gyeszly, 1991). During transient storage and distribution these products are often referred to as cool cargo. Cold chain goods, unlike other goods or merchandise, are perishable and always on the way to end-use or location, even if temporarily held in cold stores and are therefore commonly referred to as cargo throughout its logistical cycle. The logistics of the cold chain includes all the means used to guarantee a constant temperature range for products that are not heat stable, from production to consumption (Mercier, Villeneuve, Mondor, & Uysal, 2017).

1.3.1 Cold chain challenges

To detect and prevent breaches within the cold chain, there is an ongoing need for rapid, sensitive, inexpensive and reliable techniques to offset food safety risks (Wang & Duncan, 2017).

In order to avoid the spoilage of perishable food products, monitoring is necessary in every stage of the cold chain. General challenges facing most seafood cold chains is to maintain the appropriate temperature at all times from 'sea to shelf' (0 - 2 °C). Due to human or mechanical errors on-board ships, the cold chain can be readily impaired during production, transient storage and distribution. Frequent opening and closing of doors can decrease quality considerably. Liu *et al*. (2004) noted that frequent door opening / closing or prolonged door opening time will result in an increased core temperature in foodstuffs during chilled refrigeration. They investigated the opening and closing of a refrigerated chill unit set to 3.0 ± 0.5 °C containing foodstuff. The door was opened once every 12 minutes for a total of 50 times over a period of 10 hours. At an outside ambient temperature of 30 °C, they found that the effect of frequent door opening caused the temperature of the compartment containing foodstuff to rise by 14.5 °C during the 10 h chilled storage period.

However, breaches may occur in the cold chain at any stage, one of the most highlighted areas where these breaches occur more frequently is during distribution. Distribution plays a vital role in maintaining equilibrium within the cold chain, particularly at transfer

points, as the risk of temperature fluctuations are higher during distribution compared to any other stage within the cold chain (Shih & Wang, 2016). Fresh fish are commonly exported from Ireland by air and sea. The advantage of air freight is that it can be faster, however the disadvantage is that it is more expensive compared to sea export, with products often exposed to fluctuating temperatures due to loading, unloading and holding at transfer points (Magnusson, *et al*., 2010).

It has been established that chilling fish below 0 °C without freezing is essential to ensure the utmost quality of fresh fish and to make it less susceptible to fluctuations in temperature during distribution (Mai, *et al*., 2012). Many studies have suggested that superchilling has produced good results throughout the cold chain in terms of extending shelf life in fish. Superchilling (also known as partial freezing or deep chilling) can be described as the process in which food products are stored between 1 and 2 °C below the initial point of freezing (Duun & Rustad, 2007). Previous research has shown that farmed cod and salmon fillets stored at superchilled temperatures had longer shelf life compared to ice-chilled fish due to a reduced growth of sulphide-producing bacteria (DeWitt & Oliveira, 2016). This has also been explored in prior studies by Lauzon (2000), who investigated whole gutted plaice stored at -1.7 and 0.6 °C. When samples were tested at 0.6 °C they were determined to have a shelf life of 12 days, whereas samples stored at -1.7 °C had a shelf life of 14 days. Similarly, cod loins superchilled at -2 °C had an increased shelf life of 14 to 15 days in comparison to 11 days when stored at 0 °C (Lauzon H. , 2000). However, in a different study, Einarsson and Lauzon (1995) observed accelerated spoilage in cod when stored at -1.8 °C compared to samples previously stored at 0.6 °C with a combination of refrigeration and ice. In a further study, superchilled cod loins combined with modified atmosphere packaging (MAP) had an extended shelf life of 21 days compared to 11 days when stored chilled (≤ 1 °C) (Lauzon, *et al*., 2010). Superchilling however, has a relatively narrow temperature range (between 1 and 2 °C below its initial freezing), and if lowered beyond this the water in the fish may freeze, causing the concentration of solutes to increase in unfrozen solutions. This can result in the muscle proteins denaturing, along with structural damages to the membranes, which can lead to an increase of drip loss and textural changes (Valtysdottir, Margeirsson, Lauzon, & Martinsdottir, 2010).

1.3.2 Cold chain management system

A quality management system can be used to manage a cold chain. To analyse, measure, monitor, document and validate the entire cold chain, a cold chain management system is used (Zhong, Xu, & Wang, 2017). A cold chain management system (CCMS) is critical to preserving and safeguarding perishable foods as it monitors the entire cold chain (Zoller, Wachtel, Knapp, & Steinmetz, 2013). A CCMS is generally an IT-based system supported by physical sensors that are / are not wirelessly connected to the cloud. The accuracy of the cold chain can be indicated by the values obtained and any breaches or weak points within the chain can be discovered (Chandra & Lee, 2014). Safety, quality and hygiene of seafood will be compromised if at any point the cold chain is broken, thus the need for a management system that controls and documents the entire process is critical.

A CCMS is a widespread common practice in the areas of meat, fruit, vegetable and dairy products (European Union, 2012). However, there are few CCMS studies on seafood products, as the majority tend to focus on the meat and dairy industries (Giannakourou, Koutsoumanis, Nychas, & Taoukis, 2005). According to food law, traceability is compulsory in accordance with EU Regulation 178/2002 (Article 18) (European Parliament and Council, 2002). The law states that in order to ensure traceability; food, feed and food producing animals, or any substance intended or expected to be incorporated into or used as food or feed must be established at all stages of production, processing and distribution. A CCMS is vital for traceability and sustainability, which are becoming increasingly more important to consumers (Verbeke, Vanhonacker, Sioen, Van Camp, & De Hanauw, 2007). This trend has been noticeable by the public outrage at the series of food contamination scandals in recent times, particularly following the 2013 horsemeat scandal, where consumer confidence in meat manufacturers fell by 24 % in the UK (Happen Group Ltd., 2014). People are now not just concerned about the taste, they are also concerned about the origin of their food.

Fresh foods generally deteriorate due to biological, chemical and physical changes in the supply chain (Giménez, Ares, & Ares, 2012). Furthermore, the low thermal gradient of fresh food makes the food in the supply chain vulnerable. It is well known that maintaining a constant low temperature throughout the supply chain helps to maintain the

quality and shelf life of fresh food. As a direct result, the control of time and temperature in the food chain becomes a crucial component, driving demand for the management of the cold chain. One of the key activities in cold chain management is distribution planning. In order to preserve food quality during cold chain distribution, more stringent temperatures and time control are required, in addition to increased investment in temperature-controlled vehicle fleets. This can however considerably increase distribution costs (Hsu, Hung, & Li, 2007). In addition, serving customers within specific delivery time windows and meeting their food and quality expectations at the same time can increase the complexity of cold chain distribution planning and potentially lead to fractures within it (Chen, Hsueh, & Chang, 2009).

1.3.3 Time Temperature Indicators

Thermal analysis is the study of the possible effect heat may have on a product's properties (Pomeranz & Meloan, 1994). Throughout the chilled preservation of fish, there are numerous chemical and biochemical changes in the fish tissue, which ultimately lead to spoilage. The enzymatic action triggers spoilage after death, resulting in self-digestion, affecting the fish appearance, flavour and texture. The rate at which self-digestion occurs depends heavily on temperature (Ansari, Abbas, & Megat Ahmad, 2004).

Time temperature indicators (TTI) are smart devices that record the time-temperature history of a product. TTIs are commonly used in industries such as food, pharmaceutical and medical (Wang, *et al.*, 2015). The TTIs allow a product's temperature (and time at temperature) to be recorded to see if it has been exposed to any undesirable fluctuations, providing an economical and easy-to-use way to detect any chill chain breaches (Taoukis & Labuza, 1989).

Temperature has the greatest impact of environmental parameters during transport on the quality of food products. Strong coordination and cooperation are needed between all involved parties to ensure that the cold chain is maintained at a constant. It is essential to minimise delays in the cold chain during harvest as well as in the production hall, carrier depot, container packing depot and/or container terminal. In addition, it is essential to ensure that temperatures within the distribution vehicles are correct; local temperature deviations can occur in almost any transport situation. During distribution, literature

reports suggest that fluctuations of 5 °C or higher may occur (Jedermanna, Ruiz-Garcia, & Lan, 2009). Any small temperature increase during storage affects microbial growth and therefore reduces the shelf life (Ryder, Iddya, & Ababouch, 2014). In accordance with Article 4 (3) (d) of Regulation (EC) 852/2004, food business operators are required to maintain the cold chain as appropriate. Unfortunately, for assessing the effects of temperature fluctuations on fish, there is no single scale. These regulations relate primarily to fixed temperature thresholds but provide insufficient information on the effects of temperature deviations above these thresholds. It is therefore essential that quality issues are detected as soon as possible, and alarms are triggered when fluctuations in temperature exceed those that are recommended (0 to 2 °C) (Bord Bia, 2017).

As mentioned previously, it is essential to identify locations or stages within the cold chain where fluctuations in temperature may occur. Zubeldia *et al*., (2016) investigated commercial freezer cabinets in Spain and found that temperature fluctuations were extremely pronounced in the summertime, especially for foodstuffs on top racks. Remaining shelf life was decreased by 25%, 40% and 57% for fresh cheese, cooked chicken breast and smoked salmon respectively due to fluctuating temperatures. Temperature throughout the refrigeration of iceberg lettuce in Japan fluctuated between 3 °C and 15 °C during transport (Koseki & Isobe, 2005). In China, during the transportation of frozen tilapia fish fillets, cargo temperature fluctuated after 6 h, between -18.6 °C and 16.8 °C (Tingman & Xiaoshuan, 2010). These researchers concluded that a significantly shorter shelf life occurred when products were exposed to slight temperature fluctuations of 2.0 °C compared to those with temperature fluctuations of less than 0.5 ° C.

Temperature during shelf life can be determined using thermometers such as thermometer data loggers. Temperature monitoring systems on board trawlers and smaller boats are not commonly implemented at the chain's primary end. In these cases, using autonomous temperature monitoring technology can eliminate the need for time consuming manual inspections and provide a full temperature log from catch to consumer (Patel, Kar, Jha, & Khan, 2012).

Eliasson *et al*., (2019) used data loggers to determine temperature profiles of superchilled and traditionally iced cod during chilled distribution over a 16-day period. Temperature

monitoring showed that the industrial superchilling method brought the core fish temperature close to −1 °C within 1 h, demonstrating minimal changes in muscle temperature during storage. However, traditionally iced cod took longer to cool down compared to superchilled samples, with temperatures reaching above 3 °C during the chilled storage period. Derens *et al*., (2006) monitored three categories of refrigerated food products, fresh meat, meat products and yogurt, throughout the cold chain. At the end of manufacturing (before shipment through the supply chain), temperature data loggers were placed among food products. Findings from the study indicated that the final three phases of the CCMS was particularly critical, namely display racks, after-shopping transportation and home refrigeration.

Using more advanced technologies Freiboth *et al*. (2013) analysed and monitored the intermodal export of fruit, integrating wireless sensor systems with multiplexed communications, fleet management and mobile networking systems. They determined that the majority of cold chain breaches were at port, during the loading and unloading periods and also between the hours of 12 to 3 pm which measured as the hottest time of day. Similarly, Jedermann *et al*. (2006) and Behrens *et al*. (2007) introduced a smart container system that combined wireless sensor networks with radio-frequency identification (RFID) to evaluate temperatures outside and inside a container of gutted sea bass (*Dicentrarchus labrax*). Several packing methods were evaluated, with measurements indicating fluctuations during the chilled distribution of samples. Internal temperatures rose between 6 and 9 °C during transportation when outside temperatures reached above 20 °C. They determined that variations depended on the insulation characteristics of the styrofoam container and the cooling materials used (i.e. ice, dry-iced, gel pads, etc.).

1.3.4 Temperature mapping

Monitoring optimum temperature is a prerequisite for maintaining a secure chain of custody across the cold chain with the aim of supplying good quality and safe foodstuffs as well as reducing waste and economic costs (Raab, Petersen, & Kreyenschmidt, 2011). Temperature mapping is a common practice when regulating a CCMS and involves collecting temperature data over time at multiple points within an allocated space, such

as cold rooms, production halls and distribution vessels. Data loggers are customarily used at regular locations and intervals to map product and environment temperatures. Having knowledge of specific temperature profiles inside a particular chain makes it possible to identify weak temperature control points and analyse the cause of fluctuation or temperature stress. Appropriate measures can then be taken to prevent or reduce the risk of any undesirable increase in product temperature. Gudmundsson *et al*. (2013) placed fifteen temperature loggers throughout a fish processing plant to map its ambient temperature and processing time. The results indicated that ambient temperatures were mostly below 10 °C, except before and during pallet packing, where they reached temperatures of 15 °C. In accordance with the temperature mapping results, reefer doors were determined as the weakest point during the export of the fresh fish. The results indicated that the reefer doors greatly contributed to leakage and temperature fluctuations during the transport of foodstuffs (Gudmundsson, Margeirsson, & Arason, 2013). Martinsdottir *et al*. (2010) investigated the exportation of cod loins from Iceland to Germany and found that the fluctuations in ambient temperatures were much lower when transported by sea (≤ 2 °C) compared to air freight (≤ 4 °C). For 35 % of transportation time, the temperature of fish by air freight measured above 0 °C, in comparison to only 18 % by sea freight. It is well known that the quality of fresh fish will remain high until its shelf life expires by minimizing temperature fluctuations. When the temperature of a fish product increases, chemical reactions (autolysis) are stimulated, which decrease the flavour, colour and texture of the product while accelerating the growth of the bacteria (Otwell, 1997). Temperature mapping during distribution is a key component for maintaining the optimum temperature and quality of fresh fish. Even with safeguards in place, such as ice and thermometers, temperature fluctuations may still occur, as suggested in the literature studied. To date, literature has focused primarily on more popular species such as cod, salmon, and tilapia. However, the mapping of less popular species during distribution and in particular the measurement of temperature fluctuations during export, has been relatively unexplored within this field.

1.4 Spoilage of fish

Any change that makes a food product unfit for human consumption may be regarded as food spoilage (Hayes, 1985). As previously mentioned, a combination of biochemical and

microbial reactions results in the spoilage of foods. From the moment of catching, fish begin to spoil due to this complex enzyme and bacterial activity.

Temperature is a key influencer of both enzymatic and microbiological activity in foodstuffs. Freshly caught seafood can quickly spoil if temperature is not controlled. Generally, a number of stages of the cold chain, such as transfer points or storage rooms, are found to be the weakest link during chilled perishable food management, resulting in fish products that spoil in a shorter time period unless adequately packaged, stored and transported (Giannakourou, Koutsoumanis, Nychas, & Taoukis, 2005). Thermometers and humidity sensors are installed in vehicles and warehouses in traditional CCMS (Wang & Duncan, 2017). It is estimated by the United Nations that about 1/3 of all food produced for human consumption is wasted each year due to temperature mismanagement (FAO, 2011); (NRDC, 2012).

1.4.1 Microbial spoilage

Microbial growth and metabolism are the main cause of spoilage in fresh foods. Microflora type and composition in a fish product are influenced by both intrinsic factors such as species, catch location, time of season, etc. and extrinsic factors which include post-harvest handling, time/temperature of storage, processing measure, etc. The seafood environment can be favourable for bacterial growth. Microorganisms grow rapidly between 10 and 40 °C, with limited growth between 3 and 10 °C dependent on the food source. As the temperature increases from −12 to 0 °C, only specific microorganisms can develop while below −12 °C, generally no microorganism will grow (Roccato, Uyttendaele, & Membre, 2017). Other factors between interfaces may contribute to increased spoilage due to microbiological growth, in addition to neglecting appropriate storage temperatures. Low glycogen levels, the poikilothermic (varying in temperature according to the temperature of the surrounding environment) nature of fish, high moisture levels, a relatively high proportion of non-protein nitrogenous compounds and a comparatively higher post mortem pH compared to other muscle foods, all contribute to encouraging bacterial survival and growth (Gram & Huss, Microbial spoilage of fish and fish products, 1996). Certain pathogens are common between seafood and other muscle foods such as *Salmonella, Listeria monocytogenes* and *Staphylococcus aureus*. However, seafood does contain pathogens associated with its microflora such as *Clostridium botulinum Type E, Vibrio species* and *Aeromonas* which may be less

common in other muscle foods (Lampila, 2012). In freshly caught fish, microorganisms are found on all the outer surfaces (skins and gills) and in the intestines. Following fish death and the end of rigor mortis, autolysis begins to occur. This results in the production of a nutrient-rich medium on which microorganisms thrive. As a result of this microbial metabolism, amines, sulphides, alcohols, aldehydes, ketones and organic acids are formed which can result in unacceptable off-flavours, odours, colours and slime (Gram & Dalgaard, 2002). There is a distinction in the microflora present on seafood between organisms which are 'spoilage associated' and organisms which are 'specific spoilage organisms'. Although there may be a large number of total organisms, only a fraction of these may actually cause spoilage (Gram & Huss, 1996). It is possible to identify specific spoilage organisms through the qualitative ability of the organism to produce off-odours and its quantitative ability to produce spoilage metabolites (Gram & Dalgaard, 2002). Olafsdottir *et al.*, (2006) investigated the effect of storage temperature on specific spoilage organisms (SSOs) generation and changes in the quality of haddock (*Melanogrammus aeglefinus*). Fillets were stored in containers at 0, 7 and 15 °C and quality and microbial counts were monitored. Results indicated that higher levels of microbial metabolites were generated as the storage temperature increased and shelf life consequently was reduced.

1.4.2 Biochemical spoilage

Following death, a sequence of biochemical reactions takes place in fish, the rate of which is dependent on the species, condition, temperature and handling practices. As oxygen rich blood is not available post mortem, aerobic recovery of adenosine triphosphate (ATP) no longer occurs. To compensate, fish muscle glycogen is broken down to provide energy. This results in the production of lactic acid and a lowering of the pH. As the levels of ATP continue to decrease, rigor mortis sets in, causing the fish to stiffen. This usually occurs a couple of hours after death and subsides after a couple of days (Jacobson, Nielson, Jorgensen, & Nielson, 2010). Proteolysis of fish, although a desired process in pork and beef for tenderisation, causes gaping, unwanted softening of the muscle and a deterioration in protein quality. Endogenous proteolytic enzymes act on peptide bonds in the secondary structure of proteins resulting in the cleavage of peptide bonds. Protein

functionality, including solubility and water holding capacity, can be influenced by the occurrence of protein oxidation. Oxidation may occur in the protein backbone and on the side chains of amino acids and may lead to the formation of reactive species such as peroxides and hydroperoxides that may increase lipid oxidation (Davies, 2005). Lipids are also degraded enzymatically, forming di- and mono-glycerides, glycerol, nitrogen bases and free fatty acids. The latter can particularly affect the sensory properties of fish (Hyldig, Nielson, Jacobson, & Nielsen, 2012). Lipid oxidation can negatively affect odour, taste, and in some extreme cases the nutritional content in fish. Auto-oxidation, photosensitized oxidation and enzymatic oxidation are the main processes which cause lipid oxidation in fish muscle (Hyldig, Nielson, Jacobson, & Nielsen, 2012). The enzymatic degradation of trimethylamine-N-oxide (TMAO) produces dimethylamine and formaldehyde, both reactive and cytotoxic compounds (Jacobson, Nielson, Jorgensen, & Nielson, 2010).

1.4.2.1 Total Volatile Base Nitrogen (TVB-N)

The inevitable protein breakdown as the tissues of the fish are colonised by successive bacterial strains is the effect of microbial activity on fresh seafood. The decomposition rate is influenced by the initial count and strain of bacteria and storage conditions, e.g. gaseous atmosphere, humidity and temperature (Strenström & Molin, 1989). Initially, fish contain significant trimethylamine oxide levels, which in the live fish have an osmoregulatory function. Both *Pseudomonas* and *Alteromonas* species reduce the volatile base of trimethylamine oxide (TMAO) to trimethylamine (TMA), a volatile base with an extremely fishy odour (Van Spreekens, 1977). Ammonia is another undesirable volatile base produced by a non-protein-nitrogen (NPN) degrading oxidation product, such as amino acids and creatine (Bannerjee, 1967). Total volatile base nitrogen (TVB-N) is a nitrogen value that includes all volatile nitrogen compounds, including TMA and ammonia (Villemure, Simmard, & Picard, 1986). Ammonia (NH_3) is the dominant contributor to meat spoilage, while TMA is often the main base for fish deterioration.

The determination of TVB-N therefore is a widely used method of quality assessment for fish. Acceptable limits for TVBN levels in different categories of fish products are as

described in EC Directive 95/149/EC (see Table 1.1). This Directive also specifies which methods are to be used in the quantification of TVB-N.

Table 1.1: TVBN limits as set in the EU Directive 95/149/EC.

Category	Species	Limit (mg N/100 g)
A	*Sebastes* spp. *Helicolenus capensis Sebastichthys capensis*	25
B	Species member of the *Pleuronectidae* family (with the exception of halibut: *Hippoglossus* spp.)	30
C	*Salmo salar* Species member of the *Merlucciidae* family Species member of the *Gadidae* family	35

Both megrim and pollack fall into category C and have a limit of 35 mg N/100 g of flesh.

To establish whether temperature control alone is sufficient to maintain and reduce levels of ammonia and TMA, Oehlenschlager *et al*. (1992) studied TVB-N in cod and haddock to determine their freshness during chilled storage (≤ 0 °C). The concentration of TVB-N levels remained constant in the first 8 to 12 days of ice storage, then slowly increased. TVB-N values began to rise rapidly over time after 12 days of storage, exceeding the acceptable TVB-N limit of 35 mg/100 g on days 15 to 20. When limits are exceeded, members of the *Gadidae* family are considered unfit for human consumption (EC No. 2074, 2005). Similarly, a recent study by Hunag *et al*. (2019) also investigated TVB-N as a freshness indicator for Wuchang bream (*Megalobrama amblycephola*) during storage at room temperature (≤ 25 °C) and under refrigeration (≤ 4 °C). The initial TVB-N value for fresh fish at room temperature was 6.3 mg/100 g, it then increased after 20 h to 22.4 mg/100 g. According to the food standards for seafood in China (GB 2733–2015), the rejection limit of TVB-N for freshwater fish is 20 mg/100 g (NHFPC, 2015). Therefore, results implied that fish samples subjected to room temperature could not be consumed after 20 h. The TVB-N value for refrigerated samples did not exceed the threshold (20.1 mg/100 g) until day 6 of storage. This has also been explored in prior studies for hake kept in ice flakes produced by ozonized water (Petfrost) (Pastoriza, Berna´rdez, Sampedro, Cabo, & Herrera, 2008). TVB-N levels of hake from catches that spent 17 days on board, stored in ice (≤ -1 °C) were just within the legal limit (31.6 mg TVB-N/100 g), while the Petfrost treated samples were still well below the rejection limits

(22.9 mg TVB-N/100 g). When temperature stressing treatments were introduced, Pino (2018) concluded that in pollock fillets TVB-N levels increased slowly when stored at -1 °C (9.45 mg/100 g to 15.5 mg/100 g). When samples were stored at 2 °C, TVB-N levels increased rapidly in comparison, reaching 67.3 mg/100 g at the end of the storage period. This indicated that even minor temperature fluctuations had a significant impact on the volatile nitrogen in the fish samples.

In addition to temperature, researchers have also investigated the effects of different cut s on the TVB-N levels in fish. Calanche *et al*., (2019) observed TVB-N values in chilled (≤ -1 °C) seabream (*Sparus aurata*), on day 5 filleted, gutted and whole seabream measured at 24.5, 21.0 and 22.8 mg /100 g respectively. TVB-N levels increased significantly for filleted fish reaching 86.2 mg/100 g on day 18, whereas TVB-N levels did increase for both gutted and whole, however to a lesser extent (36.5 and 32.0 mg /100 g respectively). Calanche *et al*. (2019) attributed the higher values to the release of oxidative enzymes and pro-oxidants from varying ruptured cellular organelles in filleted fish.

1.5 Quality indices in fish

Fish quality is assessed using a variety of different analytical methodologies. These include the measurement of microbial and chemical quality indices, such as total volatile base nitrogen (TVB-N), and physical indices including colour, texture, temperature and sensory analysis.

1.5.1 Colour

Consumers recognise, discriminate and select nutrients with their eyes to a great extent. They expect an item of a certain shape and colour to have a particular odour, taste and texture through conditioning and association. Previous quality treatments may be identified by colour in seafood as good or bad indicators. If seafood is poorly handled and/or temperature mismanaged, this will be reflected in the colour of the skin, eye, gill and flesh (Pangborn, 1964).

In food, colour is a central parameter and is used to assess the quality, maturity and age of many foods after harvest (Gormley, 1975). Whitefish flesh when raw has a translucent quality. Once cooked it becomes opaque and whitish, as the proteins denature and coagulate (Shewan, Macintosh, Tucker, & Ehrenberg, 1953). In muscle cells of fish prior to slaughter, the protein myoglobin stores oxygen, which is used to extract the energy required for constant activity. Myoglobin is a protein that is richly pigmented. The amount of myoglobin in the cells determines the flesh redness or darkness (Chaijan, Benjakul, Visessanguan, Lee, & Faustman, 2008). Fish containing white flesh are generally those with intermittent short bursts of activity that are resting / floating or largely inactive throughout their lifetime. They may have some red meat that is used for swimming around the fins and tail. Some fish, like salmon and trout, have a red colour due to astaxanthin, a pigment that occurs naturally from the crustaceans they consume (Johnson & An, 1991). Other factors contributing to colour variations in flesh can be attributed to improper handling or unavoidable circumstances such as insufficient bleeding, bruising, processing and spoilage.

In whitefish, the effects of prolonged chilled storage on fish and seafood products are difficult to evaluate. Changes are less evident than in more pigmented species such as

salmon and mackerel, and therefore colour may be the least valuable indicator of whitefish spoilage. However, during advanced spoilage of whitefish a red colour develops in the flesh along the backbone (Tetteh, 2010). Whitefish species generally appear more yellow during chilled storage. Yellowness can be attributed to factors such as microbial growth on the surface of the fish flesh, and discoloration can occur through bleeding action of cut surfaces likely due to low pH precipitation of sarcoplasmic proteins (Statham & Bremmer, 1989).

Three physical factors are required in order to visually assess the colour of the fish; a light source, a fish sample, and a light receptor mechanism. The CIE L*a*b* scale is based on the opponent process theory, which assumes that human eye receptors perceive colour as pairs of opposites. The L* scale indicates the level of light or darkness in a sample where dark is indicated by a low value (0 - 50) and light is indicated by a high number (51 - 100). The a* scale indicates an object's redness or greenness where a positive value indicates red and green is indicated by a negative value. The b* scale shows an object's yellowness or blueness where a positive value is yellow, and a negative value is blue (Hutchings, 1999).

Different genetic backgrounds, feeding, environment and slaughter conditions can have an antagonistic effect on fish pigmentation between species (Buttle, Crampton, & Williams, 2001). Therefore, L*, a*, b* values can increase and decrease in fish depending on species, pre- and post-slaughter treatment and degree of spoilage.

Fagan *et al*. (2004) concluded that during the storage of chilled whiting (*Merlangius merlangus*), L*, a*, b* values increased gradually overtime, with measurements for L* values ranging from 44.9 to 56.4, a* values 1.69 to -2.07 and b* values 0.39 to 4.91, over a 5-day chilled (≤ 4 °C) storage period. This method has also been explored in further studies by Glover *et al*. (2009), however instead of whitefish, farmed salmon (*Salmo salar*) was investigated. Colour measurements were taken over a 6-day chilled storage period (≤ 4 °C) with L* values ranging from 49.25 to 41.84, a* values 28.32 to 32.42 and b* values 28.05 to 30.77. Whilst both species were going through spoilage, the salmon became lighter in colour as a function of both time and storage conditions, whereas whiting became more opaque with tinges of yellow.

Variations in L*, a*, b* values can also be seen in the same species dependant on the treatment. Previous research showed that certain preservation treatments such as chilled versus frozen can affect said values. This pattern has been reported by Skałecki *et al*. (2010) in cod (*Gadus morhua*) fillets that were chilled (≤ 4 °C) and analysed within 12 h compared to frozen samples stored at -20 °C analysed after 4 weeks. There appeared to be no significant change in L* values measuring between 62.72 and 62.21 respectively. However, a* values decreased from 7.42 to 3.88, as freezing caused a lower level or redness in frozen fillets, and a higher yellow value was observed in b* values, increasing from of 8.19 in fresh fillets to 10.57 in frozen, with significant difference. Skałecki *et al*. (2010) did note that the observed colour differences could be associated with mechanical damage caused by the freeze-thaw process in the frozen samples. Such damage could be mainly caused by the formation of ice crystals in fillet tissues, leading to the breakdown of cell membranes (Badii & Howell, 2002).

Total colour difference (ΔE) is defined as the numerical comparison of a sample to the standard (calibrated white plate) and/ or between treatments. Chavalier *et al*. (2001) used total colour (ΔE) to determine the effect of pressure treatments on the colour of turbot fillets. It was determined that colour parameters of fish muscle were influenced by both pressure level and pressure holding, with ΔE reaching 24.3 ± 0.5 when 200 MPa for 30 min was applied. Dowlati *et al*. (2013) evaluated the freshness of gilthead sea bream, based on the total colour (ΔE) of the gills and eyes during chilled storage. During the storage period they found a linear increase in ΔE, however after day 10 there was a rapid increase in ΔE, indicating spoilage as the colour of the eye and gill became less vibrant with more dull / grey tones. Analytically, differences in perceivable colour may be classified as very distinctive ($\Delta E > 3$), distinctive ($1.5 < \Delta E < 3$) and sight variances ($\Delta E < 1.5$) (Adekunte, Tiwari, Cullen, Scannell, & O'Donnell, 2010).

The total colour difference can be calculated by using the following equation (Francis & Clydesdale, 1975):

$$\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{0.5}$$

Once L*, a*, b* values are established, whiteness indices (WI) may be also be calculated. Whiteness indices (WI) are widely measured to yield numbers that are closely correlated with consumer white colour preferences. In a single term, it mathematically combines

lightness and red-yellow. The WI is the whiteness of food products as a whole, which may indicate decolouration during shelf life or from other process treatments (Hsu, Chen, Weng, & Tseng, 2003). As whitefish ages and if it has been exposed to temperature fluctuations, the flesh will intensify resulting in a deep white-yellow colour, thus increasing the whiteness values as the flesh of fresh whitefish is usually opaque in colour (Caballero, et al., 2009).

The whiteness is determined by using the following equation (Park & Lin, 2005):

$$\text{Whiteness} = 100 - [(100 - L^*)^2 + a^{*2} + b^{*2}]^{1/2}$$

In regard to overall whiteness, carp (*Cyprinus Carpio L.*) fillets, for example have an L* value of 49.45 ± 0.16, a* value of 9.53 ± 0.26 and b* value of 6.68 ± 0.2 on day 1 of chilled storage (Tian, Wang, Yuan, & Zhang, 2016). When the L*, a* and b* values are entered into the equation for measuring 'whiteness', the result (48.13 ± 0.14) can then be evaluated and compared to the 'whiteness' of other fish products and species such as turbot (*Psetta maxima*) fillets which were previously reported as having a whiteness value of 51.33 ± 1.02 on day 1 of chilled storage (Xu, *et al.*, 2016). Common carp fillets have a slightly pink flesh colour; therefore the WI will be darker than those obtained from turbot which have a white, opaque flesh colour when fresh. Futhermore, Hosseini-Shekarabi *et al*. (2014) determined that process treatments on the same fish species (black mouth croaker (*A.Nibe*)) can have a significant variance on WI values. A higher WI was obtained in surimi croaker (66.23 ± 0.03) compared to minced croaker (51.68 ± 0.02) ($P < 0.05$). Hosseini-Shekarabi *et al*. (2014) noted that an increase in WI may be a result of leaching of certain components, particularly blood and pigments during washing and compression, resulting in higher surimi whiteness values compared to minced fish. Researchers have also established that WI decreases/ increases gradually over time during a food products shelf life, when handled and stored correctly (Benjakula, Visessanguanb, Thongkaewa, & Tanaka, 2005). Ocaño-Higuera *et al*. (2006) detected no significant difference ($P > 0.05$) in WI for catarina scallops (*Argopecten ventricosus*), during chilled (0 °C) refrigeration over a 15-day storage period. Therefore, when temperatures were maintained, colour was not significantly affected.

1.5.2 Texture Analysis

The three main components of food acceptability are texture, appearance and flavour. Texture is as important as flavour as a freshness criterion. The degree of softness or hardness without specific instrumentation can be hard to evaluate. While comparing a small number of fish samples using a combination of sight and touch is relatively easy, comparisons of the same samples become difficult over time without the samples being assigned some kind of numerical value.

Rigor mortis after the death of fish is the most extreme change. The technological impact of this is dependent on whether the fish is filleted pre, during or post rigor. During rigor, the body of the fish becomes entirely inflexible, and filleting yield is quite poor, and gaping will result from rough handling. If the pre-rigor fish is cooked, the texture becomes soft and pasty, if cooked during rigor, the texture is hard but not dry, and the flesh becomes firm, succulent and elastic after rigor (Huss H. , 1995).

Parameters of processing such as improper handling, prolonged storage and fluctuations in temperature affect the textural properties of fish. This is inherently due to the denaturation and aggregation of myofibrillar proteins, which can lead to a hard, dry and fibrous product (Moosavi-Nasab M. , Azadian, Farahnaky, & Yousefi, 2013). A number of methods are available for evaluating texture, including sensory analysis and instrumental methods. Instrumental methods have been used since 1861 to measure texture and are commonly used as a quality check across a multitude of food and pharmaceutical industries (Sigurgisladottoir, *et al*., 1999).

Textural properties are measured based on curves of force-deformation from foodstuffs under stress (bitten, chewed, torn, etc.). Texture Profile Analysis (TPA) comprises of a two compression-relaxation cycle (Larmond, 1976). Each compression cycle measures the deformation resulting from a uniaxial compression force being applied (see Figure 1.3). To evaluate the textural properties, TPA imitates the action of the jaw with the back-and-forth movement of the two cycles (Karlovic S. , *et al*., 2009). Barroso *et al*. (1998) stressed that the TPA not only imitates jaw action, but also offers a large amount of data that correlates heavily with sensory perception.

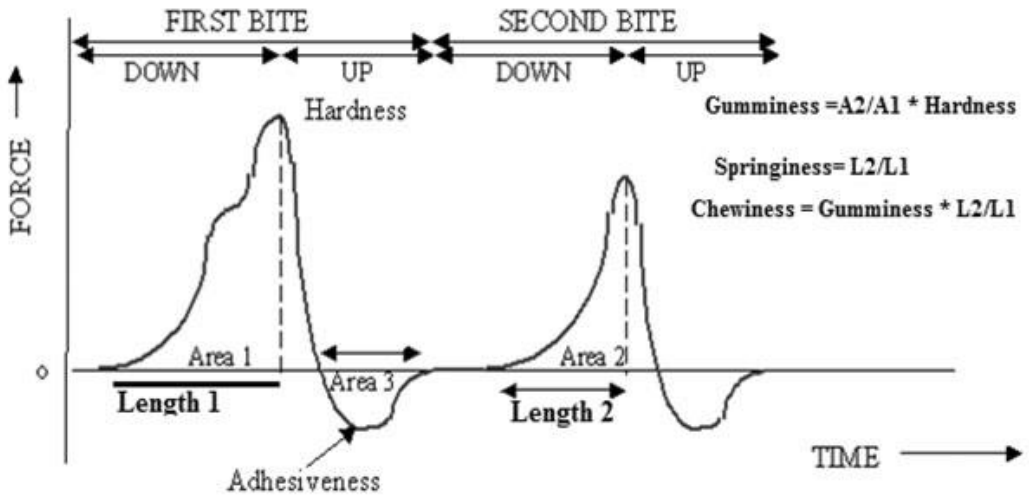

Figure 1.3: A typical texture profile analysis (two-bite force) curve (Banjare, Manikant, Geol, & Uprit, 2015).

Seven textural parameters have been developed for TPA. These parameters are defined as follows (Bourne, 2002):

- **Hardness:** The peak strength during the first cycle of compression.
- **Fracturability:** The force at the curve's first significant break.
- **Cohesiveness:** Compared to the first cycle, how well the product resists the second deformation.
- **Springiness:** How well the product springs back physically between the first and second cycles, i.e. after the first compression, the height the food recovers.
- **Gumminess:** Hardness product, multiplied by cohesiveness.
- **Chewiness:** Gumminess product, multiplied by springiness.
- **Adhesiveness:** The area of negative force for the first bite, which represents the energy required during the first compression to pull the compression.

It is well known that storage temperature generally influences the measurement of fish texture during operation and handling (Pearce, Rosenvold, Andersen, & Hopkins, 2011). The effects of different storage temperatures on the post-mortem changes in the texture of sea bream (*Pagellus bogaraveo*) were examined by Suarez *et al*. (2010). They stated

that firmness of samples stored at 1 °C was maintained longer than samples stored at 4 °C. The rate of muscle collagen degradation was faster at 4 °C compared to 1 °C, thereby affecting the cross-linkage of the connective tissue and becoming a major contributing factor to the loss of firmness in fish texture. Caballero *et al*. (2009) also reported post-mortem changes in sea bream muscle during iced storage, determining that hardness, gumminess and chewiness parameters had significant changes in quality between samples stored at varying temperatures (-1 to 6 °C) over a 7-day storage period. In an additional study, Badii and Howell (2002) assessed changes in the texture and structure of cod and haddock fillets stored at -10 and -30 °C. They observed that during storage, time and temperature increased fish muscle hardness significantly, affecting texture and structural changes during storage.

1.5.3 Sensory Analysis

The characteristic flavour of fish after catch naturally develops in the initial days of storage, but the appearance and texture are related to other sensory changes that occur during storage. Each fish species has their own characteristic sensory attribute of flavour, appearance, odour, and texture which change with time and temperature after harvest (Olafsdottir & Fleurence, 1998).

Sensory evaluation of fish products is closely linked to the perception of consumer quality (Damoglou, 1980). Sensory assessment of fish freshness and eating quality continues to be one of the most important methods of quality assessment in the seafood industry today. The sensory attributes of fish are influenced by processing measures. Prolonged storage and temperature fluctuations can lead to the development of tastelessness, off-flavours, fragility and loss of tenderness and juiciness (Pino, 2018). In fresh fish, the main sensory attributes affected by temperature fluctuations include an accelerated development of rancidity, texture hardening and toughness. While information about consumer acceptance is important, it is also crucial to know which sensory attributes consumers use to describe and discriminate between products.

Sensory analysis methods can be objective and subjective. Objective methods include descriptive tests, such as profiling and quantitative response scaling, and discriminatory

tests, such as triangle testing and forced choice. Subjective methods are aimed at testing consumers and measuring consumer responses, including attitudes and emotional responses to products. One of the most common methods of sensory analysis is the use of quantitative response scaling in which a panellist is asked on a scale or line to evaluate the intensity of a named attribute or descriptor. This line is then measured in order to obtain a numerical value proportional to the perception of the attribute intensity by the panellist (International Organistation for Standaradisation, 2003).

Chaiyapechara *et al.* (2003) observed that the presence of off-flavours, especially fishiness, were the most critical factors accountable for variations in sensory characteristics of rainbow trout (*Oncorhynchus mykiss*) fillets between treatments. The 'fishy' flavour was described by a trained panel as a blend of attributes such as flavour intensity, fishy, sour, stale, bitter and grassy. The off-flavour was negatively correlated with mild flavours such as chicken, nutty and egg / sulphur. In a sensory evaluation of cultured gilthead sea bream, Orban *et al.* (1997) concluded that panellists found that the cooked flesh of farmed fish was juicier, oilier and less meaty than that of wild fish. While the raw farmed fish had a stronger 'seaweed' odour, the intensity of aroma, taste, brightness and firmness was not distinguishable between farmed and wild once cooked. Bonilla *et al*. (2007) reported a high correlation between sensory attributes and storage time, which indicated that the attributes gradually deteriorated overtime. At the beginning of storage, small changes in positive attributes concerning odour and flavour were observed during sensory analyses. After the seventh day of storage they were hardly detectable, due to an increase of negative attributes such as a sour taste and a rotten pungent odour. Whilst both flavour and texture were evaluated in cooked cod fillets, Bonilla *et al*. (2007) concluded that panellists favoured flavour as a more important criterion of quality. Regarding consumer evaluation, Sawyer *et al*. (1988) concluded that when an untrained panel evaluates sensory properties of fish, they tend to use a relatively small set of descriptive terms for certain attributes such as appearance, flavour and texture to differentiate species compared to trained panellists. Therefore, when using a specific group of panellists, whether that be trained or untrained, it will unearth diverse information. For instance, consumer panels tend to be more market led and opinionated, whereas trained panels lean towards being more descriptive and factual based (Roberts, 2007).

To ensure the highest consumer acceptability for seafood products, it is necessary to correlate sensory acceptance with quality measurements such as colour, off-flavours, odour, TVB-N and texture.

1.6 Objectives of Research

This research endeavours to add to the limited knowledge and scientific information currently available for megrim (*Lepidorhombus whiffiagonis*) and pollack (*Pollachius pollachius*), by identifying factors within the cold chain that, when exported, may adversely affect the proximate composition, value and shelf life of fresh fish.

This will be achieved by determining the source and impact of fresh fish temperature fluctuations during chilled distribution, with emphasis on the quality of fish when storage exceeds ≥ 5 °C.

Differences in characteristics between flat and round fish will also be compared to determine such impacts on spoilage rates and general quality based on size and environmental factors among species.

Furthermore, the data obtained will be analysed to distinguish quality parameters of fish under ideal/non-ideal storage conditions and the effect these parameters have on the shelf life of the fish throughout the cold chain management system.

Regarding the seafood industry in Ireland, research results may encourage seafood processors to monitor more closely when exporting seafood, thus allowing them to keep the best quality of their product and possibly achieve higher profitability.

2 MATERIALS AND METHODS

2.1 Materials and reagents

Chemicals were obtained from Sigma Aldrich Ireland Ltd., Arklow, Co. Wicklow, Ireland and Lennox, Dublin, Ireland. All reagents were of analytical grade. These reagents included methyl red indicator, bromocresol green, boric acid, hydrochloric acid, silicone anti-foam tablets, magnesium oxide and formaldehyde.

2.2 Raw material processing and sampling

Sampling took place over an 8-month period from February to September (2018), during the conventional megrim and pollack season in Ireland (European Comission, 2001). The fish samples were landed and acquired from a local fish retailer in West Cork, Co. Cork, Ireland, and from a local fish retailer in Waterford city, Co. Waterford, Ireland. Fish were obtained from the North East Atlantic Ocean – FAO 27/ VI (a-b) and VII (a-k) (Figure 2.1). Fish spent between zero and three days in a temperature-controlled holding on ice before landing in port. Samples obtained from West Cork were transported to the Seafood Innovation Hub (SIH) in Clonakilty, Co. Cork, Ireland for further processing. Transporting the whole fresh fish took approximately one and a half hours in a temperature-controlled truck kept between 0 and 2 °C. The fish samples were retrieved whole and then gutted to minimise contamination prior to experimentation. Each fish box contained 10 whole megrim and 10 whole pollack on ice for each delivery. Samples obtained from the Waterford fish monger were landed on the day of purchase, collected and transported back to Waterford Institute of Technology laboratories in a secure iced polybox containing LogTag temperature recorders. The transit took approximately 10 minutes from the fish retailer to the campus and the fish samples maintained a temperature of 2 °C ± 0.6 °C.

Upon delivery the fish samples were measured individually for weight (megrim= 0.43 ± 0.05 kg and pollack= 4.9 ± 0.52 kg) and for total length (megrim = 26.89 ± 2.31 cm and pollack = 39.8 ± 0.50 cm). Filleting and trimming were done by hand in an air-controlled production hall (4 °C), by removing head, bones and fins from the fillet surface. The fillet sides were kept raw and stored at -0.1 to 2 °C until required for analyses.

For process treatments, the results obtained from temperature sensors (LogTag Trix-8 multi-use temperature data recorder) during the exportation trials were replicated in the laboratory using a calibrated oven (Carbolite LHT 4/30 high temperature bench top laboratory oven). Whole fish samples on ice (mimicking on-board conditions) were stressed at 5 °C, 8 °C, 15 °C and 22 °C for 2.5 h prior to testing, to determine changes in fish quality and microbial growth amongst both species.

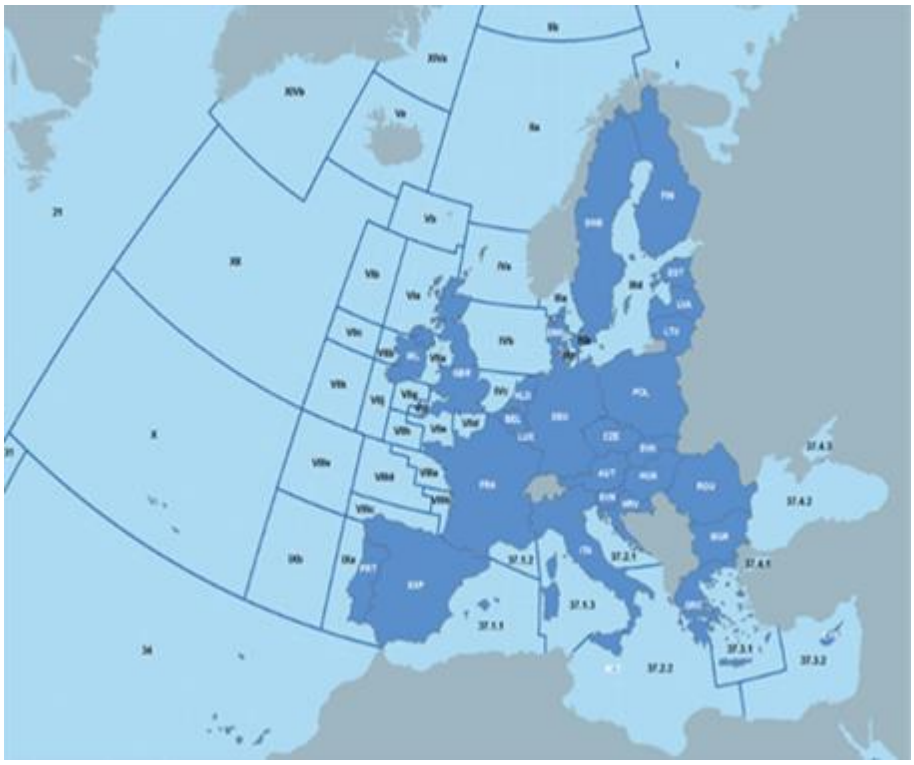

Figure 2.1: The boundaries of the Atlantic, Northeast (FAO 27 / VI (a-b) & VII (a-k)) corresponding to the ICES fishing areas for statistical purposes (FAO, 2017).

2.3 Temperature Analysis

Numbered 'L' descriptors were allocated to each Logtag dependent on its location among the fish samples. L1 was located at the top of polyethylene boxes containing iced chilled fish, L2 was in the middle of the boxes between the fish and L3 was at the bottom of the fish boxes. The temperature within the boxes containing the fish were recorded and a complete representation of the air temperature of each box was determined. The results obtained during the temperature trials were the averages of L1, L2, and L3 representing

the area in which the tags were placed, which were then graphed to determine when and where breaches occurred.

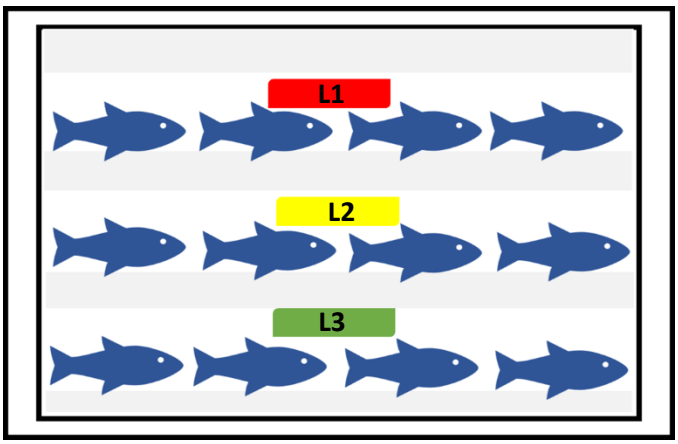

Figure 2.2: Colour coded temperature logger distribution within a polyethylene box containing fish and ice.

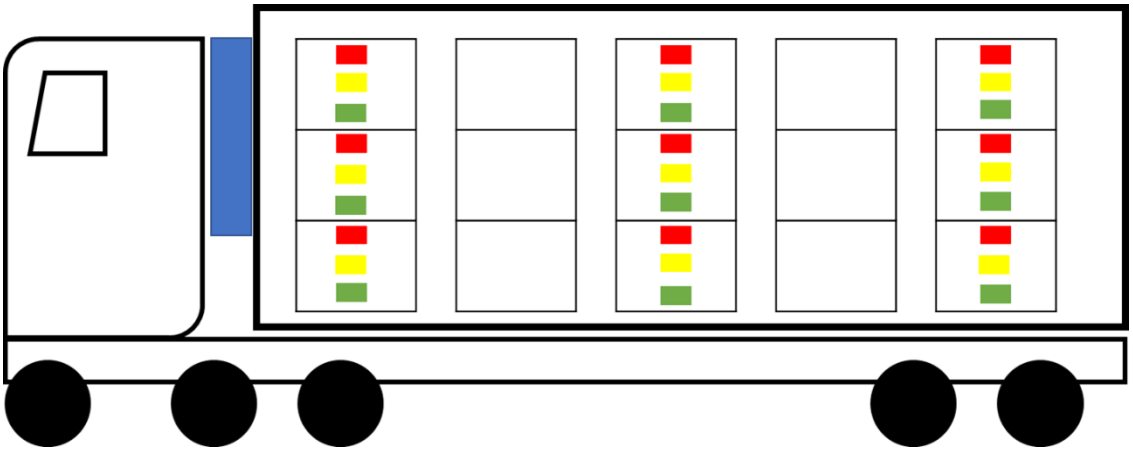

Figure 2.3: Experimental side view setup of the temperature loggers in a distribution truck.

Temperature data loggers (LogTag Trix-8 multi-use temperature data recorder) were used to monitor the temperature of exported iced crates containing whole fresh chilled megrim and pollack to France and Spain from West Cork, Ireland. Both fish species were packed on ice in polyethylene containers on-board refrigerated distribution trucks (see Figure 2.2). Throughout the cargo, the vacuum packed Logtags were placed in the front, middle and rear of the distribution trucks among the fish using colour coding zip ties to distinguish between the shipped containers, i.e. 9 Logtags per location, with 3 Logtags placed in the top, middle and bottom of each container (see Figure 2.3). The data loggers

were configured to start temperature recording once the shipment left for export and ended when the shipment arrived at its allotted destination. Once the data loggers were retrieved, the data was collected using analyser software (LogTag® Analyzer 3 software). The data retrieved from the Logtags were correlated with stoppage times, defrost cycles and the opening and closing of reefer doors to determine why, when and for what period of time, temperature fluctuations occurred.

A second temperature trial was conducted by placing temperature monitoring LogTags amongst the fish being delivered from the producer to the SIH. LogTags were packed within the iced polyboxes, to monitor temperature fluctuations during delivery. The data was analysed once retrieved and fluctuations identified.

A third temperature trial was conducted within the SIH to determine whether fish samples were affected during chilled storage when the chill door was opened and closed. LogTags were placed in polyboxes containing chilled and iced whole fish within the walk-in chill room. The temperature was recorded over a 5-day period, during which time the chill door was opened and closed for varied periods of time.

A fourth temperature trial was conducted within the SIH to determine the effect outside ambient temperatures have on chilled whole fish in iced polyboxes. Polyboxes containing iced whole fish were kept at temperatures which emulated outside temperatures, using an air-controlled production hall for 24 h on different days of shelf life. This method was used to mirror the effect of outside ambient temperatures on polyboxes left on the slip when landed after exportation. Samples were tested over a 24 h period as fish can be loaded and unloaded at any time. Results were analysed, and the fluctuations were identified.

The data logger trials indicated temperature variations during storage. These variations were replicated under controlled laboratory conditions to allow further testing.

2.3 Laboratory analysis

Analysis was conducted on both species of fish in triplicate across all process treatments over a 16-day storage period. This was achieved by using a suite of quality tests, including

nutritional, microbiological, textural, colour, organoleptic and spoilage amines (total volatile base nitrogen) determination to validate the cold chain management system.

Following temperature treatments, whole gutted fish were filleted and cut into suitable sizes for examination.

2.4 Proximate Composition

Both species were stored in chilled refrigeration (0 to 2 °C) in polyethylene boxes layered with ice over a 16-day storage period prior to testing. Samples T2 to T5 were temperature stressed for 2.5 h prior to testing.

Table 2.1: Temperature treatments of samples during shelf life and quality analysis testing.

Treatment	
T1	Control Samples stored between 0 to 2 °C
T2	Stressed at 5 °C for 2.5 h prior to testing
T3	Stressed at 8 °C for 2.5 h prior to testing
T4	Stressed at 15 °C for 2.5 h prior to testing
T5	Stressed at 22 °C for 2.5 h prior to testing

Moisture content of the fish samples across all process treatments were determined by the oven method (IAFMM, 1979). Fish samples (4 g) were dried at 103 °C until a constant weight was achieved. The moisture content was calculated as a percentage of the original samples weight.

Equation 2.1: Moisture content in fish muscle;

$$\%\text{Moisture} = \frac{W2 - W3}{W2 - W1} \times 100$$

where,

W1 = weight of container with lid;

W2 = weight of container with lid and sample before drying;

W3 = weight of container with lid and sample after drying.

Protein was determined using the Kjeldahl method. The protein determination method incorporates digestion, distillation and titration (Suhre, Corrao, Glover, & Malanoski, 1982). The protein content was determined by taking 2 g of minced fish sample, placed into digestion tubes with kjeltabs (2), adding 15 ml sulphuric acid and 10 ml of hydrogen peroxide. The tubes containing the samples were placed on a preheated digestion block (410 °C) until samples appeared clear and colourless. When the digested samples were cooled, 50 ml of water was added. The tubes were placed in a preheated distillation unit. The sample were distilled for five minutes with the addition of 7 ml sodium hydroxide. The distillate was retrieved in a conical flask containing 4% boric acid with a mixed indicator of bromocresol green (1%) and methyl red (0.7%). The distillate retrieved was titrated against 0.1 N hydrochloric acid.

Equation 2.2: Protein content in fish muscle;

$$\%\text{Protein} = \frac{(\text{Sample titration} - \text{blank titration}) \text{x } 0.0014 \text{x} 100}{\text{Sample weight (g)}} \text{ x } 6.25$$

The fat content was obtained through Low Field Nuclear Magnetic Resonance (LFNMR) spectrometry using a SMART Trac system (CEM GmbH, Kamp-Linfort, Germany). Traditional LFNMR can differentiate between fat and water (Métais & Mariette, 2003).

Ash was determined by placing 5 g of minced weighed fish sample into a crucible. The crucible containing the sample was put into a muffle furnace (500 °C) until the contents turned light grey to white (12 h). Once the crucible had cooled in a desiccator, the remaining ash content was reweighed to determine the final ash weight.

Equation 2.3: Ash content in fish muscle;

$$\%\ \text{Ash} = \frac{\text{Weight of ash x} 100}{\text{Sample weight (g)}} \qquad \text{(AOAC, 1996)}$$

The carbohydrate levels for both species was calculated by difference. Once a sample is measured for moisture, protein, fat and ash, they are subtracted from the initial total weight. The resulting value will be the sample's approximate carbohydrate value. (FAO, 2001).

2.5 Microbiological Analysis

The International Commission on Microbial Specifications for Foods (ICMSF, 1986) established a mesophilic aerobic count limit of 7.01 log cfu/ g for fish suitable for human consumption.

Micro analysis was conducted over a 16-day chilled storage period for both fish species. As shown in Table 2.1, five distinct temperature ranges (T1 to T5) were selected based on the results obtained during the temperature analysis trials. Temperatures were chosen to investigate how minor and more severe temperature fluctuations can affect the microbial load in fresh fish. T1 remained as the control throughout with samples stored at 0 to 2 °C, for the stressed temperature ranges (T2 to T5), samples were held at the higher temperatures for 2.5 h prior to testing.

Weighed minced fish samples of 10 g were homogenised with 90 ml of Ringer's solution for three minutes (Steward Stomacher 400 Lab Blender, London, UK) and 10^{-1} dilutions were used for analysis for all process treatments. Aliquots of 0.1 ml were prepared for each dilution from the prepared serial dilutions, which were plated on standard plate count agar (PCA). Mesophilic counts were calculated by incubating the plates for 48 h at 30 °C. The results for the megrim and pollack fillets were expressed as $\log_{10}$cfu (colony forming units)/ g.

2.6 Colour Analysis

The colour of the fish samples from the three process treatments were measured using a Chroma Meter CR-400 (Minolta Co. Ltd, Japan) as depicted in Figure 2.4. Colour was calculated as L* (lightness), a* (redness) and b* (yellowness) using the CIE LAB scale. The Chroma Meter was calibrated with a standard white plate: L = 96.86, a = -0.02, b = 1.99, respectively. Measurements were conducted in triplicate on fish samples from each process treatment. The values were the resulting averages of these measurements.

Equation 2.4: The total colour difference (Francis & Clydesdale, 1975):

$$\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{0.5}$$

Equation 2.5: The whiteness (Park & Lin, 2005):

$$\text{Whiteness} = 100 - [(100 - L^*)^2 + a^{*2} + b^{*2}]^{1/2}$$

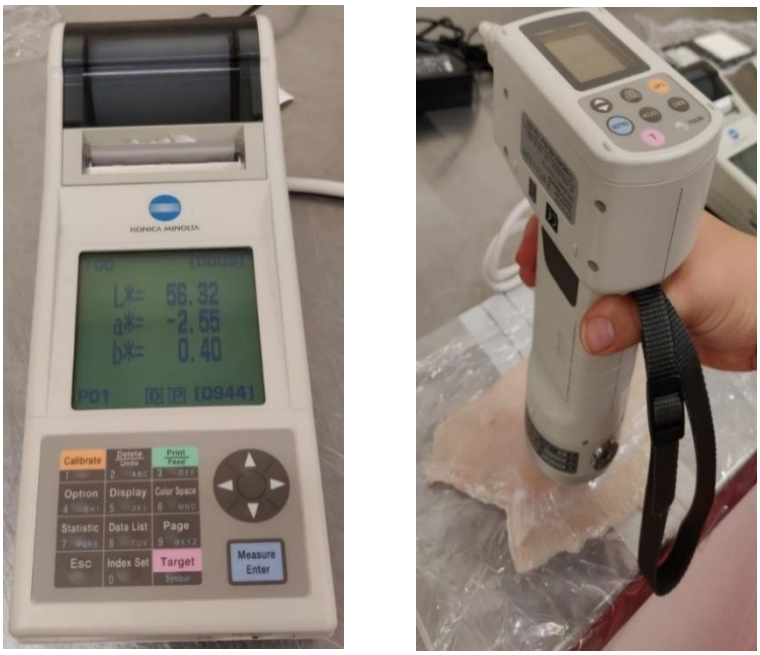

Figure 2.4: Colour analysis of megrim fillets using a Chroma Meter CR-400.

2.7 Texture Analysis

A TA.XT2i Texture analyser (Figure 2.5) (TA.XT2i Texture Analyser, Stable Micro Systems, UK) was used to carry out Texture Profile Analysis (TPA) on megrim and pollack fillets according to the method of Sigurgisladottir, *et al*. (1999). The force-time deformation curves were obtained using a 5 kg load cell with a 3 mm /s cross-head speed and a 25 mm diameter flat ended perspex cylinder probe. Samples underwent a double compression of 20% of the sample's height. The initial compression elapsed for five seconds before making the second compression (two-bite compression). All samples were tested in triplicate.

Figure 2.5: Textural analysis of pollack fillets using a TA.XT2i Texture analyser.

2.8 Total volatile base nitrogen (TVB-N)

The total volatile basic nitrogen in megrim and pollack samples were determined using a method described by Antonscopoulos & Vyncke (1989). A minced fish sample of 5.0 ± 0.2 g and 100 ml of distilled water was homogenised (Neutec 9000471 homogeniser, Neutec Group Inc., NY, USA) for two minutes and filtered through Whatman No.1 filter paper. The extract was placed in a distillation tube with 3 g ± 0.1 g of magnesium oxide and silicone antifoam. The extract was distilled for approximately four minutes in a preheated distillation unit (BÜCHI, model k-350, Switzerland). Approximately 150 ml of distillate was received into a conical flask containing 80 ml of 2% boric acid and four drops of Tashiro mixed indicator (bromocresol green and methyl red indicator). The volatile bases within the boric acid solution were determined by titration against 0.1 N HCl acid.

Equation 2.6: Total Volatile Base Nitrogen (TVBN/100 g);

$$\text{mg TVB-N} = \frac{(\text{Sample titration} - \text{blank titration}) \text{x } 0.1 \text{x} 14.008 \text{x} 100}{\text{Sample weight (g)}}$$

Due to weight variations during the megrim selection phase, two sizes were chosen for testing (725.11 ± 67.77 g for larger specimen and 130.11 ± 19.10 g for smaller specimen). All pollack catches were selected at a standard weight of 4.9 ± 0.52 kg. The rate at which a fish spoils can differ between fish, as fish with a fatty composition spoil quicker than leaner fish, larger sized fish remain fresher for longer compared to smaller fish even of the same species, cold water fish tend to spoil quicker than warm water fish and flat fish deteriorate more rapidly than round fish (Abbas, Saleh, Mohamed, & Lasekan, 2009).

2.9 Sensory Analysis

Sensory analysis using megrim and pollack samples were carried out at different stages of shelf life during fresh chilled storage from 2 to 13 days. The samples were steam cooked in a convection oven at 100 °C until an internal temperature of 75 °C was achieved. A semi-trained panel (n=10) taste tested samples in a standard sensory panel room. Samples were coded with a random three-digit identification number. Panellists were asked to score the acceptability of each sample on a 9 cm quantitative response scale from 1 (Extremely Poor) to 9 (Extremely Good) using the following sensory analysis descriptions: appearance/colour, aroma, overall taste/flavour, texture, overall acceptability. The values were averaged and acceptability during shelf life was determined.

2.10 Statistical Methods

All statistical analysis for the study was completed in MINITAB 18.1 (Minitab, Inc., 2010). Triplicate subsamples were measured for each treatment and all the analyses were carried out in duplicate. The data were assessed using one-way analysis of variance (ANOVA), with a post hoc Tukey test when significance was recognised. The data is shown as the mean value ± the standard deviation. Statistical significance level was set at $P < 0.05$.

3 RESULTS AND DISCUSSION

3.1 Temperature Analysis

3.1.1 Trial 1. Exportation to France and Spain

During exportation to France temperature spikes were apparent every 6 to 8 h, with a recovery period of approximately 3 h until unloading occurred (hour 32 to 36) during which time temperatures continuously increased above 5 °C without any significant recovery (Figure 3.1 and 3.2).

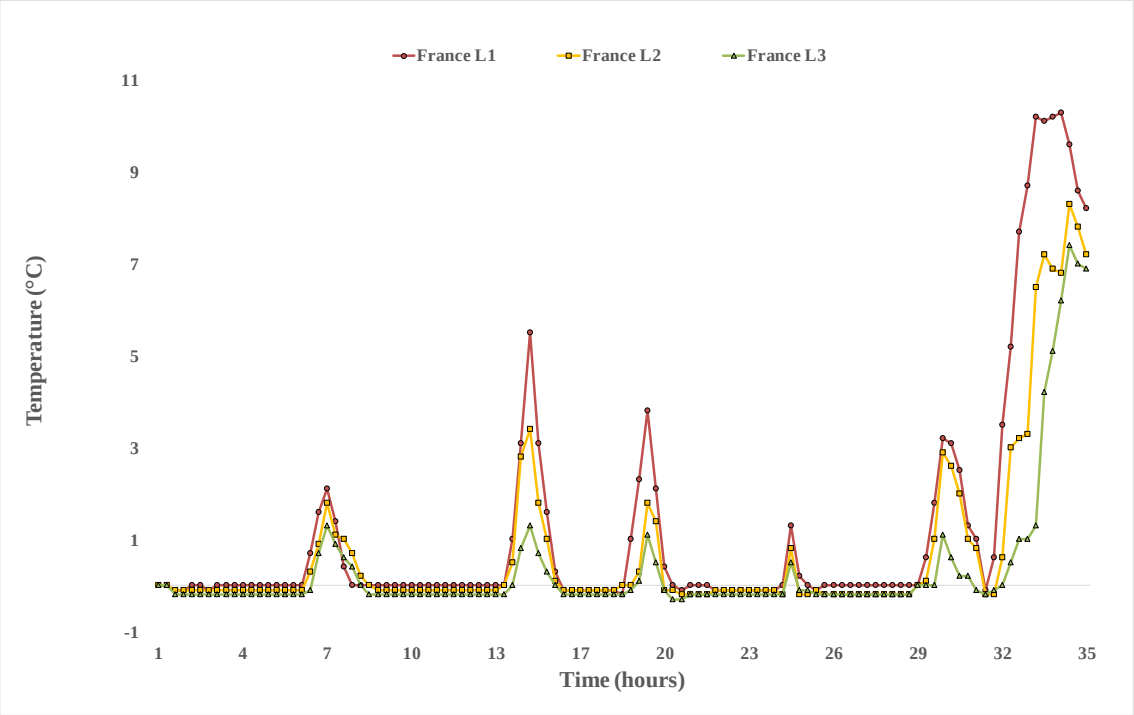

Figure 3.1: Export France – whole megrim temperature profile.

The average temperature in polyethylene boxes containing chilled megrim was 0.8 °C during its 36 h exportation to France (Figure 3.1). This was similar to the boxes containing chilled pollack samples that had an average of 0.4 °C (Figure 3.2). In the first significant breach, the boxes containing megrim had risen above its storage specifications to 5.5 °C during its 14 h of exportation. Temperatures varied from 8.2 to 10.3 °C in L1, 6.8 to 7.2 °C in L2 and 6.2 to 6.9 °C in L3 during the unloading period. Similarly, with the pollack samples, during hour 33 to 35 of unloading, temperatures began to rise rapidly from 0.9 to 9.9 °C in L1, 0.4 to 9.1 °C in L2 and 0.1 to 8.5 °C in L3.

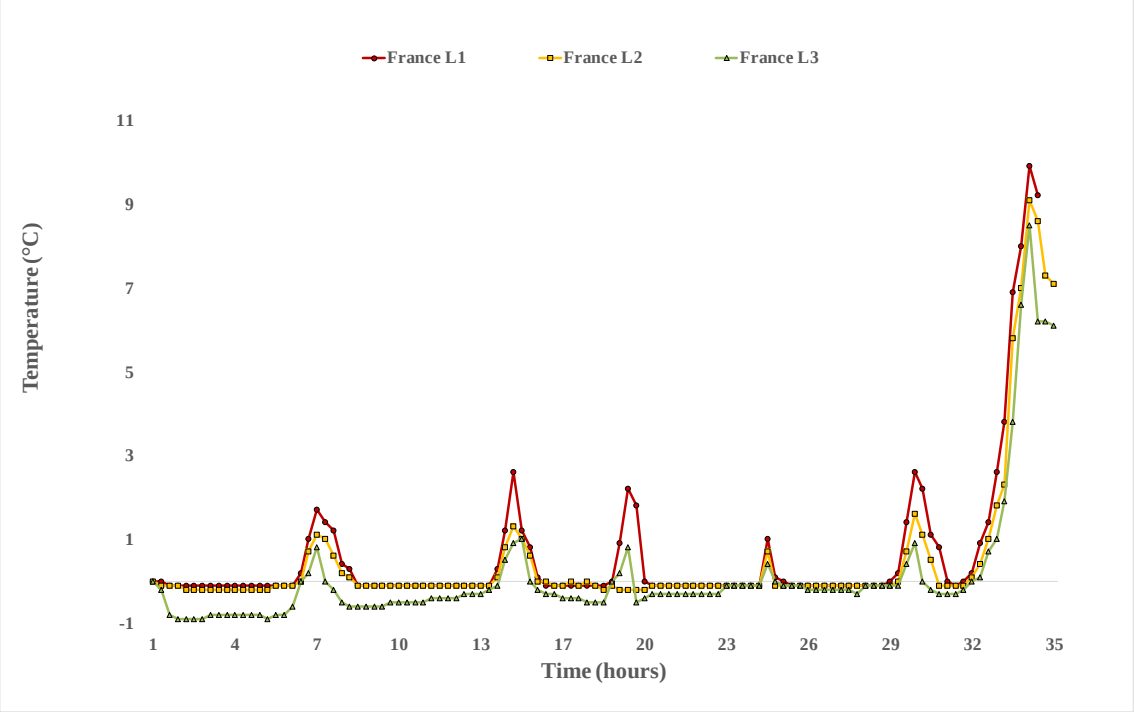

Figure 3.2: Export France – whole pollack temperature profile.

Table 3.1: Time in hours Logtag sensors recorded temperatures above the recommended temperature (0 to 2 ˚C) during a 36 h megrim export to France.

Above 2 ˚C		Above 5 ˚C	
L1	2.4	L1	3.4
L2	0.2	L2	2.2
L3	0.2	L3	1.4

As shown in Table 3.1, Logtag sensors that were less insulated and closer to the lid of the container (L1) were more susceptible to outside temperatures. This resulted in prolonged temperature fluctuations which additionally began to affect the temperature sensors in the centre (L2) and bottom (L3) of the containers containing fresh fish. Temperature sensors from L1 were out of specification for a total of 2.4 h between 2 to 5 ˚C and a total of 3.4 h above 5 ˚C in Table 3.1.

Table 3.2: Time in hours Logtag sensors recorded temperatures above the recommended temperature (0 to 2 ˚C) during a 36 h pollack export to France.

Above 2 ˚C		Above 5 ˚C	
L1	2.4	L1	2.0
L2	2.0	L2	2.0
L3	0.2	L3	1.4

A similar trend to Table 3.1 was observed with pollack samples in Table 3.2. Temperatures were significantly raised above the recommended threshold, with both L1 and L2 above 5 ˚C for 2 h and L3 above 5 ˚C for 1.4 h.

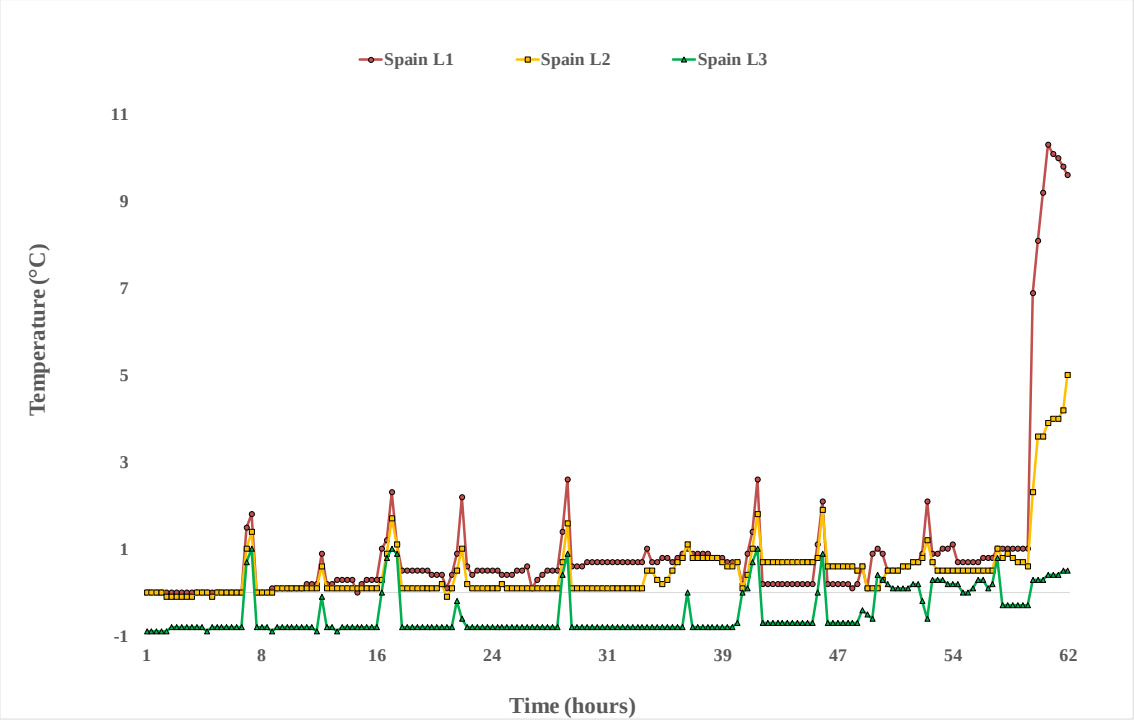

Figure 3.3: Export Spain – whole megrim temperature profile.

During the 62 h export of chilled megrim to Spain the average temperature recorded in polyethylene boxes was 0.7 °C (Figure 3.3). This was similar to that found in pollack samples having an average of 0.3 °C (Figure 3.4). Within the first significant breach megrim samples rose above its storage specifications (0 to 2 °C) to 5.7 °C in its 17 h of exportation. The second breach occurred during the 60 h of exportation, temperatures began to rise from 2.2 to 11.1 °C in L1 and 1.1 to 7.2 °C in L2. However, during this time period L3 were relatively unaffected as temperatures only rose 0.9 °C. The third breach encountered was between hours 60 and 62. Temperatures increased to 11.4 °C in L1, 11.1 °C in L2 and 5.3 °C in L3. These findings were unlike those in pollack samples which had minor fluctuations in temperature during export from 0 to 60 h, which remained acceptable as temperatures did not exceed 2.6 °C. The first major breach however did occur between the 60 to 62 h, where temperatures rose to 9.6 °C in L1, 5 °C in L2 and 0.5 °C in L3.

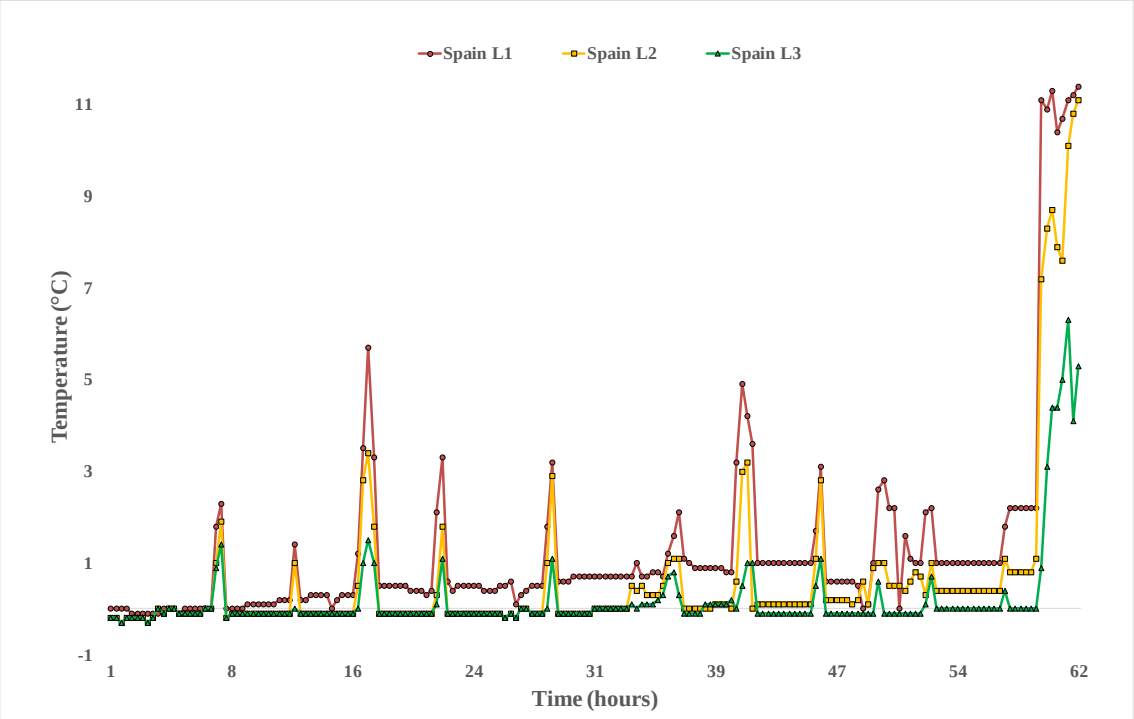

Figure 3.4: Export Spain – whole pollack temperature profile.

A potential cause of the undesirable temperature fluctuations can be due to off cycles of the refrigeration unit of the truck, as during certain time periods the trucks were being defrosted. All distribution trucks were equipped with a Hubbard 720 Alpha transport refrigeration unit (Hubbard Products Ltd, Ipswich), which had a fixed defrost cycle every 6 to 8 h. This coincided with temperature fluctuations in both export trials to France and Spain as seen in Figures 3.1 to 3.4. The temperature fluctuations also occurred during the unloading period, when boxes were being transferred into the auction warehouse. The Spanish cargo had higher temperature fluctuations in comparison with the French cargo. This could be a result from longer exportation time, higher outside temperature, frequent breaks by the driver and more defrost cycles.

Table 3.3: Time in hours Logtag sensors recorded temperatures above the recommended temperature (0 to 2 ˚C) during a 62 h megrim export to Spain.

Above 2 ˚C		Above 5 ˚C	
L1	4.4	L1	2.4
L2	2.4	L2	0
L3	0	L3	0

Table 3.3 displayed temperature fluctuations during exportation to Spain. L1 and L2 were affected above 2 to 5 ˚C. Only L1 was affected above 5 ˚C for 2.4 h during the exportation.

Table 3.4: Time in hours Logtag sensors recorded temperatures above the recommended temperature (0 to 2 ˚C) during a 62 h pollack export to Spain.

Above 2 ˚C		Above 5 ˚C	
L1	8.0	L1	3.0
L2	2.2	L2	2.4
L3	1.2	L3	0.4

For temperatures between 2 and 5 ˚C, Table 3.4 had the largest temperature variations totalling 8 h in L1. Consequently, L2 and L3 were impacted for 2.2 and 1.2 h respectively. Fluctuations above 5 ˚C also occurred, with L1 being out of specification for 3 h, L2 for 2.4 h and L3 for 0.4 h.

Of all the temperature variations listed above 5 ˚C, Table 3.1 had the most significant fluctuations. As shown in Table 3.1, L1 remained out of specification for 3.4 h, L2 for 2.2 h and L3 for 1.4 h.

3.1.2 Trial 2. Domestic Analysis

Temperature was monitored at 20 minute intervals over a 4 h period during domestic distribution of whole chilled fish in a medium sized refrigerated van using a XARIOS 150 transport refrigeration unit (TSS Ltd.). Temperatures did not exceed 2.5 °C (Figure 3.5). The temperature peaks in L1 correlated with delivery stops. This indicated that the temperature of the fish on the top of the boxes was affected by the opening and closing of the truck doors. However, fish in the middle and bottom compartment of the boxes maintained a relatively constant temperature of 0.2 °C for L2 and 0 °C for L3, remaining in the recommended chilled storage range of 0 to 2 °C for seafood.

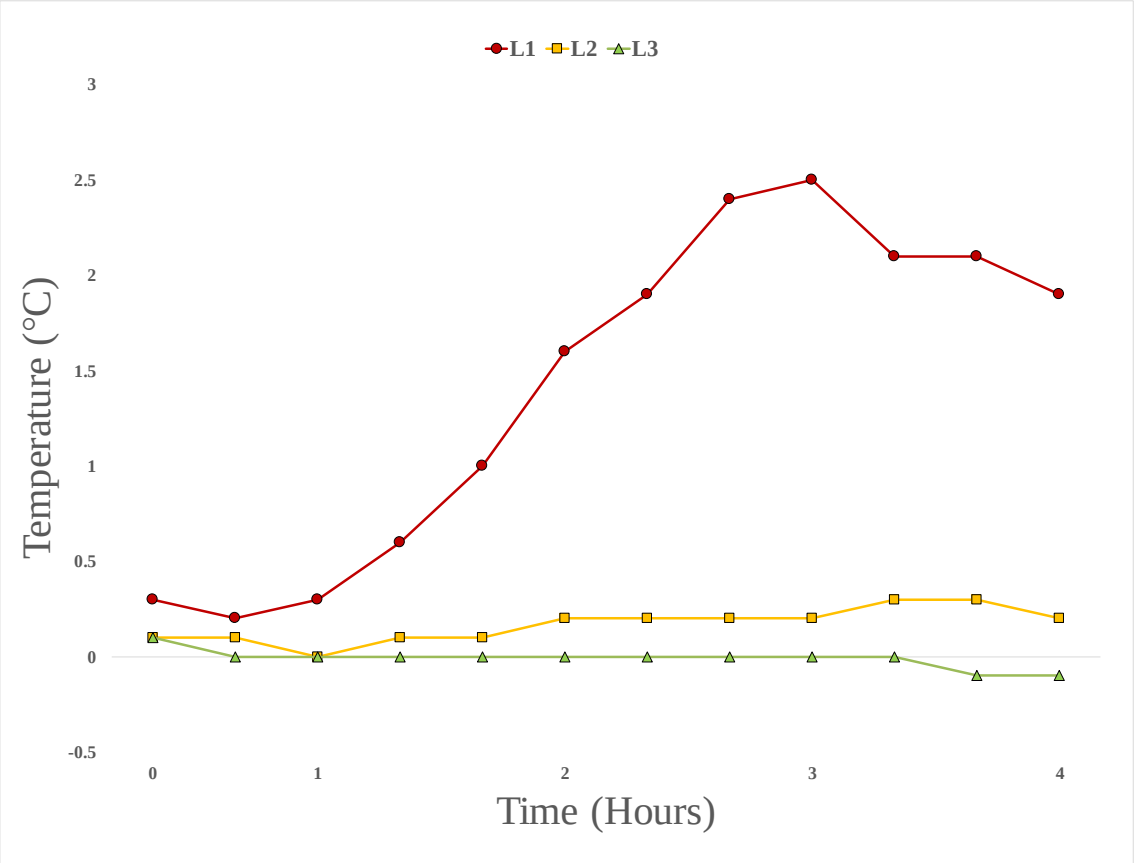

Figure 3.5: Domestic distribution – Whole megrim and pollack temperature profile.

3.1.3 Trial 3. Chill Storage

Temperature data points were recorded every 10 minutes, then averaged after 6 h intervals for 4 days using whole fish during chilled storage (Figure 3.6). Over the 4 day storage period for L2s and L3s, temperatures exceeded 0 to 2 °C recommended for the whole trial but remained below 4 °C. Temperature peaks in L1s on day 3 were noted to exceed 5 °C and reached 5.9 °C by day 4 in tags that were closer to the surface of the box with less of a barrier between that of the outside temperature. After each temperature peak in L1 tags the temperature decreased again to below 4 °C. Although the temperature of L2 and L3 Logtags were higher than the recommended (0 to 2 °C), they did not exceed 5 °C and stayed somewhat constant throughout the 4-day storage period. The temperature peaks in L1 Logtags correlated with the opening and closing times of the walk-in chill door during refrigeration.

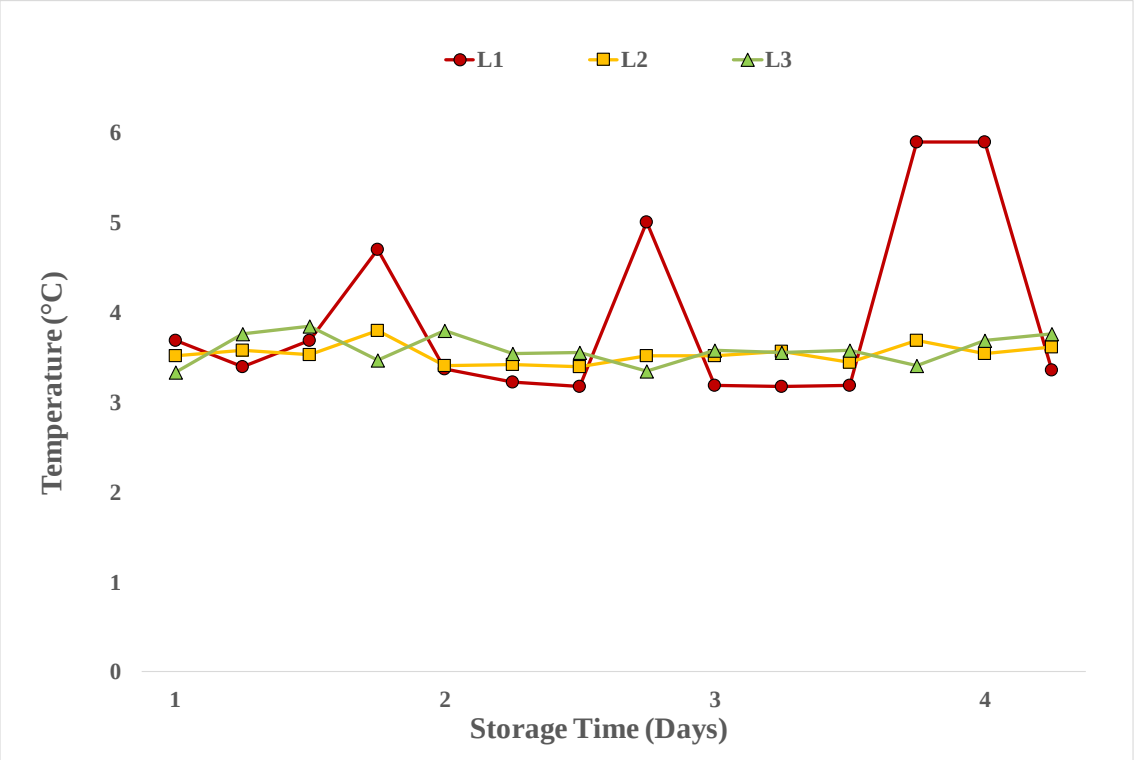

Figure 3.6: Chilled storage – Whole megrim and pollack temperature profile.

3.1.4 Trial 4. Ambient Temperatures

Iced polyboxes containing whole fish at different stages of shelf life were held at temperatures to emulate the more severe temperatures that can be encountered when products are left at ambient temperatures in areas such as unloading docks or placed in insufficient refrigerated storage. The outside air temperature at its highest was 18 °C on the 29/08/18 at 4 pm GMT in West Cork. There was a total of 13.5-day light hours on said day. An air-controlled production hall was used to emulate the outside temperatures of said day, over a 24 h period using an adjustable thermostat. The Logtags were placed on the top (L1), middle (L2) and bottom (L3) of the polyboxes and temperatures were taken at 20-minute intervals.

Temperatures were recorded at their peak between 16.30 and 17.30 pm, when the ambient temperature was at its highest (as per previous paragraph). The Logtags on top of the polyboxes (L1) recorded temperatures at 10.3 °C (Figure 3.7) during this time. The Logtags placed in the middle among the chilled fish recorded temperatures up to 8.1 °C (L2) and temperature at the bottom of the polyboxes (L3) went from 0.1 to 4.1 °C. The high temperature peaks correlated with the changing ambient temperature throughout the 24 h time period. When outside temperatures were below 10 °C, temperatures within the polyboxes containing fish remained below 5 °C and within legal refrigeration limits, however still above the recommended limit for seafood of 0 to 2 °C.

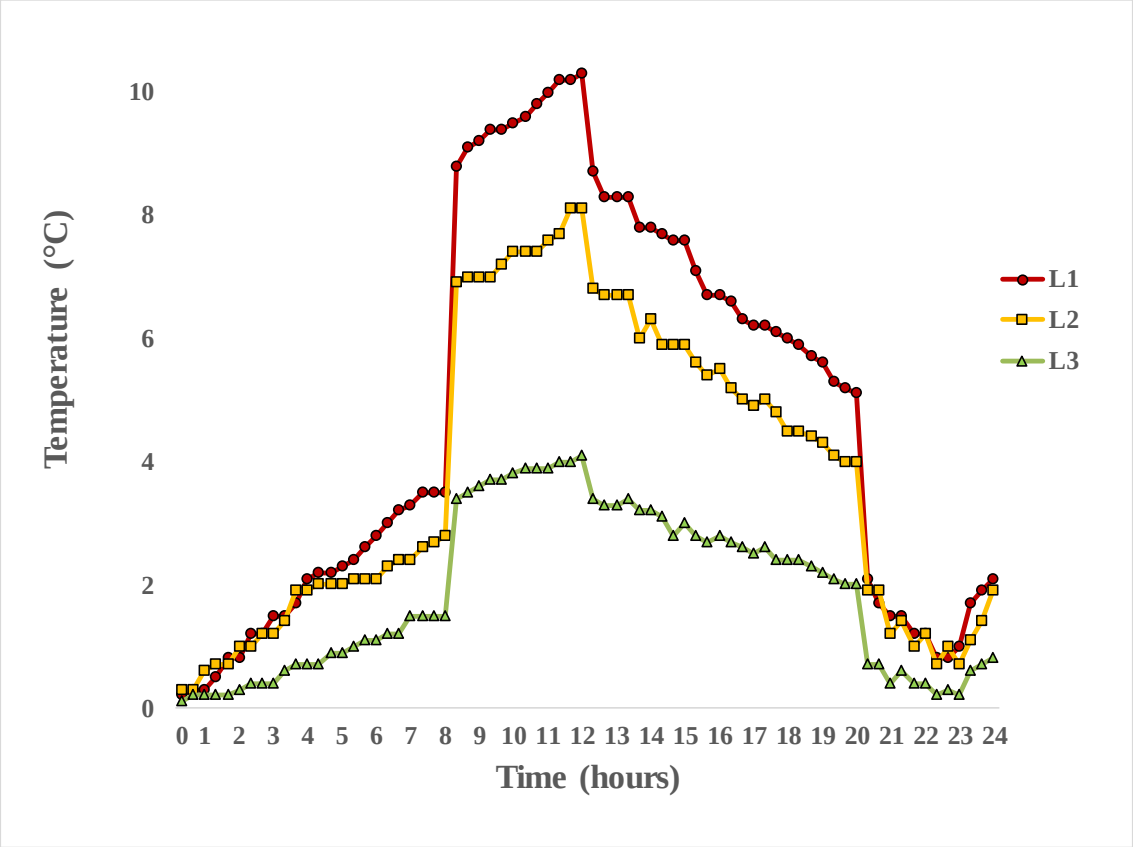

Figure 3.7: Ambient Temperature – Whole megrim and pollack temperature profile.

3.1.5 Chapter summary

Both fish species experienced temperature breaches during the export trials to France and Spain (Figures 3.1 to 3.4 respectively). In an ideal scenario the cargo would remain at a constant control temperature of 0 to 2 °C throughout the entire distribution process, therefore anything above this is considered a significant temperature breach. Ice was uniformly spread throughout the polyboxes between each layer of fish. The rationale behind the temperature effect on each layer is due to the proximity to the outside temperature and the quality of insulation between each layer. As L3s were on the bottom of polyboxes, it not only had a layer of ice below the fish, it also had the impact chill from the ice above on the other layers. L2s were compacted between the ice layers, while L1s had the ice layers below but only one layer of ice above and between the polybox cover.

The breaches ranged from a minimum of ≤ 5 °C to the more severe of 11.4 °C in L1, as shown in Figure 3.3 for megrim exported to Spain. When further investigated the temperature breaches coincided with the defrost cycles (every 6 to 8 h) and during unloading periods. Other fluctuations can be attributed to breaks when the driver opened the reefer doors to check on the cargo, which was verified with the production company. It took approximately 1 to 3 h for the cargo to recover fully to acceptable temperatures of 0 to 2 °C after each temperature breach. During the second trial the opening and closing of reefer doors during distribution had an effect on the temperature of the fish on top (L1) of the polyboxes closest to the van door (Figure 3.5). However, fish in the middle (L2) and bottom (L3) compartments remained relatively unaffected. Similar to the other trials, L1s were the only Logtags significantly affected by the opening and closing of doors in chilled storage (Figure 3.6). This further indicated that the fish on top of the polyboxes were most affected by the outside ambient temperatures during each temperature analysis trial. Trial 4 (Figure 3.7) was carried out to emulate the more severe temperature breaches that may be experienced during unloading or when products are placed in insufficient refrigerated storage. This demonstrated how prolonged exposure to outside ambient temperatures can have a significant impact on the temperature of the fish within the iced containers. The trial displayed similarities to the other trials where the fish closest to the top (L1) of the polyboxes were less insulated and were first susceptible to temperature fluctuations.

Temperatures and exposure times were chosen to reflect the breaches that occurred during distribution for conducting further laboratory analysis. Based on the results obtained during the temperature analysis trials, temperatures were chosen ranging from a moderate 5 °C to a more extreme 22 °C to show the minor and extreme effects that limited temperature exposure can have on the fish quality. An exposure time of 2.5 h was chosen as it took 1 to 3 h for temperature to stabilise after heat exposure occurred.

3.2 Shelf Life and Quality Analysis

The temperature fluctuations obtained above 5 °C were replicated in the laboratory to determine any significant deterioration in shelf life and quality. Fish samples were tested to determine the changes in fish quality and microbial growth amongst both species, by mirroring temperature fluctuations which occurred during trials. As shown in Table 2.1, five distinct temperature ranges were chosen to investigate the impact that minor and more significant temperature fluctuations might have on the shelf life and quality of fresh fish.

3.1.1 Nutritional Analysis

3.1.1.1 Proximate composition of Megrim

Megrim stored at 0 to 2 °C without temperature stressing (T1) had moisture levels that ranged from 79.8 to 86.1 % over the 16-day storage period (Figure 3.8). These levels were comparable to previously reported values for megrim of 80% (Barbosa, Trigo, & Prego, The chemical composition of different edible locations (central and edge muscles) of flat fish (Lepidorhombus whiffiagonis), 2017). Samples that were temperature stressed at 15 °C and 22 °C (T4 and T5) showed a significant decrease in moisture content during the storage period ($P < 0.05$) (Figure 3.8). The protein levels in megrim kept refrigerated at 0 to 2 °C (T1) ranged from 14.20 to 17.77% as shown in Figure 3.9, which were marginally lower than previously reported in studies on megrim (17.8%) (FAO, 2001). This was the same for the samples T2 and T3 which ranged from 14.2 to 17.8 for T2 and 14.4 to 17.6 for T3 respectively. There was no significant change in protein across all temperature treatments. The fat levels for all samples were slightly lower (0.2 to 2.7 %, see Figure 3.10) than those reported of between 1.0 and 3.9 % by the FAO (2001) and were also lower than previously reported levels of 3.9 % by Careche and Tejada (1991). Carbohydrate concentrations were calculated by difference throughout all treatments and were negligible. Carbohydrate levels are usually too small to be of any significance in the proximate composition of whitefish; hence no values are shown. Results indicated that for the megrim samples, both temperature and duration had no significant effect on ash measurements (Figure 3.11). Results ranged between 0.17 and 2.20 throughout all treatments.

Reasons for the lower levels of moisture, protein and ash may be attributed to some samples being caught during spawning season (January to April) therefore a slight depletion in those levels can be expected. However, some of the lower levels could also indicate leaching of protein and minerals through moisture as the samples dried out in T4 and T5 (Fagbenro & Jauncey, 1995). The lower levels of fat could be due to spawning. Prior to spawning the female fish will not eat, therefore once the carbohydrates are depleted, the fish's body will then start to break down fats and proteins for energy resources. Therefore, the proximate composition can vary from the reported studies conducted by the FAO (2001).

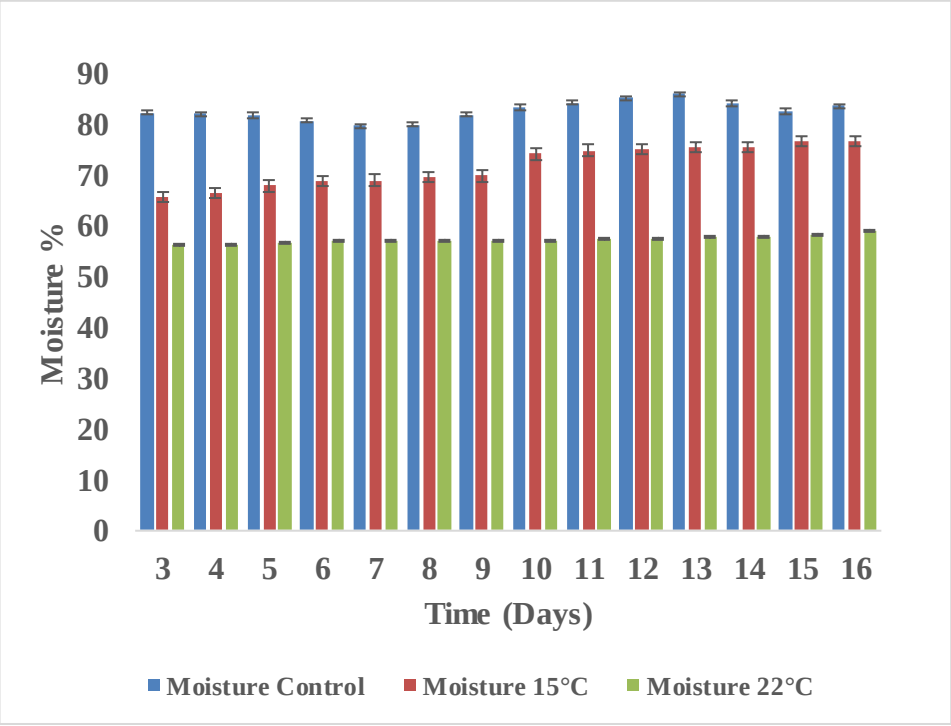

Figure 3.8: Moisture % for megrim temperature profile.

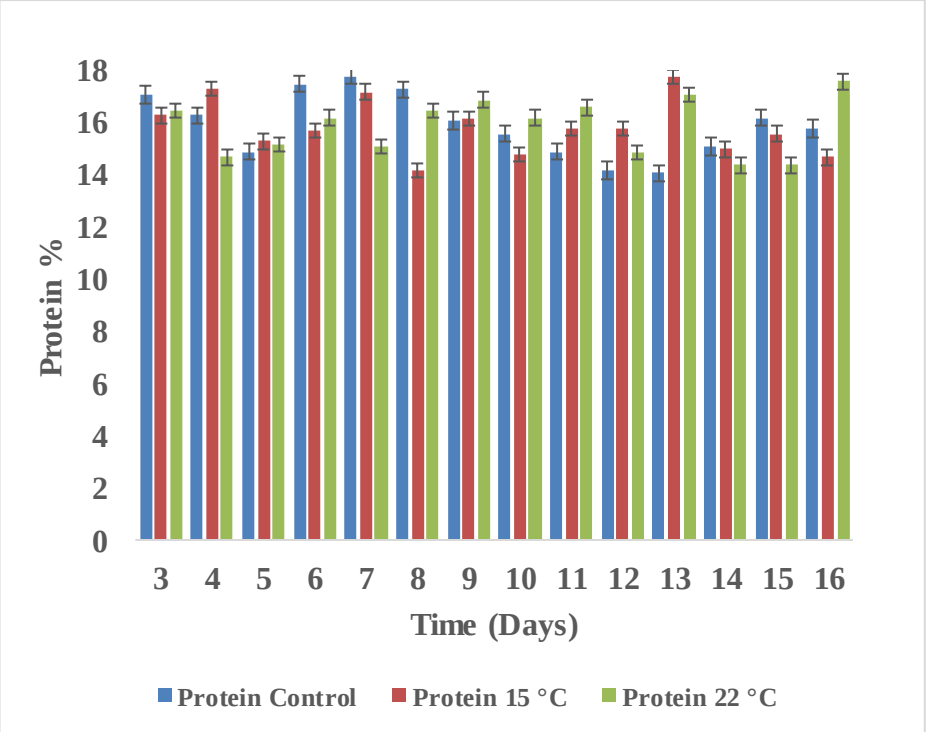

Figure 3.9: Protein % for megrim temperature profile.

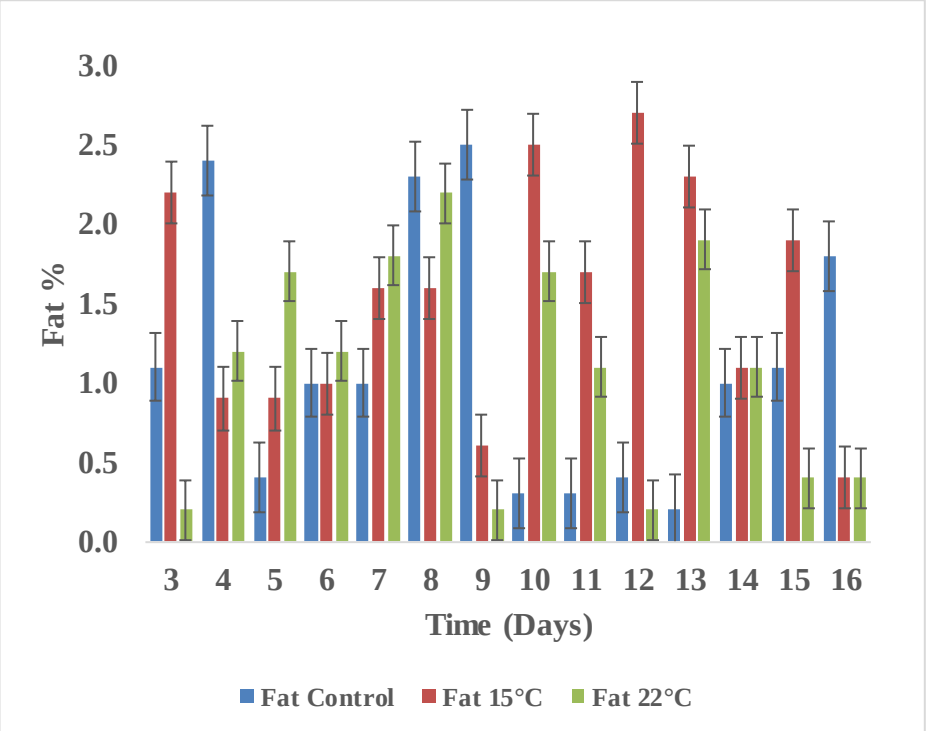

Figure 3.10: Fat % for megrim temperature profile.

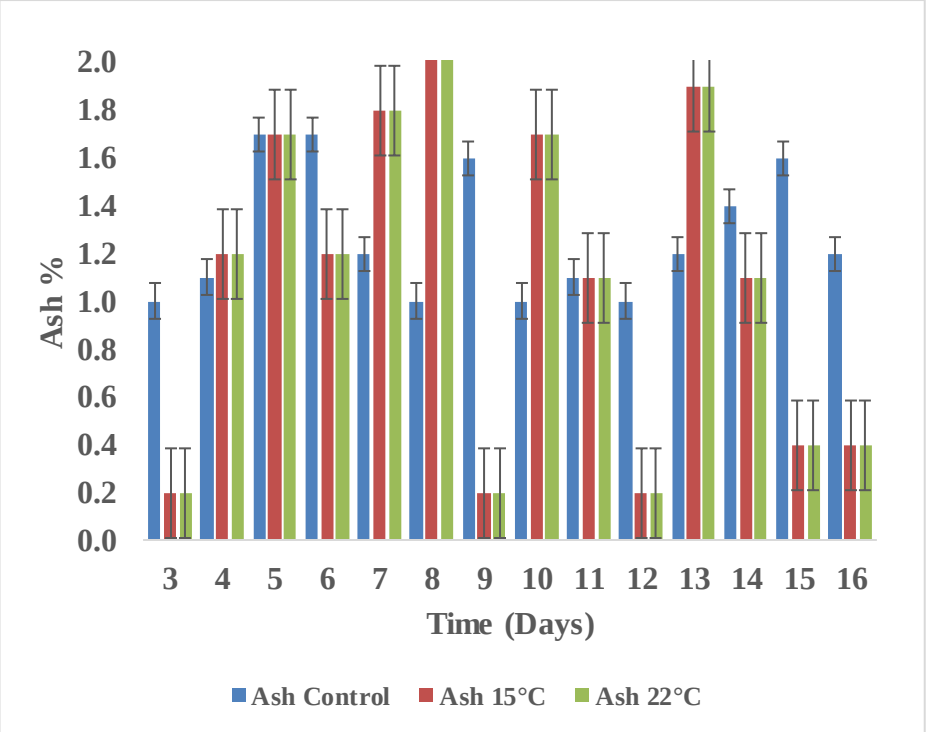

Figure 3.11: Ash % for megrim temperature profile.

3.1.1.2 Proximate composition of Pollack

The moisture levels in the pollack ranged between 77.9 and 85.1% (Figure 3.12) and were comparable with previously reported moisture contents of 79% for pollack (Tamarit, 2018). The temperature stressed samples had significantly lower moisture levels ($P < 0.05$) (Figure 3.12), similar to megrim results. Protein levels in the pollack control (0 to 2 °C) ranged from 17.1 to 19.8% (Figure 3.13) and were within the range previously reported for pollack (16.4 to 20.3%) (FAO, 2001). Protein levels did not differ significantly when temperature stressed (Figure 3.13). Fat levels ranged between 0.2 and 0.7% in all treatments (Figure 3.14) and were consistent with those previously reported by the FAO (2001), which reported that fat values for pollack should reach levels between 0.6 to 0.8%. Ash content did not show any significant fluctuations with or without temperature treatments throughout the 16-day test period, ranging from 1.17 to 2.73 (Figure 3.15).

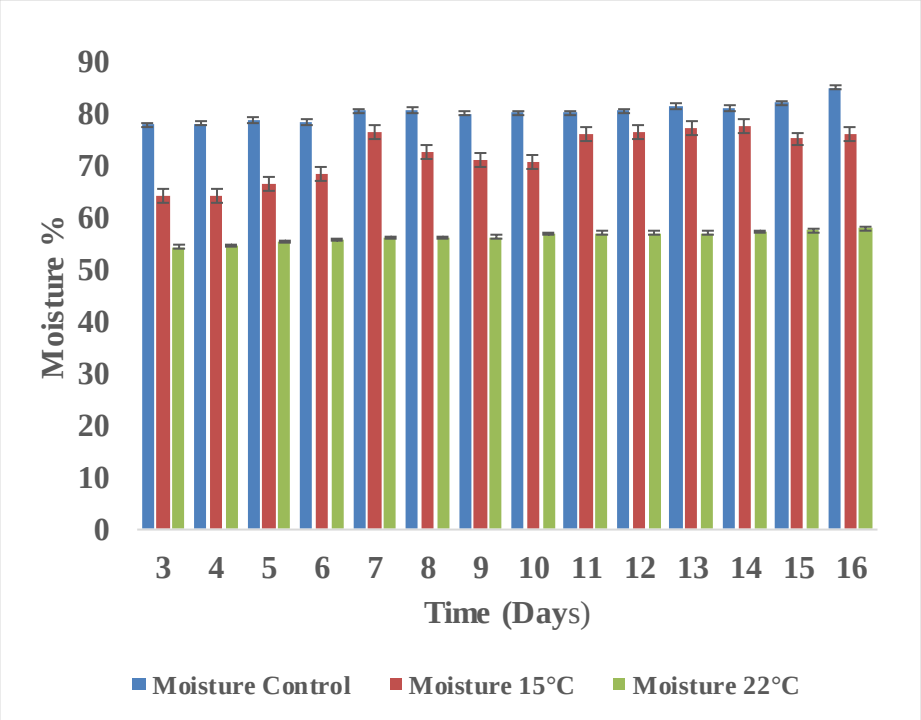

Figure 3.12: Moisture % for pollack temperature profile.

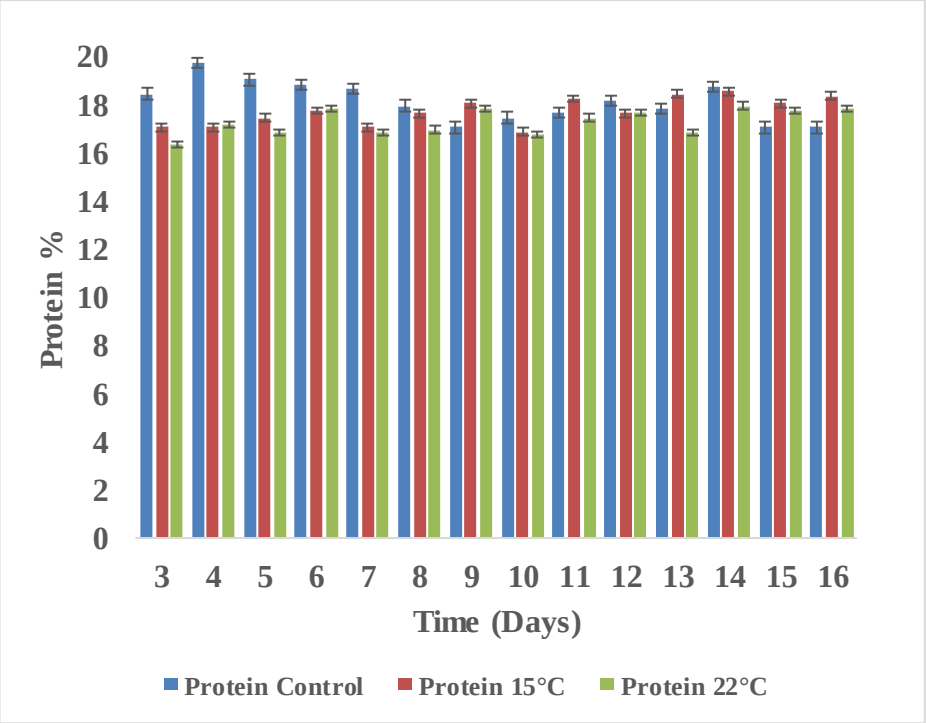

Figure 3.13: Protein % for pollack temperature profile.

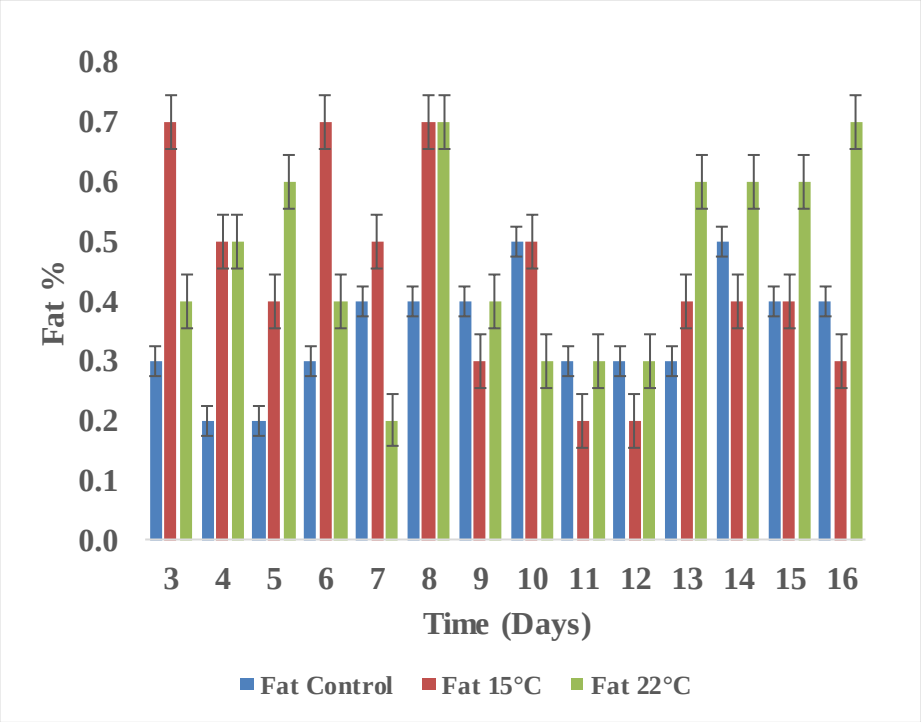

Figure 3.14: Fat % for pollack temperature profile.

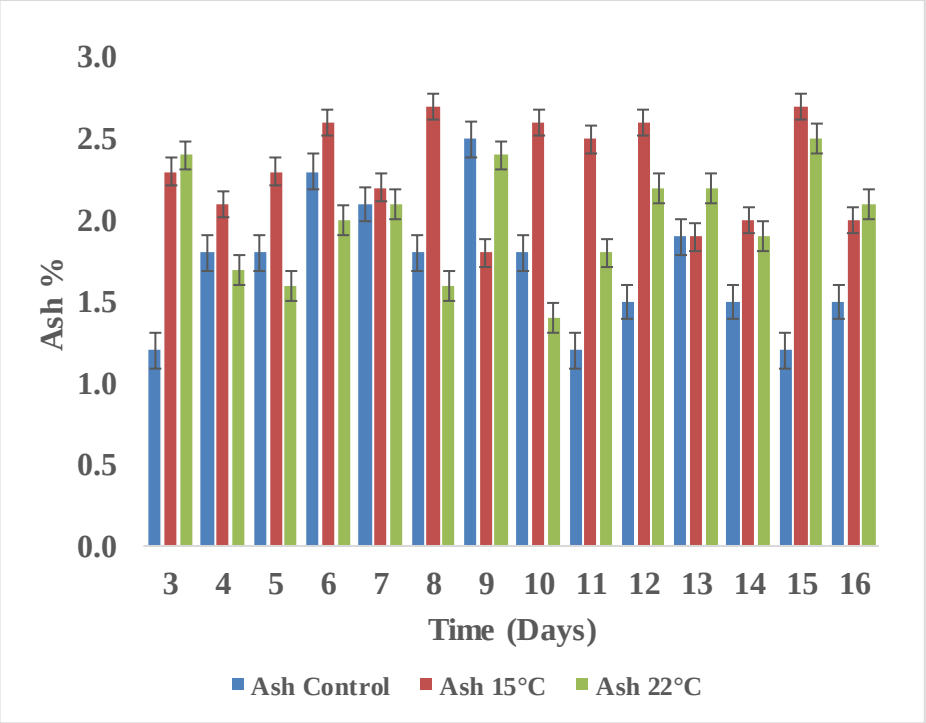

Figure 3.15: Ash % for pollack temperature profile.

3.1.2 Microbial Analysis

The effects of stressed temperature treatments on the total viable counts (log_{10} cfu/ g) of megrim and pollack during chilled storage are shown in Figure 3.16 and Figure 3.17 respectively. Total viable counts (TVC) for T1 (0 to 2 °C) increased steadily over the 16-day storage period for both species. The initial TVC on day 2 for megrim was 3.5 log cfu/ g and increased to 8.3 log cfu/ g over the 16-day chilled storage period (Figure 3.16). The limit of mesophilic aerobic counts is set at 7.01 log cfu/ g by the ICMSF, therefore these limits had been exceeded by day 16 in T1 for the megrim control. This was similarly the situation for pollack T1, where initial counts began at 3.1 log cfu/ g and reached 8.07 log cfu/ g on day 16 (Figure 3.17). Comparable values were reported by Xu *et al*. (2016) for turbot, with initial TVCs of 3.15 log cfu/ g reaching 6.6 log cfu/ g by day 16 and Paleologos *et al*. (2004) reported initial TVCs of 3.5 log cfu/g and 7.8 log cfu/ g by day 16 for sea bass.

When exposed to temperature stresses (T2 to T5) both species reached unacceptable TVC levels sooner than T1 (Fig 3.16, 3.17). Findings suggested that T2 and T3 were not suitable for human consumption on day 12 for both species reaching counts of 8.1/8.87 log cfu/ g and 7.73/8.24 log cfu/ g for megrim and pollack respectively. T4 and T5 were not acceptable for consumption after day 6 of storage, reaching 7.65/7.91 log cfu/ g and 6.94/8.18 log cfu/ g.

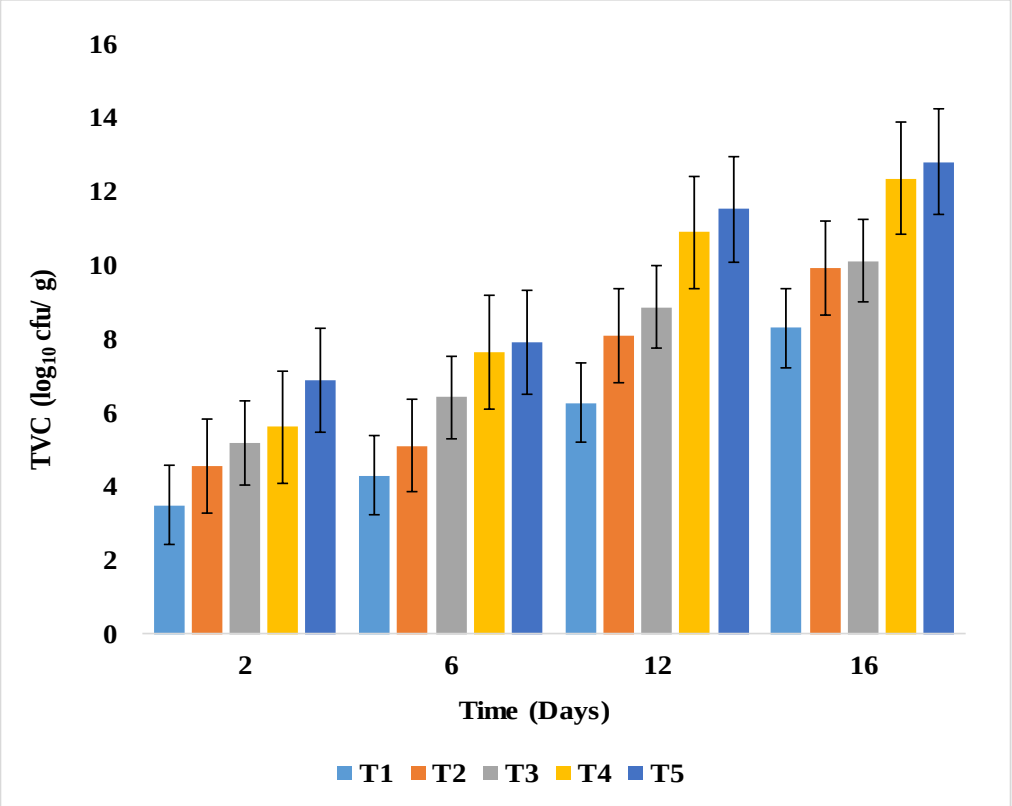

Figure 3.16: Total viable counts for megrim temperature profile.

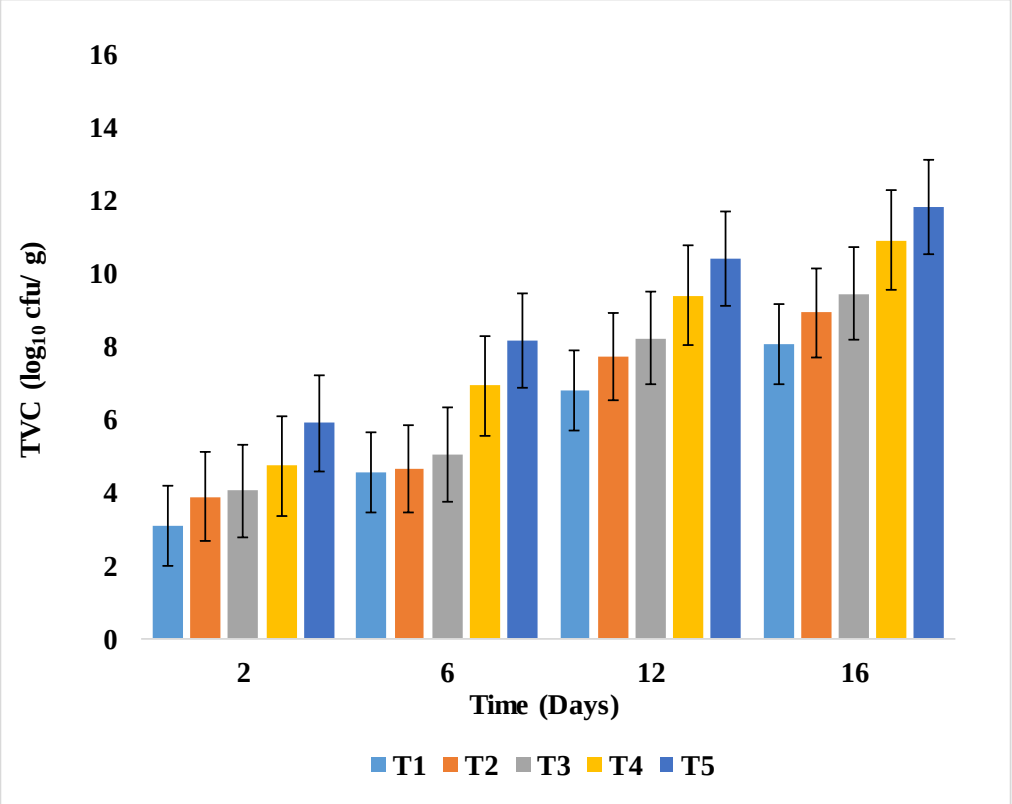

Figure 3.17: Total viable counts for pollack temperature profile.

3.1.3 Colour Analysis

The megrim muscle had a bright, smooth, white and translucent appearance before each treatment, which was characterised by a moderate value of L* (50.44 ± 0.45) and a value of a* (-1.33 ± 0.52) and b* (1.11±1.36) as shown in the control (Figure 3.18). Values are similar to those previously reported for fresh turbot, L* 52.0 ± 0.35, a* -2.5 ± 0.19 and b* 1.63 ± 0.31 (Regost, *et al*., 2011). The results for megrim indicated that the stressed fillets had an increase in L* values ranging from 57.54 to 65.91 for T4 (Figure 3.19) and 69.46 to 75.25 for T5 (Figure 3.20) during the 16-day storage period. The indices for a* and b* also increased, ranging from 1.55 to 3.67 for T4 and 2.88 to 5.49 for T5 and 2.61 to 10.86 for T4 and 11.66 to 13.59 for T5 respectively. Data points are the mean of n=6 for each sampling day. Bars represent the standard deviation.

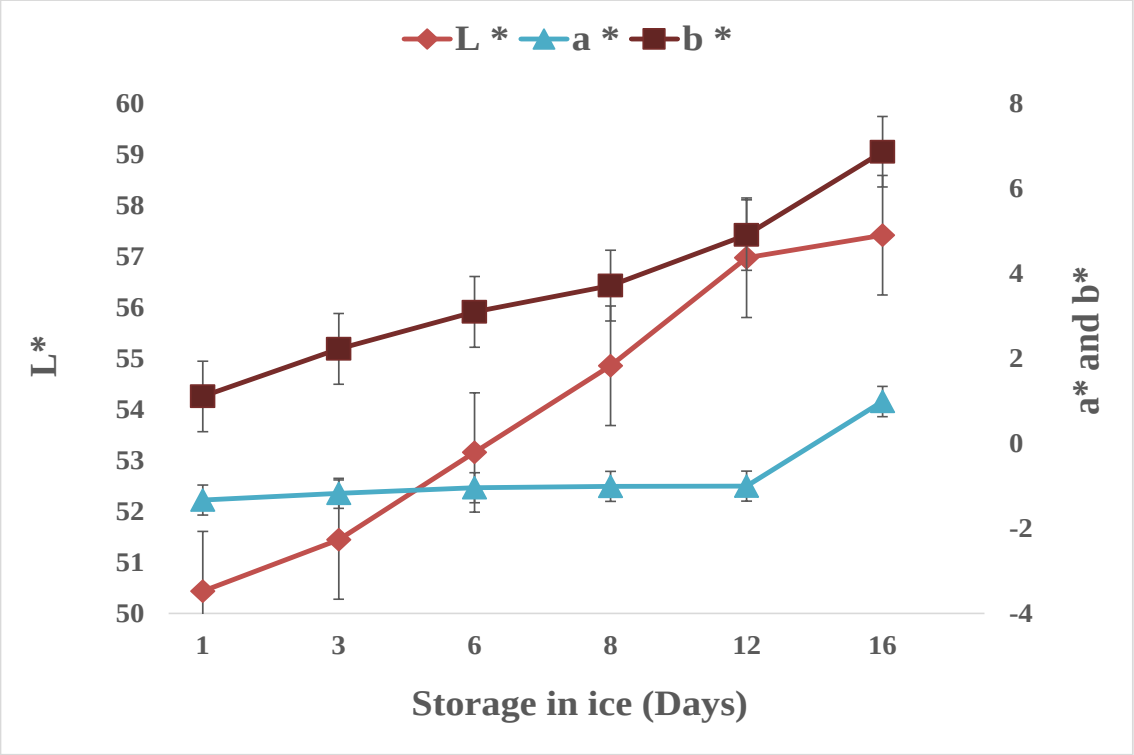

Figure 3.18: Post-mortem changes in colour parameters for raw megrim fillets T1 (0 to 2 °C).

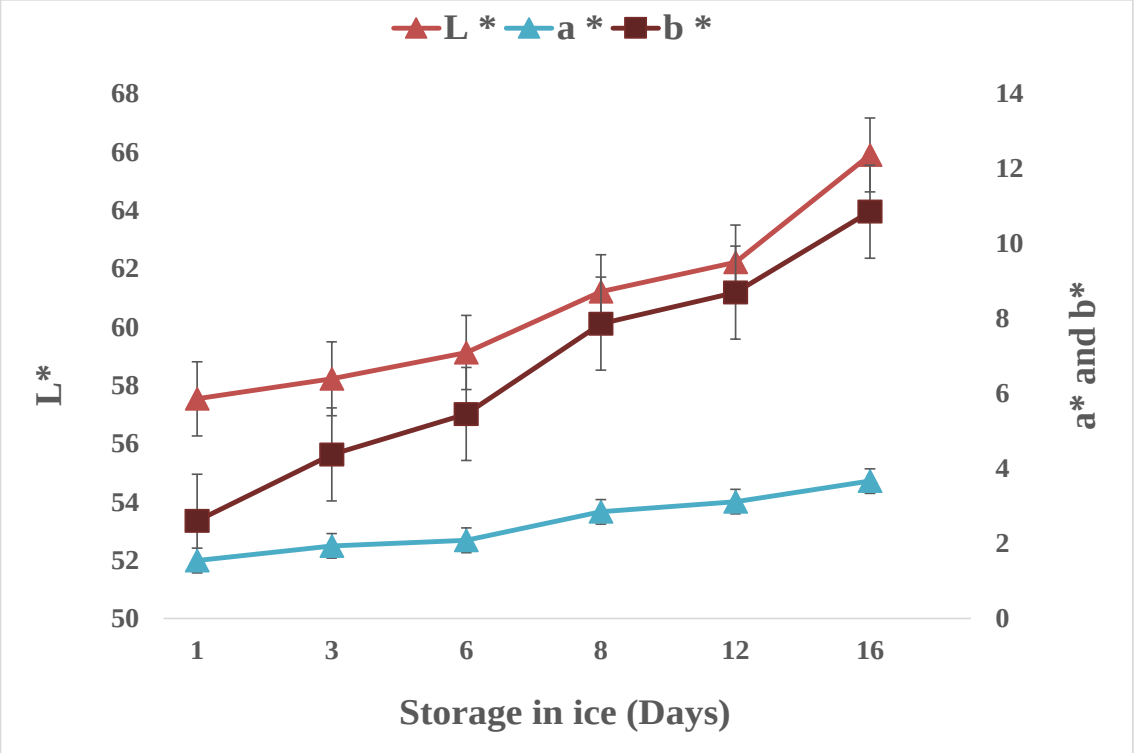

Figure 3.19: Post-mortem changes in colour parameters for raw temperature stressed megrim fillets T4 (15 °C).

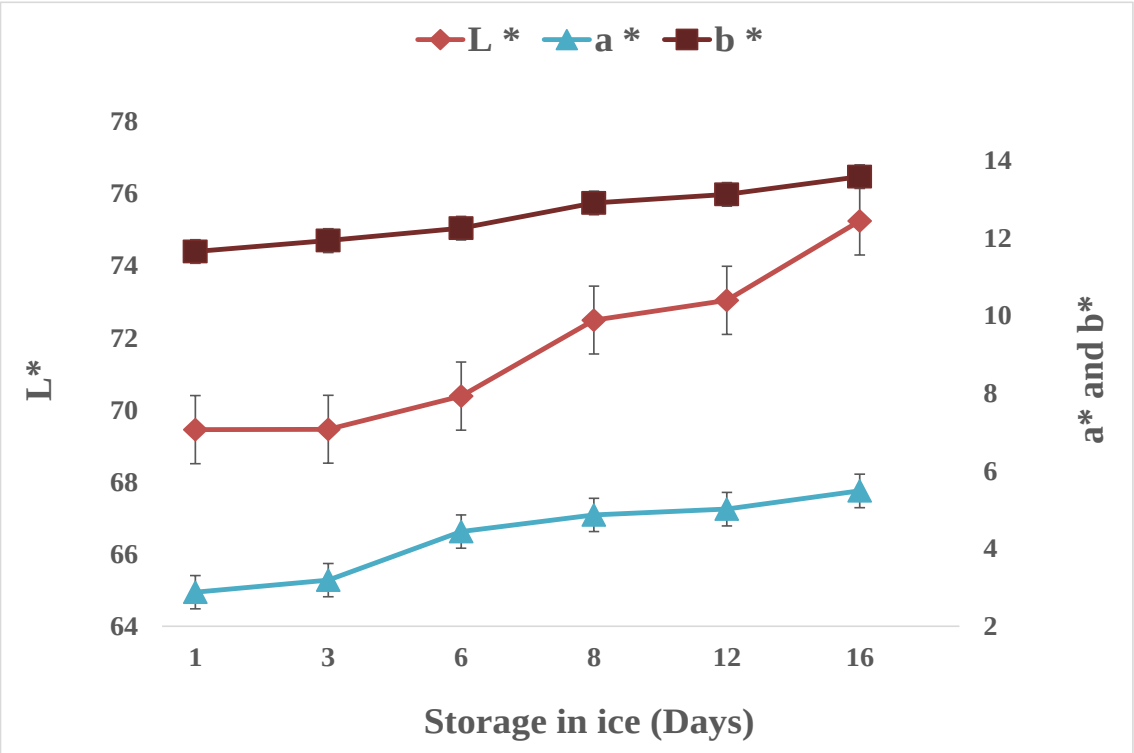

Figure 3.20: Post-mortem changes in colour parameters for raw temperature stressed megrim fillets T5 (22 °C).

A similar trend was also found with the pollack fillets (Figure 3.21 to 3.23 respectively). The pollack muscle was slightly darker and fleshier in appearance when fresh which resulted in a darker initial L* value of 42.76 ± 2.10, moderate a* value of -1.09 ± 0.65 and a deeper b* value of 4.08 ± 1.56 (Figure 3.21), in comparison to the megrim fillets. Once stressed to temperatures above the desired temperatures of 0 to 2 °C, the L* value for both T4 and T5 rapidly increased as seen in Figures 3.22 and 3.23 respectively. Once spoilage became apparent, in addition to colour changes, both species developed an off-odour, became sticky to the touch and established a slimy exterior.

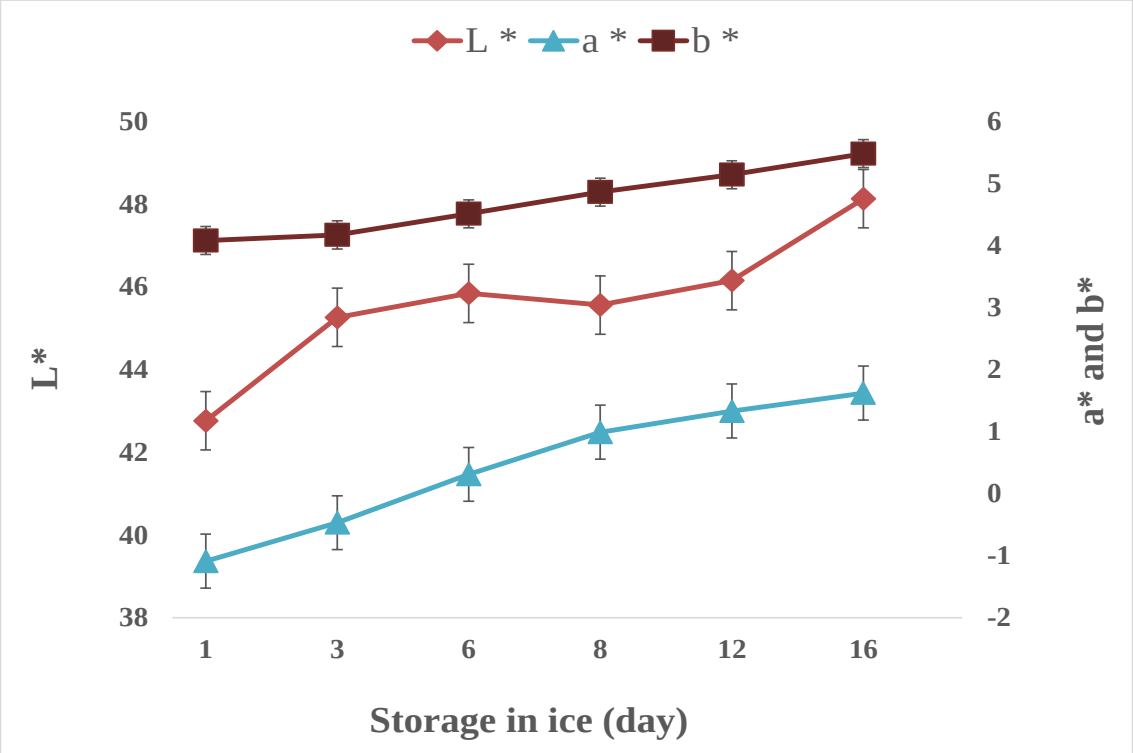

Figure 3.21: Post-mortem changes in colour parameters for raw pollack fillets T1 (0 to 2 °C).

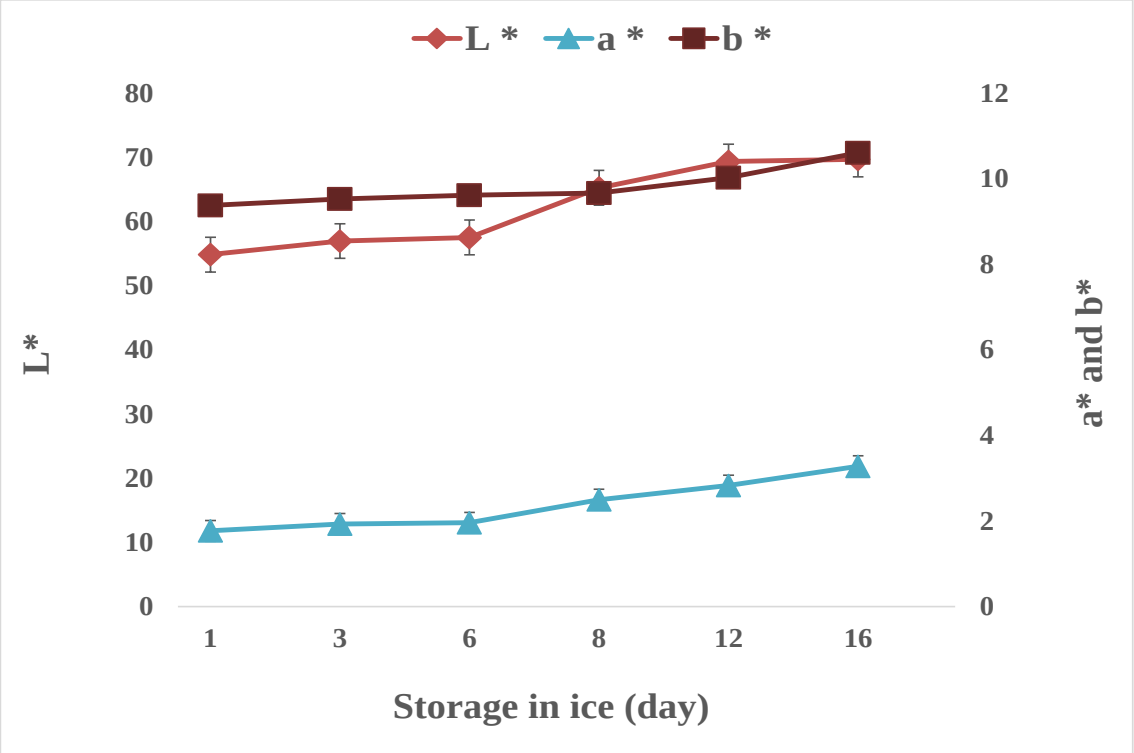

Figure 3.22: Post-mortem changes in colour parameters for raw temperature stressed pollack fillets T4 (15 °C).

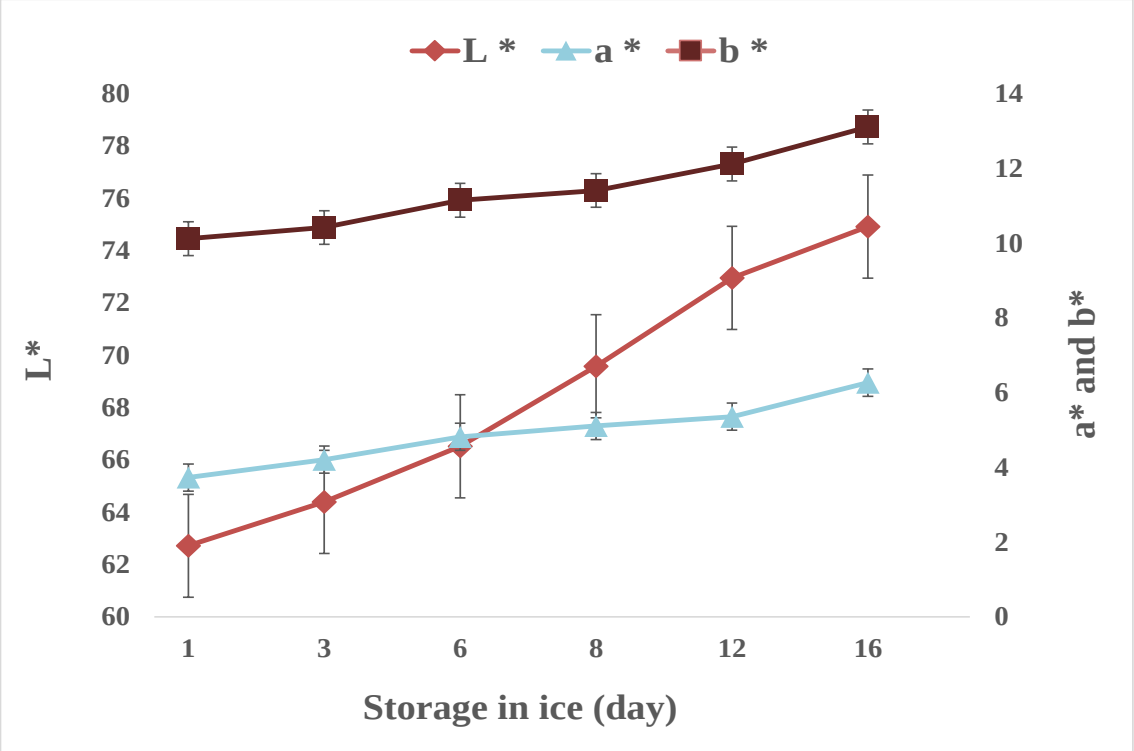

Figure 3.23: Post-mortem changes in colour parameters for raw temperature stressed pollack fillets T5 (22 °C).

In this study the colour of both species was influenced by general spoilage and was further accelerated by temperature treatments. The appearance of fish fillets for both species became whiter and less grey during storage, which corresponded to the increase in L* values. The L* value is the approximate level of luminosity within a product with accordance to a black and white scale.

The increase in a* value was due to the red colour of the lateral muscle becoming more intensified as spoilage progressed owing to the heme pigment myoglobin. The decomposition of the fish muscle through oxidation occurs due to the pigment-forming myoglobin protein, oxymyoglobin, which contributes to the flesh's reddish brown discolouration. During refrigeration this can be a regular occurrence if inadequately stored, as light and oxygen exposure triggers oxidation, causing the coloured pigments that form during temperature breaches to break down.

The increase in b* value was expected as spoilage occurred. Robb *et al*. (2000) found that when fish is stored, it lost acidity and the white pigment of flavone became yellow. This could be seen when yellow tinges of colour developed around the edges of the fillet muscle as spoilage progressed. One of the most common colour impairment pigments is fat-soluble (yellow) flavonoids. Yellowness is linked to scorching, soiling and overall product degradation due to chemical, light and processing exposure.

Another factor contributing to the discoloration could be lipid oxidation, which is influenced by both intrinsic and extrinsic factors such as temperature. As with the majority of chemical reactions, rates of lipid oxidation can increase due to time and temperature. Polyunsaturated fatty acid levels are found in high concentrations within fish, leaving products susceptible to oxidation. During the postmortem storage of fish muscle, lipid oxidation is likely to occur thereby causing discoloration.

3.1.3.1 Total colour difference

Delta E (ΔE) was used as a single value that represents the differences between the L*, a* and b* values, over the 16-day storage period. Total colour difference is expressed using ΔE; the greater the colour difference between samples, the higher the ΔE value (Checmarez, Casales, & Yeannes, 2017). Total colour difference values were calculated

using equation 2.4, as the difference between the results derived from the control T1 results and the values obtained for both species during T4 and T5. ΔE results indicated a progressive increase over the storage period and between treatments for both species when temperature was applied (Table 3.5 and Table 3.6). Increasing temperatures between T4 and T5 led to a twofold change during the initial days of storage for megrim as depicted in Table 3.5. For T4 ΔE ranged from 13.23 to 12.87 between D1 and D6, whereas for T5 ΔE ranged from 21.02 to 22.18 during the same storage period. There was a significant increase in colour difference between day 6 and day 8 in T4 (Table 3.5). However, both treatments had little variations between ΔE from D8 to D16. This correlates with results obtained during microbiological analysis in megrim (Figure 3.16), as samples that were exposed to 15 °C after day 6 reached TVC levels of 7.65 ± 0.02 log cfu/ g and on day 8 reaching 8.93 ± 0.03 log cfu/ g, both at and above the legal limit, therefore indicating spoilage.

Pollack total colour difference (ΔE) resulted in a threefold difference as seen in Table 3.6, where ΔE ranged from 7.26 to 9.76 for T4 over the 16-day storage period, compared with T5 which ranged between 21.81 and 19.51 during the same storage period. T5 was significantly higher than T4 on each storage day, in contrast to megrim samples, where a significant difference was only apparent in day 1 through day 6. This indicated that in both species limited temperature exposure had a significant effect on the overall colour of the fish flesh ($P < 0.05$). The colour difference between the control samples and the treatments indicates that temperature had a substantial impact on the fish flesh.

Table 3.5: Effect of temperature treatments on the total colour difference (ΔE) of megrim fillets over 16-day storage period.

Treatments	ΔE as a function of storage days					
	1	3	6	8	12	16
T4	13.23^{b}	13.01^{b}	12.87^{b}	20.35^{a}	23.77^{a}	22.23^{a}
T5	21.02^{a}	20.48^{a}	22.18^{a}	25.24^{a}	27.99^{a}	28.24^{a}

$\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{0.5}$ where ΔL, Δa and Δb are the differences between the L*, a*, b* values from T1.[1] Means that share the same superscript are not significantly different from one another (Tukey's HSD, $P<0.05$).

[1] L*, the level of light or darkness in a sample (+ = lighter, - = darker). a*, an object's redness or greenness (+ = redder, - = greener). b*, an object's yellowness or blueness (+ = yellower, - = bluer).

Table 3.6: Effect of temperature treatments on the total colour difference (ΔE) of pollack fillets over 16-day storage period.

Treatments	ΔE as a function of storage days					
	1	3	6	8	12	16
T4	7.26^{b}	7.15^{b}	6.50^{b}	7.81^{b}	6.81^{b}	9.76^{b}
T5	21.81^{a}	20.57^{a}	19.81^{a}	20.26^{a}	18.49^{a}	19.51^{a}

$\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{0.5}$ where ΔL, Δa and Δb are the differences between the L*, a*, b* values from T1. Means that share the same superscript are not significantly different from one another (Tukey's HSD, $P<0.05$).

3.1.3.2 Whiteness index

As shown in Table 3.7, on day 1 of storage the whiteness index (WI) was found to be 50.41 for T1, T4 was 57.43 and T5 was 67.18. There was a slight increase in WI values for T1 as storage progressed. There was a similar trend for T4 and T5, but it was more pronounced in these treatments as heat was applied and accelerated the spoilage process, resulting in a faster colour change compared to T1. As storage progressed T1 differed significantly from T4 and T5, however results of singular treatments also increased during storage but not significantly.

There was a similar trend for the pollack samples in Table 3.8, where during the storage period WI increased steadily for each treatment. This could be related to various factors such as samples selected on the day, environmental impact or previous processing, etc. As with megrim, pollack WI levels continued to increase over time as natural and heat-accelerated spoilage progressed.

Table 3.7: Effect of temperature treatments on the whiteness index (WI) of megrim fillets over 16-day storage period.

Treatments	WI as a function of storage days					
	1	3	6	8	12	16
T1	50.41	51.38	53.05	54.71	56.71	56.86
T4	57.43	57.96	58.71	60.33	61.12	64.03
T5	67.18	67.06	67.65	69.23	69.6	71.24

$WI = 100 – [(100 – L^*)^2 + a^{*2} + b^{*2}]^{1/2}$

Table 3.8: Effect of temperature treatments on the Whiteness Index (WI) of pollack fillets over 16-day storage period.

Treatments	WI as a function of storage days					
	1	3	6	8	12	16
T1	42.6	45.11	45.65	45.33	45.98	47.82
T4	53.86	55.89	56.42	63.87	67.64	67.73
T5	61.19	62.67	64.38	67.11	69.89	71.01

$WI = 100 – [(100 – L^*)^2 + a^{*2} + b^{*2}]^{1/2}$

3.1.4 Texture Analysis

During the iced storage of both species of raw fish, the textural attributes of the fish muscle deteriorated, as shown in Tables 3.9 and 3.10 respectively. Hardness generally decreased over time, which has been reported previously for cod and sea bream (Schubring, 2002; Alasalvar, *et al*., 2002). Schubring (2002) and Alasalvar *et al*. (2002) both reported a significant decrease in hardness for cod and wild sea bream fillets over a 24-day chilled storage period on ice. They suggested that the fish became softer due to proteolysis caused by endogenous and microbial enzymes. Over the 16-day chilled storage period hardness ranged from 862.50 ± 35.05 to 816.00 ± 24.44 in megrim fillets for the T1 treatment. Pollack followed the same trend, with hardness levels for T1 between 897.97 ± 16.04 and 823.46 ± 19.37 during the same storage period. The initial few days after slaughter showed a slight increase in hardness which is likely due to rigor mortis for both species (Table 3.9 and Table 3.10); hardness decreased with further storage. The gradual loss of hardness (firmness) over time was expected as general spoilage occurred as a consequence of shelf life loss and was not majorly affected when kept at an appropriate temperature between 0 and 2 °C. Temperature stressed fish (T4 and T5) developed rigor mortis faster after slaughter in both species and then rapidly decreased in hardness over time. These results were considerably lower than those in T1 treatments, which indicated that exposure to greater temperatures made the fillets much softer, which has also been previously reported for other foodstuffs such as beef and pork (Karlovic S. , *et al*., 2009).

Springiness over the 16 days of storage ranged from 1.06 ± 0.03 to 0.84 ± 0.03 for T1 of megrim in (Table 3.9) and between 0.94 ± 0.03 and 0.86 ± 0.03 for T1 of pollack (Table 3.10). Similar to the hardness characteristics for T1 treatments of both fish, there was a gradual loss of springiness over time, which was not majorly affected when samples were kept at an appropriate temperature between 0 and 2 °C. Once temperature treatments were applied springiness values dropped for T4 and T5 for both species (Tables 3.9, 3.10). This indicated that temperature stress caused a greater decrease in the fillets' ability to regain shape and structure over time when compressed in comparison to the control (T1).

The cohesiveness in both species was not significantly impacted by storage time or temperature treatments (Table 3.9 and 3.10). Both species showed a general trend of

decreasing values for cohesiveness; this could be associated with general spoilage. Therefore, temperature treatment cannot be determined as having a significant effect on cohesiveness for both species.

Similar to hardness and springiness, during the 16-day storage period, gumminess values decreased. Values ranged from 701.25 ± 37.27 to 611.06 ± 19.72 for megrim T1 (Table 3.10) and from 587.19 ± 11.53 to 435.19 ± 3.73 for pollack T1 (Table 3.11). This indicated general spoilage where the fillets became mushy and less palatable over time. For megrim both T4 and T5 decreased two-fold, whereas for pollack the decrease in values for T4 and T5 were less pronounced. Due to megrim being a flatfish and pollack being a round-fish, megrim tended to spoil faster. This may indicate why findings were not as comparable to one another. In megrim, the spoilage rate was higher and the overall gumminess values were more pronounced.

The chewiness in both species was vastly different from one another, with T1 for megrim ranging from 245.82 ± 5.43 to 203.31 ± 11.53 over the 16-day chilled storage period (Table 3.10) and pollack having chewiness values between 552.09 ± 10.36 and 472.41 ± 6.86 (Table 3.11). However, the same trend for both species occurred once the samples were temperature treated. Both megrim and pollack fillets experienced a significant decrease in chewiness for T4 and T5. The samples for T4 reduced two-fold, whereas the samples for T5 reduced three-fold. The loss of this characteristic is mostly linked to the staling of the fish fillets. Therefore, this further indicates that the temperature treatment processes decreased the elastic properties of both megrim and pollack fillets to some extent.

Table 3.9: Effect of temperature stressing on the texture of megrim during chilled storage

		Time (days)					
Treatment		**1**	**3**	**6**	**8**	**12**	**16**
Hardness	T1	$862.50 \pm 35.05^{a,A}$	$888.16 \pm 52.55^{a,B}$	$843.80 \pm 35.61^{a,A}$	$829.80 \pm 18.47^{a,A}$	$826.30 \pm 23.08^{a,A}$	$816.00 \pm 24.44^{a,A}$
	T4	$748.43 \pm 25.44^{b,A}$	$531.23 \pm 18.37^{b,B}$	$486.60 \pm 14.73^{b,BC}$	$452.53 \pm 16.79^{b,CD}$	$431.05 \pm 27.66^{b,CD}$	$391.22 \pm 37.63^{b,D}$
	T5	$673.90 \pm 15.82^{c,A}$	$410.11 \pm 16.63^{c,A}$	$366.73 \pm 19.96^{c,BC}$	$326.20 \pm 18.14^{c,CD}$	$290.87 \pm 25.21^{c,D}$	$273.60 \pm 19.03^{c,D}$
Springiness	T1	$1.06 \pm 0.03^{a,A}$	$0.84 \pm 0.08^{a,B}$	$0.90 \pm 0.06^{a,B}$	$0.87 \pm 0.01^{a,B}$	$0.89 \pm 0.02^{a,B}$	$0.84 \pm 0.03^{a,B}$
	T4	$0.86 \pm 0.05^{b,A}$	$0.87 \pm 0.32^{a,A}$	$0.73 \pm 0.05^{b,A}$	$0.70 \pm 0.08^{b,A}$	$0.57 \pm 0.06^{b,A}$	$0.55 \pm 0.1^{b,A}$
	T5	$0.71 \pm 0.08^{c,AB}$	$0.77 \pm 0.03^{a,A}$	$0.64 \pm 0.02^{c,BC}$	$0.57 \pm 0.01^{c,CD}$	$0.55 \pm 0.03^{b,CD}$	$0.49 \pm 0.06^{b,D}$
Cohesiveness	T1	$0.77 \pm 0.01^{a,A}$	$0.57 \pm 0.03^{a,B}$	$0.57 \pm 0.04^{a,B}$	$0.59 \pm 0.03^{a,B}$	$0.63 \pm 0.01^{a,B}$	$0.63 \pm 0.06^{a,B}$
	T4	$0.74 \pm 0.09^{a,A}$	$0.55 \pm 0.06^{a,B}$	$0.53 \pm 0.07^{a,B}$	$0.53 \pm 0.02^{b,B}$	$0.52 \pm 0.02^{b,B}$	$0.48 \pm 0.06^{b,B}$
	T5	$0.58 \pm 0.03^{b,B}$	$0.51 \pm 0.03^{a,B}$	$0.43 \pm 0.01^{a,B}$	$0.51 \pm 0.01^{b,B}$	$0.49 \pm 0.06^{b,A}$	$0.47 \pm 0.04^{b,A}$
Gumminess	T1	$701.25 \pm 37.27^{a,A}$	$669.49 \pm 29.33^{a,AB}$	$674.23 \pm 27.64^{a,AB}$	$664.22 \pm 15.56^{a,AB}$	$658.11 \pm 16.06^{a,AB}$	$611.06 \pm 19.72^{a,B}$
	T4	$317.82 \pm 34.47^{b,A}$	$256.77 \pm 34.71^{b,AB}$	$239.80 \pm 22.71^{b,BC}$	$246.52 \pm 21.96^{b,B}$	$234.99 \pm 12.64^{b,BC}$	$175.82 \pm 19.92^{b,C}$
	T5	$292.99 \pm 37.60^{b,A}$	$242.50 \pm 25.40^{b,AB}$	$225.30 \pm 23.10^{b,BC}$	$203.63 \pm 11.53^{c,BC}$	$175.19 \pm 3.73^{c,CD}$	$135.89 \pm 14.73^{c,D}$
Chewiness	T1	$245.82 \pm 5.43^{a,A}$	$241.05 \pm 15.72^{a,B}$	$238.60 \pm 7.22^{a,AB}$	$215.16 \pm 11.73^{a,BC}$	$203.63 \pm 6.86^{a,C}$	$203.31 \pm 11.53^{a,C}$
	T4	$202.54 \pm 7.89^{a,A}$	$198.32 \pm 10.24^{b,A}$	$187.87 \pm 11.32^{b,A}$	$173.18 \pm 15.78^{b,AB}$	$151.32 \pm 13.14^{b,B}$	$144.78 \pm 10.22^{b,B}$
	T5	$187.35 \pm 9.46^{b,A}$	$168.35 \pm 10.85^{b,AB}$	$144.76 \pm 10.32^{c,BC}$	$124.69 \pm 25.30^{c,CD}$	$109.52 \pm 8.12^{c,CD}$	$97.40 \pm 9.89^{c,D}$

Values are means ± standard deviation (n=8). Different superscripts (a, b, c) represent statistical differences between different temperatures within the same storage day. Parameter means followed by the same letter in a column are not statistically different at probability $P < 0.05$. Different superscripts (A, B, C, D) represent statistical differences between storage days within the same temperature range. Means followed by the same letter on a row are not statistically different at probability $P < 0.05$.

Table 3.10: Effect of temperature stressing on the texture of pollack during chilled storage

		Time (days)					
Treatment		**1**	**3**	**6**	**8**	**12**	**16**
Hardness	T1	$897.97 \pm 16.04^{a,A}$	$912.36 \pm 17.07^{a,A}$	$884.26 \pm 20.28^{a,AB}$	$845.67 \pm 12.84^{a,BC}$	$831.42 \pm 15.12^{a,C}$	$823.46 \pm 19.37^{a,C}$
	T4	$797.97 \pm 20.15^{b,A}$	$668.41 \pm 22.08^{b,B}$	$603.05 \pm 12.46^{b,C}$	$587.36 \pm 10.97^{b,CD}$	$536.82 \pm 25.13^{b,DE}$	$495.62 \pm 21.17^{b,E}$
	T5	$687.92 \pm 9.26^{c,A}$	$513.90 \pm 13.58^{c,B}$	$492.88 \pm 15.97^{c,BC}$	$473.26 \pm 10.29^{c,CD}$	$451.15 \pm 7.91^{c,D}$	$376.80 \pm 8.78^{c,E}$
Springiness	T1	$0.94 \pm 0.03^{a,B}$	$1.12 \pm 0.08^{a,B}$	$0.97 \pm 0.06^{a,A}$	$0.92 \pm 0.01^{a,B}$	$0.89 \pm 0.02^{a,B}$	$0.86 \pm 0.03^{a,B}$
	T4	$1.04 \pm 0.21^{a,A}$	$0.91 \pm 0.17^{a,A}$	$0.87 \pm 0.10^{a,A}$	$0.81 \pm 0.18^{a,A}$	$0.75 \pm 0.05^{ab,A}$	$0.66 \pm 0.08^{b,A}$
	T5	$0.93 \pm 0.08^{a,A}$	$0.81 \pm 0.03^{a,A}$	$0.69 \pm 0.04^{b,AB}$	$0.67 \pm 0.10^{a,AB}$	$0.59 \pm 0.12^{a,B}$	$0.54 \pm 0.06^{b,B}$
Cohesivenes s	T1	$0.79 \pm 0.02^{a,A}$	$0.74 \pm 0.06^{a,AB}$	$0.71 \pm 0.03^{a,AB}$	$0.68 \pm 0.03^{a,B}$	$0.56 \pm 0.06^{a,C}$	$0.52 \pm 0.01^{a,C}$
	T4	$0.65 \pm 0.05^{b,B}$	$0.59 \pm 0.02^{b,B}$	$0.63 \pm 0.07^{ab,B}$	$0.57 \pm 0.04^{b,B}$	$0.55 \pm 0.14^{a,A}$	$0.51 \pm 0.03^{a,A}$
	T5	$0.57 \pm 0.06^{c,A}$	$0.56 \pm 0.04^{b,AB}$	$0.56 \pm 0.02^{b,AB}$	$0.54 \pm 0.02^{b,AB}$	$0.50 \pm 0.03^{a,AB}$	$0.47 \pm 0.02^{a,B}$
Gumminess	T1	$587.19 \pm 11.53^{a,A}$	$542.50 \pm 25.40^{a,A}$	$489.80 \pm 23.10^{a,A}$	$439.80 \pm 23.10^{a,A}$	$405.89 \pm 14.73^{a,A}$	$435.19 \pm 3.73^{a,A}$
	T4	$393.45 \pm 10.25^{b,A}$	$358.96 \pm 11.32^{b,B}$	$308.11 \pm 7.55^{a,C}$	$298.32 \pm 9.41^{b,D}$	$273.84 \pm 7.89^{b,DE}$	$261.34 \pm 10.22^{b,E}$
	T5	$292.99 \pm 16.68^{c,A}$	$254.04 \pm 10.94^{c,ABC}$	$287.10 \pm 31.40^{a,AB}$	$264.20 \pm 24.40^{b,AB}$	$236.89 \pm 2.71^{c,BC}$	$205.90 \pm 20.10^{c,C}$
Chewiness	T1	$552.09 \pm 10.36^{a,A}$	$537.70 \pm 10.27^{a,A}$	$511.06 \pm 7.55^{a,B}$	$500.07 \pm 12.64^{a,BC}$	$483.40 \pm 5.43^{a,CD}$	$472.41 \pm 6.86^{a,D}$
	T4	$309.63 \pm 10.24^{b,A}$	$289.52 \pm 13.14^{b,A}$	$238.60 \pm 7.22^{b,B}$	$219.94 \pm 6.31^{b,B}$	$180.07 \pm 11.73^{b,C}$	$167.87 \pm 11.32^{b,C}$
	T5	$271.99 \pm 15.34^{c,A}$	$193.89 \pm 15.80^{c,B}$	$188.76 \pm 15.56^{c,B}$	$171.01 \pm 9.66^{c,BC}$	$162.67 \pm 9.34^{c,BC}$	$140.53 \pm 16.06^{b,C}$

Values are means ± standard deviation (n=8). Different superscripts (a, b, c) represent statistical differences between different temperatures within the same storage day. Parameter means followed by the same letter in a column are not statistically different at probability $P < 0.05$. Different superscripts (A, B, C, D, E) represent statistical differences among storage days within the same temperature range. Means followed by the same letter on a row are not statistically different at probability $P < 0.05$.

3.1.5 Total Volatile Base Nitrogen (TVB-N)

Total viable base nitrogen levels increased over the storage period for chilled fish stored between 0 and 2 °C. For larger megrim TVB-N values ranged from 7.84 to 45.39 mg N/100 g (Figure 3.24), for smaller megrim the TVB-N values ranged from 23.25 to 75.64 mg N/100 g (Figure 3.25) and pollack ranged from 12.61 to 51.83 mg N/100 g (Figure 3.26) over the 11 to 13-day storage period. However, only fish that remained within the limits defined by the EU Directive 95/149/EC limit of 35 mg N/100 g (Table 1.1) for unprocessed fish (European Comission, 1995) were acceptable for human consumption. The tolerance limits were reached by day 9 for larger megrim and day 8 for both the smaller megrim and pollack samples respectively.

When samples were subjected to 15 °C for 2.5 h prior to being tested, larger megrim on day 3 was the only sample to have a TVB-N level (34.74 mg N/100 g) within the acceptable limit. The highest TVB-N levels for each sample were reached on day 11 (129.71 mg N/100 g) for larger megrim (Figure 3.24), day 13 (288.84 mg N/100 g) for smaller megrim (Figure 3.25) and on day 11 (145.96 mg N/100 g) for pollack (Figure 3.26). Regardless of the fish species, all samples exceeded the TVB-N limit after day 3 of storage, indicating spoilage had occurred. The shelf life of the fish was therefore significantly reduced and unfit for human consumption.

When samples were maintained at 22 °C for 2.5 h prior to testing, all samples exceeded the legal limit indicating severe spoilage, regardless of species, and increased further over time. Large megrim had levels ranging from 75.92 to 262.79 mg N/100 g (Figure 3.24), smaller megrim ranged from 220.77 to 528.94 mg N/100 g (Figure 3.25) and pollack ranged from 78.72 to 259.15 mg N/100 g (Figure 3.26) during the 11 to 13-day storage period.

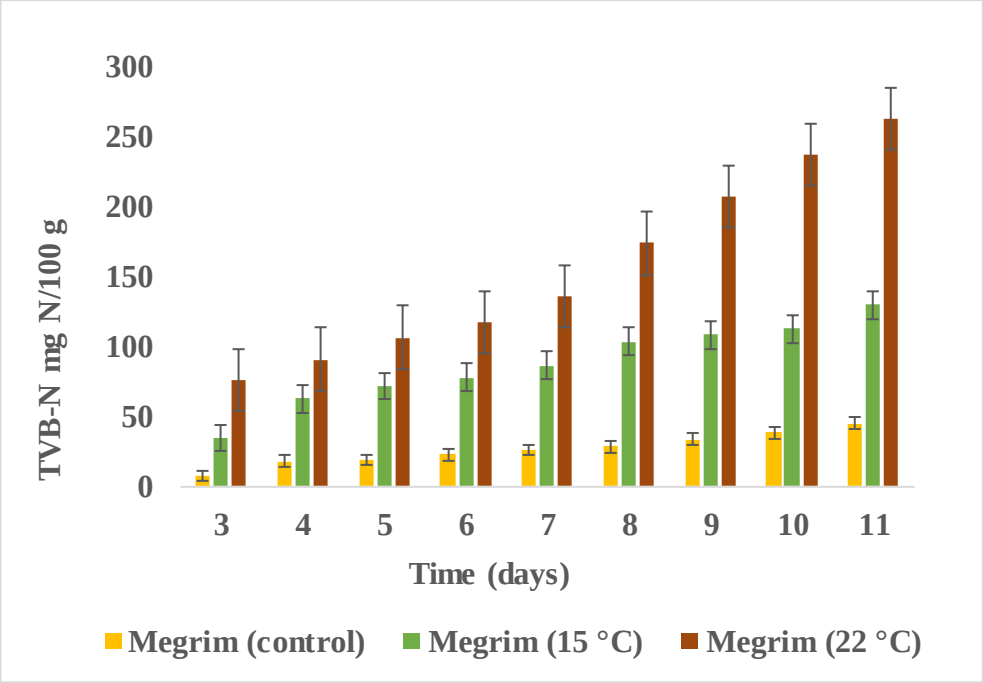

Figure 3.24: TVB-N content for large megrim temperature profile.

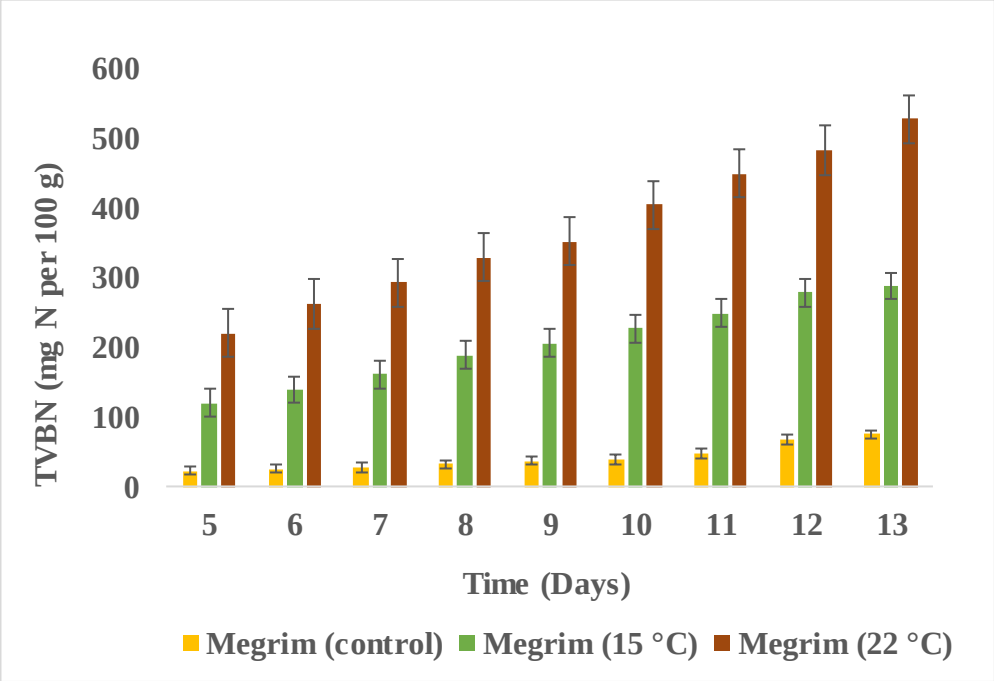

Figure 3.25: TVB-N content for small megrim temperature profile.

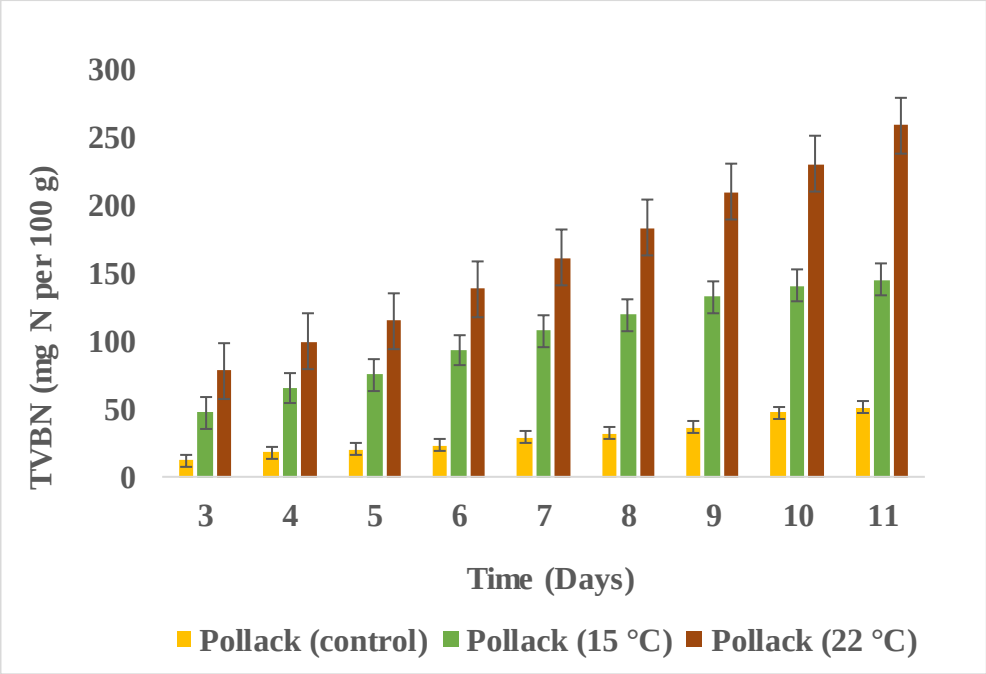

Figure 3.26: TVB-N content for medium pollack temperature profile.

3.1.6 Sensory Analysis

All treatments for both species experienced degradation in sensory attributes over the 8-day storage period. Appearance and aroma were the attributes most impacted during the storage of megrim (Table 3.11). In the control treatment (T1), the appearance on day 2 scored 8.13 ± 0.64 and by day 8 decreased to a score of 5.38 ± 1.30. This was comparable for all other attributes, resulting in a decrease in acceptability scores over the chilled storage period of 8 days. This was a natural decline in sensory attributes as spoilage progressed due to the end of the sample's shelf life, even when kept in chilled iced storage between 0 and 2 °C. This was a consistent trend in the other treatments (T2 to T5) but was more pronounced when the temperature was greater (≥ 15 °C). T2 and T3 samples scored slightly below the control, while T4 and T5 samples scored 5.75 ± 1.39 and 5.13 ± 1.13 respectively on day 2. While results showed a decrease during the storage period across all treatments in Table 3.11, there was no significant difference from T1 to T3 compared to T4 and T5.

For pollack there was a two-fold decrease in all sensory attributes over the 8-day storage period for the control (T1), as shown in Table 3.12. T2 and T3 decreased at a faster rate, reaching a two-fold sensory loss across all attributes by day 6. T4 did not display a major difference in sensory attributes until day 6, where it scored lowest in aroma and flavour (2.25 ± 1.04 and 2.63 ± 1.19 respectively). On day 2 T5 showed minimal acceptance across all attributes and was deemed unacceptable for sensory evaluation thereafter.

The loss in overall acceptability was accelerated when the fish was subjected to temperatures above the recommended of 0 to 2 °C prior to sensory analysis. Megrim acceptability of T1 (0 to 2 °C) on day 2 scored 7.3 out of 9 for overall acceptability, compared to T5 (22 °C) which scored 4.6 on the same day. That was approximately a loss of a third in overall acceptability between T1 and T5 as a result of temperature exposure. T2 scored an overall acceptability of 4.8, whereas T3 scored 3.9, with the temperature variation of 3 °C. This indicated that results between treatments did not vary significantly when temperatures were kept below ≤ 8 °C.

Acceptability for pollack is shown in Table 3.12. T1 (0 to 2 °C) on day 2 scored 7.8 for overall acceptability, compared to T5 (22 °C) which scored 5.0 on the same day. Similar

to megrim samples, with increasing temperature exposure pollack also experienced a substantial loss in overall acceptability between T1 and T5. On day 4, T3 scored an overall acceptability of 6.8, whereas T4 scored 5.8, with a 7 °C variance. The depletion in overall acceptability continued as natural spoilage took place and accelerated spoilage due to temperature stressing.

Further samples were not analysed by the sensory panel after day 6 for T2 and T3 in both species and T4 in pollack, day 2 for T4 in megrim and both T5 on day 2, as the TVC levels exceeded the acceptable TVC limit (7.01 log cfu/ g) when microbially tested.

Table 3.11: Effect of storage days and temperature fluctuations on sensory attributes of cooked megrim fillets.

Sensory scores						
Treatment		**Appearance**	**Aroma**	**Flavour**	**Texture**	**Overall Acceptability**
Day 2	T1	$8.13 \pm 0.64^{a,A}$	$8.00 \pm 0.076^{a,A}$	$7.88 \pm 0.84^{a,A}$	$7.63 \pm 0.74^{a,A}$	$7.30 \pm 0.71^{a,A}$
	T2	$7.38 \pm 0.92^{ab,A}$	$6.25 \pm 1.28^{ab,A}$	$7.13 \pm 1.25^{a,A}$	$7.13 \pm 0.99^{a,A}$	$6.90 \pm 0.99^{a,A}$
	T3	$6.13 \pm 0.99^{bc,A}$	$6.13 \pm 1.36^{ab,A}$	$6.38 \pm 1.69^{ab,A}$	$6.25 \pm 1.49^{ab,A}$	$6.40 \pm 1.36^{a,A}$
	T4	$5.75 \pm 1.39^{c,A}$	$5.50 \pm 2.07^{b,A}$	$6.13 \pm 1.89^{ab,A}$	$6.25 \pm 1.49^{ab,A}$	$6.50 \pm 1.60^{ab,A}$
	T5	$5.13 \pm 1.13^{c,A}$	$4.63 \pm 1.69^{b,A}$	$4.75 \pm 1.58^{b,A}$	$4.88 \pm 1.25^{b,A}$	$4.6 \pm 1.07^{b,A}$
Day 4	T1	$7.38 \pm 0.92^{a,A}$	$6.25 \pm 1.28^{a,AB}$	$7.13 \pm 1.25^{a,A}$	$7.13 \pm 0.99^{a,A}$	$5.30 \pm 0.99^{a,AB}$
	T2	$6.13 \pm 0.99^{ab,AB}$	$6.13 \pm 1.36^{a,A}$	$6.38 \pm 1.69^{ab,AB}$	$6.25 \pm 1.49^{a,A}$	$5.10 \pm 1.36^{a,AB}$
	T3	$5.75 \pm 1.49^{ab,A}$	$5.63 \pm 2.13^{a,A}$	$6.25 \pm 1.98^{ab,AB}$	$6.08 \pm 1.26^{a,A}$	$5.00 \pm 1.58^{a,A}$
	T4	n/a	n/a	n/a	n/a	n/a
	T5	n/a	n/a	n/a	n/a	n/a
Day 6	T1	$5.75 \pm 1.49^{a,B}$	$5.63 \pm 2.13^{a,B}$	$6.25 \pm 1.98^{a,AB}$	$6.25 \pm 1.49^{a,AB}$	$5.10 \pm 1.69^{a,AB}$
	T2	$5.50 \pm 1.07^{a,B}$	$5.50 \pm 1.41^{a,A}$	$5.13 \pm 1.46^{ab,AB}$	$6.00 \pm 1.41^{a,A}$	$4.80 \pm 0.99^{ab,AB}$
	T3	$5.00 \pm 1.07^{a,A}$	$5.00 \pm 1.69^{a,A}$	$4.75 \pm 1.49^{ab,AB}$	$5.50 \pm 1.51^{a,A}$	$3.90 \pm 1.06^{ab,AB}$
	T4	n/a	n/a	n/a	n/a	n/a
	T5	n/a	n/a	n/a	n/a	n/a
Day 8	T1	5.38 ± 1.30^{B}	5.25 ± 1.83^{B}	5.13 ± 1.46^{B}	6.00 ± 1.41^{A}	4.60 ± 1.04^{B}
	T2	n/a	n/a	n/a	n/a	n/a
	T3	n/a	n/a	n/a	n/a	n/a
	T4	n/a	n/a	n/a	n/a	n/a
	T5	n/a	n/a	n/a	n/a	n/a

Values are means ± standard deviation (n=10).

Different superscripts (a, b) represent statistical differences between different temperatures within the same storage day. Means followed by the same letter on a column are not statistically different at probability $P < 0.05$.

Different superscripts (A, B, C) represent statistical differences between storage days within the same temperature range. Means followed by the same letter within a column are not statistically different at probability $P < 0.05$.

Table 3.12: Effect of storage days and temperature fluctuations on sensory attributes of cooked pollack fillets.

Sensory scores						
Treatment		**Appearance**	**Aroma**	**Flavour**	**Texture**	**Overall Acceptability**
Day 2	T1	$8.69 \pm 0.82^{a,A}$	$7.25 \pm 1.28^{a,A}$	$7.32 \pm 2.32^{a,A}$	$7.74 \pm 1.89^{a,A}$	$7.80 \pm 0.71^{a,A}$
	T2	$7.52 \pm 1.49^{ab,A}$	$6.89 \pm 1.20^{a,A}$	$6.88 \pm 1.13^{ab,A}$	$7.21 \pm 1.19^{a,A}$	$7.10 \pm 0.99^{ab,A}$
	T3	$6.93 \pm 2.47^{b,A}$	$6.50 \pm 0.93^{a,A}$	$6.18 \pm 0.84^{ab,A}$	$6.46 \pm 1.60^{a,A}$	$6.90 \pm 0.74^{ab,A}$
	T4	$6.13 \pm 1.36^{b,A}$	$6.38 \pm 0.74^{a,A}$	$6.00 \pm 0.76^{b,A}$	$6.01 \pm 1.60^{a,A}$	$6.50 \pm 0.71^{ab,A}$
	T5	$5.16 \pm 1.72^{b,A}$	$5.50 \pm 2.07^{a,A}$	$5.13 \pm 1.9^{b,A}$	$5.25 \pm 1.49^{a,A}$	$5.00 \pm 1.60^{b,A}$
Day 4	T1	$6.25 \pm 1.91^{a,AB}$	$5.50 \pm 1.77^{a,AB}$	$5.06 \pm 1.74^{a,B}$	$6.82 \pm 2.00^{a,B}$	$7.10 \pm 2.25^{a,A}$
	T2	$5.75 \pm 1.58^{a,AB}$	$5.38 \pm 1.69^{a,A}$	$4.88 \pm 3.88^{a,AB}$	$5.33 \pm 1.77^{a,B}$	$6.90 \pm 2.10^{a,AB}$
	T3	$5.13 \pm 2.19^{a,AB}$	$5.00 \pm 1.41^{a,AB}$	$4.38 \pm 2.38^{a,B}$	$5.03 \pm 2.57^{a,AB}$	$6.80 \pm 1.85^{a,AB}$
	T4	$4.93 \pm 1.13^{a,AB}$	$4.63 \pm 1.99^{a,AB}$	$4.75 \pm 2.75^{a,AB}$	$4.88 \pm 1.25^{a,AB}$	$5.80 \pm 1.07^{a,AB}$
	T5	n/a	n/a	n/a	n/a	n/a
Day 6	T1	$4.88 \pm 2.55^{a,B}$	$5.00 \pm 2.07^{a,AB}$	$4.13 \pm 1.25^{a,B}$	$4.88 \pm 1.36^{a,B}$	$6.60 \pm 1.60^{a,B}$
	T2	$4.76 \pm 1.78^{a,B}$	$4.63 \pm 1.51^{a,A}$	$3.97 \pm 1.58^{ab,B}$	$4.38 \pm 1.60^{ab,B}$	$5.90 \pm 1.69^{a,B}$
	T3	$4.75 \pm 1.49^{a,AB}$	$3.75 \pm 1.39^{ab,B}$	$3.66 \pm 1.89^{ab,B}$	$3.91 \pm 2.18^{ab,B}$	$5.40 \pm 1.36^{a,B}$
	T4	$3.75 \pm 1.04^{a,B}$	$2.25 \pm 1.04^{b,C}$	$2.63 \pm 1.19^{ab,C}$	$3.75 \pm 1.04^{ab,B}$	$5.10 \pm 0.54^{a,B}$
	T5	n/a	n/a	n/a	n/a	n/a
Day 8	T1	4.88 ± 1.55^{B}	4.63 ± 1.51^{B}	4.22 ± 1.79^{B}	4.63 ± 1.19^{B}	5.80 ± 1.69^{B}
	T2	n/a	n/a	n/a	n/a	n/a
	T3	n/a	n/a	n/a	n/a	n/a
	T4	n/a	n/a	n/a	n/a	n/a
	T5	n/a	n/a	n/a	n/a	n/a

Values are means ± standard deviation (n=10).

Different superscripts (a, b) represent statistical differences between different temperatures within the same storage day. Means followed by the same letter on a column are not statistically different at probability $P < 0.05$.

Different superscripts (A, B, C) represent statistical differences among storage days within the same temperature range. Means followed by the same letter on a column are not statistically different at probability $P < 0.05$.

3.1.7 Chapter summary

Both species of fish underwent various temperature treatments that mirrored those experienced during distribution. Samples were kept refrigerated on ice prior to testing, however those exposed to varying temperatures were stressed at the assigned temperatures for 2.5 h prior. Findings indicated that both fish species experienced shelf life and quality degradation naturally throughout the 16-day storage period which were further intensified due to temperature stress.

During shelf life and quality evaluation, there was no significant effect on the proximate composition of the two species with temperature stress, except for moisture content.

As the heat increased, the ice around the samples for both species melted. The heat treated samples began to soak up the melted ice water, resulting in higher levels of water. Consequently, as shown in Figure 3.8 for megrim and Figure 3.12 for pollack, the higher the temperature when fish is placed on ice, the higher the moisture content.

This was not the case in regard to total viable counts. Both species did initially follow a natural degradation pattern, with T1 (0 to 2 ˚C) not exceeding microbial limits (7.01 cfu/ log) until day 16 of storage. However, the temperature stressed treatments reached these thresholds faster in comparison. T2 and T3 (5 and 8 ˚C) reached unacceptable limits by day 12 and T4 and T5 (15 and 22 ˚C) by day 6 respectively in both species (Figure 3.16 and Figure 3.17). This degradation of shelf life and quality due to temperature stress continued when samples were tested using other quality parameters. Throughout experimentation, the colour of samples was the most noticeable indicator of spoilage. Visually and measurably using the Lab scale, the findings for both species indicated that temperature stressed fillets experienced an increased spoilage rate. The fillets had changed from shiny, smooth, white and translucent to a white/ cream, fleshier appearance with a deep reddening of the lateral line. During natural spoilage, the increase in the Lab scale was expected to rise and further increased with the addition of temperature stress. In Figure 3.19 and Figure 3.20, all megrim values for T4 and T5 rose compared to that in Figure 3.18. In Figure 3.22 and Figure 3.23, the Pollack samples increased more significantly compared to Figure 3.21.

These results may be linked with the results of the microbiological analysis in Chapter 3.1.2, where by day 6 microbial levels for fish stressed by temperature had reached unacceptable limits, as did color measurements during the same time period. In terms of texture, temperature-stressed fish (T4 and T5) developed rigor mortis faster after slaughter in both species and then rapidly decreased across all textural attributes, as shown in Table 3.9 and Table 3.10. It was clear that once the fish had encountered temperature stress, the fillets started to stale and had a decline in elastic properties. As a result, this contributed in the failure of the fillets to recover form and springiness relative to the control (T1). The TVB-N was also impacted by temperature stressing. Regardless of the fish species, all samples exceeded TVB-N limits after three days once stressed to 15 ˚C, indicating spoilage had occurred. This was even more pronounced when samples were maintained at 22 ˚C as shown in Figures 3.24, Figure 3.25 and Figure 3.26. Furthermore, there was a natural decline in all sensory attributes as spoilage progressed due to the end of the samples' shelf life as seen in Table 3.11 and Table 3.12. Scoring of attributes were vastly affected once temperatures above 15 ˚C were introduced.

Increased temperatures for a limited period may have a major impact on the quality of fish. While fish may be stored on ice at low temperatures, irreversible decay will occur if there is some disturbance during distribution, e.g. delay, equipment breakdown and/or human error.

Temperature stressed samples were significantly affected by the introduction of temperatures above 15 ˚C for 2.5 h (reflecting temperature variations experienced during distribution), which subsequently influenced shelf life and quality degradation in both species. It is therefore evident from the results that keeping fish at an optimum temperature of between 0 and 2 ˚C is the best practice for preserving the highest quality and longevity fresh fish.

4 GENERAL DISCUSSION

Chilling fish involves establishing and maintaining consistent chilled storage (cold chain) of between 0 and 2 °C using a combination of ice and refrigeration. A cold chain management system (CCMS) is a process that monitors the cold chain and offers logistical advantages to fish producers as it allows the fish to retain high quality and freshness for longer during distribution by identifying breaches that enable corrective actions to be developed where necessary for a streamlined system.

Megrim and pollack landed in Ireland and then further distributed are proven to be prone to temperature fluctuations which can affect the composition of the flesh. In such instances proteins and lipids can break down resulting in the production of new compounds causing changes in meat flavour, tenderness, juiciness, odour and texture (Berkel, *et al*., 2004). Chilling helps prevent protein denaturation which may lead to bacterial attacks, as bacteria are more drawn to denatured proteins than to original proteins.

Testing was carried out in order to display not only discrepancies between species but also the effect of subjecting species to undesirable temperatures (≥ 5 °C). Once data was retrieved from the data loggers it was apparent that breaches had occurred and did influence the core temperature of the fish even when stored in various compartments of the distribution trucks, as seen in Chapter 3.1. An important point to note is that breaches were correlated with break times, defrost cycles, the opening of reefer doors and landing times at auction warehouses by the producers. This is suggestive that these breaches are certainly avoidable, thus potentially addressing significant product losses. Further analyses were conducted within the lab to mimic not only temperature breaches which occurred during distribution but also to investigate the effects more severe temperature breaches (≥ 15 °C) have on fish.

Over the course of the 16-day storage period microbial (TVC) results between treatments were significantly different with the introduction of temperatures which were experienced during the distribution trials. Results for T1 were as expected, with counts not exceeding the microbial limit for either species until day 16. However, when separate temperature treatments (T2 to T5) were used, microbial counts increased, which indicated that the

temperature led to increases in TVCs to unacceptable levels even by day 6 of testing. Findings indicated that T2 and T3 were not suitable for human consumption after day 12 for both species, whereas T4 and T5 were not suitable for consumption after day 6 of testing, reaching 7.91 log cfu/ g and 8.18 log cfu/ g for megrim and pollack respectively. Any small increase in temperature during storage affected microbial growth and consequently decreased the shelf life. Changes in the freshness of fish can be accelerated or retarded by physical conditions like temperature, physical damage to fish, pollution and contamination by bacterial flora. Of all the physical and chemical factors influencing spoilage, temperature is the primary control parameter in fish spoilage as it directly affects the development of microorganisms. Connell (1990) observed that raising the temperature from 0 °C to 5 °C at least doubled the spoilage level of cod and other related species. This is due to chemical reactions, enzymatic activity and bacterial multiplication requiring an optimal temperature range. This was reflected in this study during quality testing, i.e. colour, texture, TVB-N and sensory, all of which showed the same trend in terms of results when temperatures were varied within the two species.

This study found that there was a significant advantage in the use of temperature logging statistical software during the chilled distribution of fresh fish. The software allowed breaches to be identified during all trials which, when further replicated within the laboratory, produced results which indicated that the temperature fluctuation ≥ 5 °C significantly lowered the acceptability of both species.

Upon further analysis, it was found that maintaining chilled fish at a temperature of 0 to 2 °C was the optimum temperature range for both species to maintain adequate quality and shelf life. Additionally, even short-term temperature breaches impacted all quality parameters (opening and closing or reefer doors, inadequate packing, changes in temperature etc.), with experiments indicating the same pattern of reduced shelf life and a loss of quality in both species, as can be seen in Chapter 3 results. Tests conducted both in the laboratory and during sensory trials correlated with each other, emphasising that temperature variation can affect the overall shelf life and fresh fish quality at any point during exportation.

Findings of this research suggest that breaches can easily occur from several sources within a commercial cold chain which may reduce seafood quality and shelf life; e.g.,

initial condition, ice packing, product location, temperature consistency etc. Therefore, appropriate monitoring procedures, quality parameters and stable composition can help preserve seafood during chilled storage and throughout its relatively short shelf life.

However, the largest cold chain breaches during this research occurred throughout the unloading of fish at the warehouse. This may be attributed to reefer doors remaining open for prolonged periods of time, product left on the slip during transition which could potentially lead to increased exposure to higher outside temperatures and not being stored in sufficiently cooled areas.

Regarding the seafood industry in Ireland, research results may encourage seafood processors to monitor more closely during export, thus ensuring optimum freshness and high quality for longer facilitating higher profitability.

Further research is necessary to assess the entire distribution system from 'ocean to oven' investigating key stages such as on-board fishing vessels before landing, production halls as well as auction halls. The potential to increase the shelf life in both species by superchilling prior to distribution also merits investigation in the future.

5 CONCLUSION

The objective of the study was to determine the effects of temperature fluctuations during chilled distribution on selected quality parameters of megrim and pollack. Temperature was monitored during chilled export to France and Spain. Results indicated that temperature fluctuations commonly occurred during chilled exportation. It was concluded that there are several factors involved in correct cold chain management. Correct ice packing of the product prior to transportation is an important factor in maintaining cold chain efficiency. Product location within a shipment unit was also important as it was shown that products stored closer to the top of the container in comparison to those stored on the bottom experienced less temperature stability during transport. In terms of the transportation it is important to ensure that the refrigerator defrosting cycle not occur (where possible) when product is being transported. Similarly, it is important that the truck driver limit the number of times they open the container during the shipment process as in many cases a recovery time of approximately 3 h is required after such breaches. Collectively all these factors have implications in the quality of fish and overall shelf life. These trials confirmed breaches within the CCMS occurred, therefore further investigation was required into the effect of these breaches on the quality of fish.

A range of temperatures were selected to replicate those identified during the export trials and the resultant quality parameters were measured. When stored under the ideal storage conditions (0 – 2 °C) both species of fish were found to have a shelf life of 12 to 14 days. This was measured using microbial, chemical and textural parameters. Once fish was exposed to increased temperatures its shelf life was decreased. This effect became more pronounced with a temperature increase of ≥ 15 °C for 2.5 h, reducing the shelf life to 2 days for both megrim and pollack.

The results highlight the importance of the CCMS. Breaches in the CCMS whether minor (~ 5 °C) or more severe (≥ 15 °C) can lead to a decrease in fish quality and shelf life. Recommendations would be to reduce the temperature stress on-board trawlers prior to distribution with monitored controls along the route, which could significantly increase the quality of raw fish. The long-term imposition of strict quotas on commercial species has made it necessary for boats to spend more time at sea in order to make fishing trips viable. However, the handling and packaging of raw fish on-board Irish fishing vessels

does not adequately safeguard the fish against additional delays between landing and processing. This study indicates that current fish storage practices using ice alone may not be an effective way to maximise quality during chilled exportation and must be addressed by investment in the infrastructure of modern fishing vessels and technology. Using monitored on-board technology, fish can be kept close to 0 °C by highlighting temperature breaches, thereby slowing spoilage components. The main limitation on the preservation of quality and the decrease in shelf life is the initial freshness of the fish and natural biodegradation during storage. The former can be controlled by treatment at sea, the latter slows down if the initial quality is optimised, which would lead to an overall increase in shelf life.

The largest cold chain violations occurred during unloading at the warehouse in the research conducted. It is recommended that producers conduct periodic reviews of temperatures during transit and during unloading. Compartmental air temperatures should be kept under control in real time. If the air temperature is properly regulated the product temperature will not exceed the required threshold. This research may encourage future producers to develop and implement written procedures to ensure that the product is distributed at sufficient temperatures that enables the quality of the product to remain higher for longer.

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