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## **THE QUALITY CHANGES AND SHELF LIFE OF THAWED RAPID AND SLOW FROZEN WHOLE COD FISH (*Gadus morhua*)**

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### **ABSTRACT**

The main focus for this study was to compare and evaluate the quality changes and to determine the shelf life of previously frozen whole cod fishes (*Gadus morhua*) using rapid and slow freezing methods. Whole cod fishes were frozen to an internal temperature of  $-18^{\circ}\text{C}$  using an air blast freezer (rapid freezing) and a freezer (slow freezing) then kept in the freezer at  $-18^{\circ}\text{C}$  for two weeks. After which all fishes were thawed quickly at ambient temperatures, cut into slices, air package and store at  $4^{\circ}\text{C}$  for ten days. Chemical, microbiological and basic nutritional analyses and sensory evaluations were used to determine the quality changes and shelf life of the thawed fishes. The data and statistical test ( $p < 0.05$ ) showed no significant differences between the two methods of freezing and sensory evaluations determined the shelf life to have ended after three days in chilled storage.

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## 1 INTRODUCTION

Globally, there is an increased demand for ready-made foods from fin fish, crustaceans and other fishery. Today, the main focus is on fresh fishery products, although, the fishing industry has seen a large increase in the production of frozen fish products, mainly due to short shelf-life of fresh fish (Magnussen *et al.*, 2008). This global mandate for fishery products has realised an increased demand for semi-frozen and frozen fish products which are thawed and processed before been sold on the markets (Magnussen *et al.*, 2008).

Commercially, fillets that were previously frozen are often referred to as chilled or defrosted on the markets, since the word fresh is generally not accepted for thawed fish products (Martinsdóttir & Magnússon, 2001). Fresh fish supplies are often insufficient or irregular which offsets the demand for a steady price. Fillets that were frozen at sea are generally frozen soon after catch, however, some consumers have criticised about toughness and dryness of fillets frozen on board vessels. Perchance, previously sea frozen fillets can be offered as a similar or better quality than unfrozen fillets to consumers, better prices could be obtained for these thawed fillets (Martinsdóttir & Magnússon, 2001). Additionally, fillets frozen on board vessels are at all times frozen prior to the onset of rigor mortis and microbial spoilage, hence, the freshness of previously sea frozen fillets could brand them as a speciality on the markets (Martinsdóttir & Magnússon, 2001).

Freezing minimizes microbial and enzymatic activity and hence preserves the flavour and the nutritional properties better than chilled storage (Sampels, 2014). However, the formation of ice crystals during freezing is a critical point, and the larger the ice crystals are formed, the higher is the risk of texture damage and membrane disruption resulting in increased oxidation (Sampels, 2014). Protein aggregation in frozen fish depends on various factors such as the fish species, storage temperature, temperature fluctuation, storage time and enzymatic degradation (Badii & Howell, 2002). The susceptibility of fish species to changes induced by frozen storage is significantly different. In frozen fatty fish, oxidative changes in lipids and pigments affect the odour and colour as well as proteins, while in lean fish the main changes are reported to involve aggregation of proteins which alter muscle texture (Badii & Howell, 2002).

Frozen stored gadoid fish such as cod, hake, pollack and whiting produce high levels of dimethylamine (DMA) and formaldehyde (FA) due to the breakdown of trimethylamine-N-oxide (TMAO) by the action of trimethylamine oxide demethylase (Badii & Howell, 2002). The formaldehyde produced has been proposed to form cross-links with proteins thus resulting in aggregation and toughening of fish muscle (Badii & Howell, 2002). However, this reaction is very temperature dependent as the enzyme activity can be inhibited if the storage temperature is near negative twenty nine degree Celsius (-29°C) or lower (Martinsdottir & Magnússon, 1995). Protein changes in fish frozen under poor conditions can be recognised after the fish is thawed (Johnston *et al.*, 1994). The normally bright, firm and elastic product becomes dull and spongy. The flesh will tend to sag and break and there will be substantial loss of fluid, which can be squeezed out easily and the fish can become dry and fibrous after cooking (Johnston *et al.*, 1994).

St. Vincent & the Grenadines (SVG) fish processing sector is extremely important to SVG fisheries, although it is primarily small scale and utilizes very little machinery. This sector supports about five hundred vendors, exporters, gutters and processors, most of which are cottage industries.

In 2013, fish and fishery product exports contributed 0.3 Million USD to the economy of SVG. Of that 0.3 Million USD, 6.1% was exported as fresh, whole fish, 4.9% was exported as frozen processed fishery products, see Figure 1 (SVG Fisheries Division, 2013). Thus, giving an indication to the magnitude of fish processing in SVG. The high value off-shore pelagic species such as mahi-mahi, wahoo and tunas and some demersal species are usually gutted before sold to local consumers as fresh or chilled on ice, whole or sliced and occasionally as fillets. In some instances, freezing of whole gutted fish is executed by processors. However, it is only performed when there is a large supply and/or landing of the particular fish species. Freezing of fishery products is very important to SVG, as it is the main method of preserving fishery products. Although, other methods of preservation such as smoking, drying and salting are utilized, freezing is the primary method of choice.

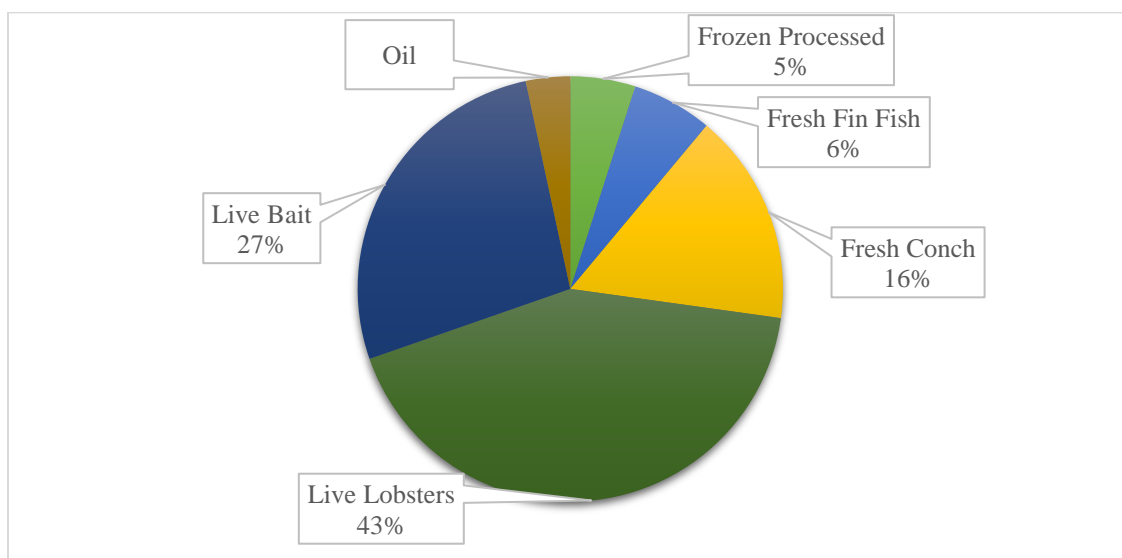


Figure 1: Percentage value of fish & fish products exports in 2013 (SVG Fisheries Division, 2013).

The freezing of whole gutted fish, mostly the pelagic species, is mainly done at the National Fisheries Marketing Limited (NFML), which is the principal and foremost landing and processing facility in SVG. It has the capability and capacity to freeze fish and fishery products to negative eighteen degree Celsius (-18°C) or lower using a blast freezer followed by storage in a freezer at the same temperature or lower. However, this is not usually performed because of the extremely high energy cost associated with using a blast freezer in SVG, limited availability of large amounts fishery products and local consumer's misconceptions of frozen fishery products. Whenever fishery products are to be frozen at the NFML, it is usually placed in the freezer for several days to achieve a frozen product, thus indicating a slow method of freezing. This therefore raises questions of this frozen product quality such as, does the air blast freezing of whole fishes give better product quality than freezing in storage freezer and how should whole fishes be frozen for best possible quality?

For possible assessment of the freezing method quality utilized at the NFML, rapid and slow methods of freezing were selected for study. The comparison should give an indication of the effects on the quality of slowly frozen fishery products produced at the NFML in SVG, giving

guidance for improvement. The production of good quality frozen fishery products in SVG could open up new possibilities for fish processors. New and improved fishery products might be produced which are not season dependent, when the prices and demands are high. Additionally, this might give new and existing processors opportunities for market differentiation and increased revenue.

The off shore pelagic fish species caught throughout SVG are not available in Iceland, which is where the study was executed, Atlantic cod (*Gadus morhua*) was selected as it is a lean fish species similar to the species mentioned above. The division of fish into lean and fatty species is based on the way the different species deposit their lipids. Lean species deposit lipid in the liver and fatty species in the fat cells (Burgaard, 2010). Additionally, scientific research have shown that marine fish have TMAO (trimethylamine-N-oxide) naturally present in its muscle tissue which is broken down to TMA (trimethylamine) by bacteria during ice or chilled storage and to DMA (dimethylamine) and FA (formaldehyde) by enzymes during frozen storage. The production of TMA is mainly responsible for the odour of rotting fish, while, FA causes texture changes to the fish muscle. Thus, analysis of these chemical compounds gives an indication of the quality of lean fish species been frozen and chilled. Therefore, the results on the quality changes in frozen and thawed cod can be compared to the above mentioned off-shore pelagic species caught throughout SVG because of these similar characteristics.

The main focus for this study is to compare and evaluate the quality changes of thawed frozen whole cod fish (*Gadus morhua*) using rapid and slow freezing methods. To achieve this the following objectives should be attained throughout this study. Freeze whole cod fishes to  $-18^{\circ}\text{C}$  in an air blast freezer (fast freezing) and in a cold storage (slow freezing). Conduct quality evaluation using methods of chemical, microbiological and basic nutritional analyses and sensory evaluation on chilled cutlets produced from thawed fishes. Determine the shelf life of the chilled cutlets from the rapid and slow frozen whole fishes through the aforementioned analyses.

## **2 LITERATURE REVIEW**

### **2.1 Freezing, frozen storage and rigor mortis**

Freezing should take place as soon as possible after the fish is slaughtered, preferably before the onset of rigor mortis, subsequently, all intermediate storage will result in lower quality and reduced shelf life after freezing (Magnussen *et al.*, 2008). If immediate freezing is not possible for reasons such as transport, filleting and processing, chilling should take place immediately after catching and product temperatures is quickly reached to below  $0^{\circ}\text{C}$  (Magnussen *et al.*, 2008). Rigor mortis is defined as the stiffening of the muscles of an animal shortly after death. In fish, rigor usually starts at the tail and the muscles harden gradually along the body towards the head until the whole fish is quite stiff (Stroud, 2001). It is possible for the fish to remain rigid for an hour to three days, depending on a number of factors such as the species, its physical condition, the degree of exhaustion before death, its size, the amount of handling during rigor, and the temperature at which it is kept (Stroud, 2001).

Rigor mortis can affect the quality of frozen whole fish in three main ways by causing gaping, toughness and excessive drip loss on thawing of fish (Stroud, 2001). Fish that goes into rigor at



higher temperatures, will have greater drip loss on thawing and when the fish is cooked and eaten, it will be tough and stringy. However, rigor alone is not the only cause of toughness and high drip loss in thawed frozen fish, since, the flesh may be intrinsically tough or it may have been toughened by incorrect freezing, cold storage or thawing. Whole fish frozen pre-rigor tend to have a higher drip loss than similar fish frozen in rigor or post-rigor. Freezing followed by cold storage is an efficient method of fish preservation, nevertheless, the method does not improve product quality. The final quality depends on the quality of the fish at the time of freezing as well as other factors during freezing, cold storage and distribution (Johnston *et al.*, 1994). The deterioration in fish quality during frozen storage is unavoidable. However, if the effects are to be reduced, it is essential that fish be of good quality prior to freezing.

The initial quality of the fishery product prior to freezing and cold storage is of utmost importance as well as the freezing time of the product is also fundamental. The freezing time is the time taken to lower the temperature of the product from its initial temperature to a given temperature at its thermal centre (Arason, 2014). The thermal centre of a particular product is usually in the middle or the thickest part of the product. The freezing time depends on the required final temperature, the physical properties of the product and the freezer specifications (Arason, 2014). Therefore, it is important to be aware of these parameters prior to an attempt of freezing a particular product. Moreover, the fish tissue fluid contains various salts and other compounds in solution, hence the fish muscle freezes in rather a different manner from water. Therefore, the initial temperature of fish has to be reduced to a much lower temperature than water before most of the heat is removed and the fish is completely frozen (Nicholson, 2001).

Freezing and frozen storage of fishery products may lead to denaturation and aggregation of myofibrillar proteins within the fish muscle. These changes within the fish muscle result in altered functional properties, changed textural attributes and reduced water holding capacity and juiciness (Burgaard, 2010). The result is a hard, dry and fibrous fish product which is not appealing to consumers. Moreover, during frozen storage, lipid oxidation occurs in lean as well as fatty species. Oxidation of the phospholipids in lean species results in cold-store flavour and oxidation of triglycerides in more fatty species results in a rancid taste and odour (Burgaard, 2010).

Rapid (fast) freezing may result in small ice crystals distributed inside the cells of the frozen product. The effect of rapid freezing rate on practical food quality is however still discussed as the freezing rate is related to the size of the product, its form or thickness, the amount of product being frozen at the same time, how it is distributed in the freezer and the efficiency of the refrigeration systems (Magnussen *et al.*, 2008). The freezing rate required for fast freezing such as in an air blast freezer is very high and can only be achieved with very efficient heat transfer and large temperature differences between the air temperature of the freezer and the fishery product to be frozen. Multi-purpose air freezers can be used for most fishes, finished or semi-finished fish products, with acceptable results due to the air flow characteristics (Magnussen *et al.*, 2008). Slower rates of freezing have been traditionally found to result in larger extracellular ice crystal formation, which can cause severe tissue damage in frozen foods. Thus, improvement in the freezing process is often related to increasing the freezing rate that may be achieved by more efficient refrigeration systems (Alizadeh *et al.*, 2007).

## 2.2 Thawing

Thawing is the process of changing a product from frozen to unfrozen state. It involves transferring heat to a frozen product to melt the ice that was formed within the flesh during the freezing process (Archer *et al.*, 2008). The thawing time is referred to as the time required to melt all the ice present in the frozen seafood and it occurs at the point where ice crystals are converted back to water completely and the temperature throughout the seafood reaches  $-1^{\circ}\text{C}$  (Archer *et al.*, 2008). Similar to freezing, thawing should be carried out as quickly as possible to maintain product quality, however, it should not be so quick that it adversely affects the product (Archer *et al.*, 2008).

Thawing generally occurs more slowly than freezing which can potentially cause further damage to frozen fishery product texture. However, less attention has been paid to enhancing thawing rates or employing innovative thawing techniques to preserve the tissue structure and product quality (Alizadeh *et al.*, 2007). The thawing rate during conventional thawing processes is controlled by two main parameters outside the product which are the surface heat transfer coefficient and the surrounding medium temperature (Alizadeh *et al.*, 2007). The surrounding medium temperature is supposed to remain below  $15^{\circ}\text{C}$  during thawing, to prevent development of microbial flora. Therefore, the heat transfer coefficient is the only parameter affecting the thawing rate at atmospheric pressure. Indeed, due to microbial and enzymatic activity, a minimal ambient temperature must be ensured for the thawing process (Alizadeh *et al.*, 2007).

The methodologies and techniques used for freezing and thawing processes are important in the preservation of the quality of frozen fish and fishery products. Retention of fresh-like quality such as the colour, texture and juiciness is the primary focus of freezing preservation and expectation of consumers (Alizadeh *et al.*, 2007). Furthermore, it is very important that the product surface does not get too warm during thawing, as this can accelerate spoilage. Thus, once the fishery product is thawed, it must be kept chilled or processed immediately (Archer *et al.*, 2008).

## 2.3 Drip loss

Drip loss is considered to be a form of moisture migration. It is known that fish usually lose weight on thawing and it may be up to 5% of the original product weight for properly frozen and cold stored whitefish, though it can be more if the thawing process is uncontrolled (Archer *et al.*, 2008). The factors that influence thawing drip loss are many and complex. However, it is determined by a number of factors intrinsic to the food product, the conditions of freezing and the thawing process and conditions. Thawing drip loss is visually unattractive, soluble nutrients are lost from the fishery product and it represents a significant economic loss to the processor (Archer *et al.*, 2008). It is stated that the freezing process is generally much more important than thawing for drip loss of fishery products. Drip loss is considered to be most associated with the occurrence of large intracellular ice crystals, which cause maximum damage to the walls of individual cells. The formation of large ice crystals is considered to be associated with slow freezing rates, high and fluctuating temperatures during frozen storage and long frozen storage times (Archer *et al.*, 2008). The role of thawing in minimising drip loss is that very rapid thawing has generally been found to increase drip loss, possibly because of the reduced time for reabsorption of drip. Reabsorption of water is a slow process and can take several hours, particularly for seafood whose muscle structure

has less capacity for reabsorption after it was frozen. However, it is unlikely that long thawing times will be practically beneficial for the majority of fish species (Archer *et al.*, 2008). Consequently, the use of rapid freezing methods, well controlled frozen storage conditions and good temperature control throughout all stages of handling and processing is undoubtedly the best way of minimising thawing drip loss in fishery products (Archer *et al.*, 2008).

A major problem arising from improper freezing and storage procedures is excessive thaw exudates. Thaw exudate (drip loss) is the weight loss during thawing calculated as a percentage:  $100 \times (\text{IFW} - \text{TFW})/\text{IFW}$ , where IFW is the initial fillet/fish weight recorded after filleting and TFW is the weight of the thawed fillet/fish (Mørkøre & Lilleholt, 2007). When muscle tissue is rapidly frozen, small ice crystals form both intra- and extra-cellularly, whereas ice crystals form first in the extracellular space when muscle is frozen slowly (Mørkøre & Lilleholt, 2007). During further cooling, water inside the fish muscle fibres may migrate to ice crystals that already exist in extracellular areas, eventually resulting in very large extracellular ice crystals, compacted muscle fibres and extensive denaturation of the muscle proteins. Loss in liquid-holding capacity of the proteins along with mechanical damage to cells by ice crystals are proposed as the main reasons for the higher amount of thaw exudates from fishery product frozen at higher temperatures (Mørkøre & Lilleholt, 2007).

#### **2.4 Factors influencing shelf life of thawed fish**

Shelf life is defined as the period of time under defined conditions of storage for which a food product remains safe and fit for use (Guizani *et al.*, 2005). In other words, during that period of time the fishery product should retain its desired sensory, chemical, physical, functional or microbiological characteristics. The deterioration in quality of frozen seafood is caused by physical, enzymatic and chemical factors. Quality loss in frozen fish has been attributed to protein denaturation which correlates strongly with loss of sensory quality such as taste, and texture characteristics over the frozen storage period (Martinsdottir & Magnusson, 1995). When the fish is thawed, its storage life will depend upon a large number of conditions such as species, biological condition, freezing method, storage temperature and time, thawing methods, and storage condition of thawed products (Martinsdottir & Magnusson, 1995). The main aims for post-freezing processes are to prevent fall in quality and protect the products during storage and distribution. Correct post-freezing treatment will also reduce decolourisation of the fish flesh. Efficient packing is essential to reducing microbial and chemical contamination, dehydration and mechanical damage from the surroundings. Thus, the typical fish smell will be reduced by packaging and liquid loss from the product, especially after thawing, will also be reduced (Magnussen *et al.*, 2008).

Gaping occurs in the flesh of fishery products when the collagenous microtubules connecting the myotomes and myocommata within the fish muscle breaks. Thus, the formation of large ice crystals has been proposed to explain breakdown of the myocommata that is seen as a physical separation of the myotomes in fillets/slices of fish (Mørkøre & Lilleholt, 2007). The texture of fish is an important contributor to palatability and in turn can influence its purchase and wide use. Therefore, proper control of textural attributes will result in better quality products and will improve consumer acceptance of the fishery product (Mørkøre & Lilleholt, 2007). Fish muscle tissue that is frozen and stored in the frozen state inevitably loses some of its appeal to consumers, usually observed as a loss in juiciness and an increase in toughness after cooking. In Atlantic cod,

formaldehyde (FA) and dimethylamine (DMA) are breakdown products from trimethylamine-N-oxide (TMAO). The formation of FA may cause denaturation and aggregation of the myofibrillar proteins, resulting in irreversible changes in the texture, which becomes harder and less juicy (Mørkøre & Lilleholt, 2007). Furthermore, weight loss in terms of drip losses from fish products may represent direct economic losses. The amount of liquid retained in fish is also important for the general appearance and moistness of the flesh (Mørkøre & Lilleholt, 2007).

## 2.5 Quality evaluation methods for fish quality

The quality of fish degrades because both microbial spoilage and biochemical reactions occur during handling and storage (Guizani *et al.*, 2005). Many methods have been used for the assessment of fish quality during storage. Such methods include changes in the microbial population, total volatile basic nitrogen (TVB-N), and sensory evaluation and in the case of frozen fishery products the changes in the protein and water content for the reasons that protein denatures during frozen storage and drip losses upon thawing. These analysis would give a good indication on the influence of freezing, thawing followed by chilled storage on the quality of the fishery product as it relates to its nutritional properties.

Among the chemical indices of spoilage assessed are trimethylamine (TMA), total volatile bases (TVB) and hypoxanthine contents of the flesh. TMA is the best known compound produced during fish spoilage and it is mainly derived from bacterial breakdown of trimethylamine oxide (TMAO) which is an osmolyte naturally found in marine fish (Lauzon *et al.*, 2010). TMA does not increase much during the early stages of spoilage. It is therefore not considered suitable for discriminating fish stored less than 6 days in ice or chill storage. TVB-N content is an alternative to measuring TMA, and includes ammonia, dimethylamine (DMA) and TMA (Lauzon *et al.*, 2010).

## 3 METHODS

### 3.1 Experimental design

The main focus for this study would be to compare and evaluate the quality changes of frozen whole cod fish (*Gadus morhua*) using both rapid and slow freezing methods as well as evaluating the shelf life of products kept under chilled storage conditions which would be produced from thawing of the whole fish. Therefore, it is important to use a variety of analytical methods to cover different aspects of quality deterioration. Below, the different processes that have been conducted during the project are briefly described as well as a flow chart of the activities (Figure 2).

#### 3.1.1 Receive raw materials

Eight fresh, whole cod fishes were obtained from the Icelandic company *Toppfiskur*. The fishes received were two days old and still in rigor. Therefore, the fishes were placed in chilled storage with ice before commencing the study the following day. One fish was used for obtaining samples to determine the initial TVB-N, protein, fat, ash and water content of the fish and were recorded as day zero (Day 0). The remaining fishes were divided into two groups, slow and fast freezing.

Each fish was weighed and assigned a colour coded tag number, where blue tags were chosen for those to be fast frozen and pink tags for those that would be slow frozen. The largest and smallest fish in each group were inserted with a coded temperature logger, also see [3.2](#).

### 3.1.2 Freezing experiments

Seven whole cod fishes were frozen using both fast and slow freezing method. Approximately four fishes were placed on hooks in a 20 trays harmony blast chiller shock freezer manufactured by ILSA spa set at -30°C with an air speed of 5m/s for three hours followed by frozen storage in an Isocab modular cold room manufactured by ThyssenKrupp for two weeks set at -18°C. Additionally, the three fishes that were slow frozen were placed in the frozen storage for two weeks.

### 3.1.3 Thawing experiments

The whole frozen fishes were thawed quickly at ambient temperature overnight. The fishes were weighed, cleaned and cut into cutlets approximately one inch in thickness, then air packaged in preparation for chilled storage. After this was completed, samples for chemical, microbiological and basic nutritional analyses and sensory evaluation were taken from three different fishes (to avoid individual differences) and were recorded as day one (Day 1).

### 3.1.4 Chilled storage experiments

After fishes were thawed, they were cleaned and sliced into cutlets, approximately one inch in thickness, air packaged and kept in chilled storage for about ten days. Samples were taken from three different fishes (to avoid individual differences) at 3, 6 and 9 days of chilled storage for chemical, microbiological and basic nutritional analyses and were recorded as Day 3, 6 and 9 respectively.

### 3.1.5 Chemical analyses

Samples collected on Day 0, 1, 3, 6 and 9 were analysed for total volatile bases of nitrogen (TVB-N) and water content using the methods: *Determination of TVB-N in seafoods* and *Determination of moisture (water) content in foods and animal feeding stuffs* respectively. The TVB-N measurement was determined in duplicate by direct steam distillation into boric acid using a Kjeldahl-type distillatory. Approximately, 50g of sample was weighed into a blender cup, to which 100mL of 7.5% aqueous trichloroacetic acid was added then homogenized for one minute. The mixture was filtered through fluted filter paper, then duplicate volumes were transferred to distillation flasks with 6mL of sodium hydroxide each. A beaker containing 10mL of boric acid with an indicator was placed under the condenser. After distillation was completed, the boric acid was back-titrated with diluted sulphuric acid solution (0.032N). The TVB-N content was calculated using the equation given and expressed in mgN/100g.

Moisture (water) content method was adopted from the international standard, ISO 6496 (Technical Committee ISO/TC 34, 1999). Into a ceramic crucible containing a thin layer of sand and a glass rod, approximately 5.0g of sample was weighed to the nearest 1mg. The crucible with its contents were placed into an oven set at 103°C ±2°C for four hours after which it was allowed

to cool in a desiccator for 30 minutes and re-weighed to the nearest 1mg. The percentage by mass moisture content was calculated using the equation given in ISO 6496. Additionally, samples collected on Day 0, 1 and 9 were analysed for protein, fat and ash content using the methods: *Determination of crude protein content by block digestion/steam distillation*, *Determination of fat and oil content by Soxtec method* and *Determination of crude ash content in foods and animal feeding stuffs* respectively. Crude protein content method was adopted from the international standard, ISO 5983-2 (Technical Committee ISO/TC 34, 2005). The crude protein content measurement in duplicate was performed using block digestion followed by steam distillation in a distillation unit capable of steam distillation and auto-titration. Approximately 1.0g of sample was weighed to the nearest 0.1mg then placed into a digestion tube with two Kjeldahl catalyst tablets (comprising of 3.5g potassium sulfate and 0.4g copper (II) sulfate pentahydrate per tablet). After which 17.5mL of sulphuric acid (97% purity) was added to the digestion tube followed by digestion at 420°C for three hours. This was followed by steam distillation in the distillation unit capable of steam distillation and auto-titration. The percentage by mass crude protein was calculated using the equation given in ISO 5983-2.

Fat content was determined by utilizing the sample which was used for water content determination, which was finely grounded and mix thoroughly for digestion. A cotton ball was placed in the socket containing the sample. Two boiling stones were placed in a dry, pure aluminium cup, which had been heated in the oven and cooled in the desiccator. The cups were weighed accurately. About 80mL of petroleum ether was placed in aluminium cup. The socket containing the sample was placed in the *Soxtec* device along with aluminium cup containing the petroleum ether, following which the pre-set program was run for about 1hour and 35 minutes. After this, the sample (which is now contained in the aluminium cup) was dried in an oven at 103°C ±2°C for 30 minutes. Then the sample was cooled in a desiccator and the aluminium cup re-weighed. The fat was calculated as percentage of sample.

Crude ash content method was adopted from the international standard, ISO 5984 (Technical Committee ISO/TC 34, 2002). Into duplicate platinum incineration dishes, approximately 2.0g of sample was weighed to the nearest 0.001mg. The incineration dishes containing the samples were heated on a hot plate until the sample was carbonized, after which they was placed into a muffle furnace at 550°C for four hours. It was allowed to cool in a desiccator for 30 minutes and re-weighed to the nearest 0.001mg. The percentage by mass crude ash content was calculated using the equation given in ISO 5984.

### 3.1.6 Microbiological analyses

Samples collected on Day 1, 3, 6 and 9 were analysed for total viable psychrotrophic count (TVC) and hydrogen sulphide (H<sub>2</sub>S)-producing bacteria using the method: *Determination of total viable psychrotrophic count (TVC) and hydrogen sulphide (H<sub>2</sub>S)-producing bacteria by spread plate method*. Total viable psychrotrophic counts and counts of spoilage (H<sub>2</sub>S-producing) bacteria were performed on Iron Agar using the spread-plate method which was adopted from modified Gram, *et al.*, 1987 method. Approximately, 20g of sample was mixed with 180g of cooled Maximum Recovery Diluent (MRD, Oxoid, UK) in a stomacher for 1 minute. Successive 10-fold dilutions were done as required. Total viable psychrotrophic counts (TVC) and counts of H<sub>2</sub>S-producing bacteria were performed on iron agar (IA) with spread-plate method. After which, the plates were

incubated at 17°C for 5 days. Counts of all colonies both white and black on IA give the number of total count and counts of black colonies give the number of H<sub>2</sub>S-producing bacteria. The spoilage bacteria are those which formed black colonies on this medium. Total number of colonies were counted by colony counter and calculated the total viable bacteria and H<sub>2</sub>S producing bacteria by CFU/g.

### 3.1.7 Sensory evaluation

Samples collected on Day 1 and 3 were evaluated for sensory parameters of cooked fish according to the method: *Generic Descriptive Analysis (GDA) for cooked cod fillets*. This method was developed from the Quantitative Descriptive Analysis (QDA) for cooked fish samples description, which establishes a detailed description and quantify product sensory aspects (Meilgaard, *et al.*, 1999). Samples were prepared by taking portions of the cutlets from each freezing method and placing them in coded aluminium boxes, which were then cooked in a steam cooker for 5 minutes. Each freezing method was evaluated in duplicate with different codes. GDA panellists received portions of similar thickness and evaluated products one at a time in separate booths to reduce distraction and panellist interaction. Panellists entered the data into a computer according to the GDA scale, see Appendix 1 for GDA scale used. The Torry freshness scheme was also used for measuring sensory parameters of cooked fish products which starts at a score of 10 (very fresh) and goes down to 3 (very spoiled). A score of 7 indicates that the freshness characteristics of the fish product are no longer detected, marking the end of the freshness period. The end of shelf life is reached at a score of 5.5, where the fish is considered unfit for human consumption.

### 3.1.8 Drip loss

After thawing, the whole fishes were weighed and the weights recorded according to the assigned colour coded tag as indicated in [3.1.1](#). Upon cutting into slices, the total weight of three slices from different fishes were taken and recorded on the respective trays for each freezing method. Throughout, the study a tray containing slices from three fishes were weighed on Day 3, 6 and 9 for each freezing method and the drip loss calculated as a percentage using the equation:  $(ICW - FCW)/ICW \times 100$ , where ICW is the initial cutlet weight recorded after slicing and FCW is the final weight of the cutlet on the specific day.

### 3.1.9 Cooking yield

Samples were taken on Day 1, 3, 6 and 9 for each freezing method for determination of the cooking yield. The total weight of three cutlets for each freezing method was first recorded then cutlets were placed on a rack to be cooked in a steam cooker for about six minutes. After which the samples were reweighed and the cooking yield calculated as a percentage using the equation:  $(ICW - FCW)/ICW \times 100$ , where ICW is the initial weight of the cutlets and FCW is the weight of the cutlet after cooking.

### 3.1.10 Pictures of cutlets

On Day 0, 1, 3, 6 and 9 pictures of uncooked (raw) cutlets were taken for a visual comparison throughout the study.

### 3.2 Temperature profiles

Five *iButton*® temperature loggers (DS1922L/T) were utilized in this study. One temperature logger was placed in the largest and smallest weighed fish for the fast and slow freezing methods. The remaining logger was placed in the cold storage freezer to record the air temperature of the cold storage. After thawing, the temperature loggers were placed in the trays of the air packaged cutlets for the respective freezing method that were kept under chilled storage conditions for ten days.

### 3.3 Statistical analysis

Statistical analysis of the data obtained from this study was carried out by using Microsoft Excel 2013. Analysis of variance (ANOVA) was used to find significant differences of the samples. Additionally, significance of differences was defined at the 5% confidence level ( $p < 0.05$ ).

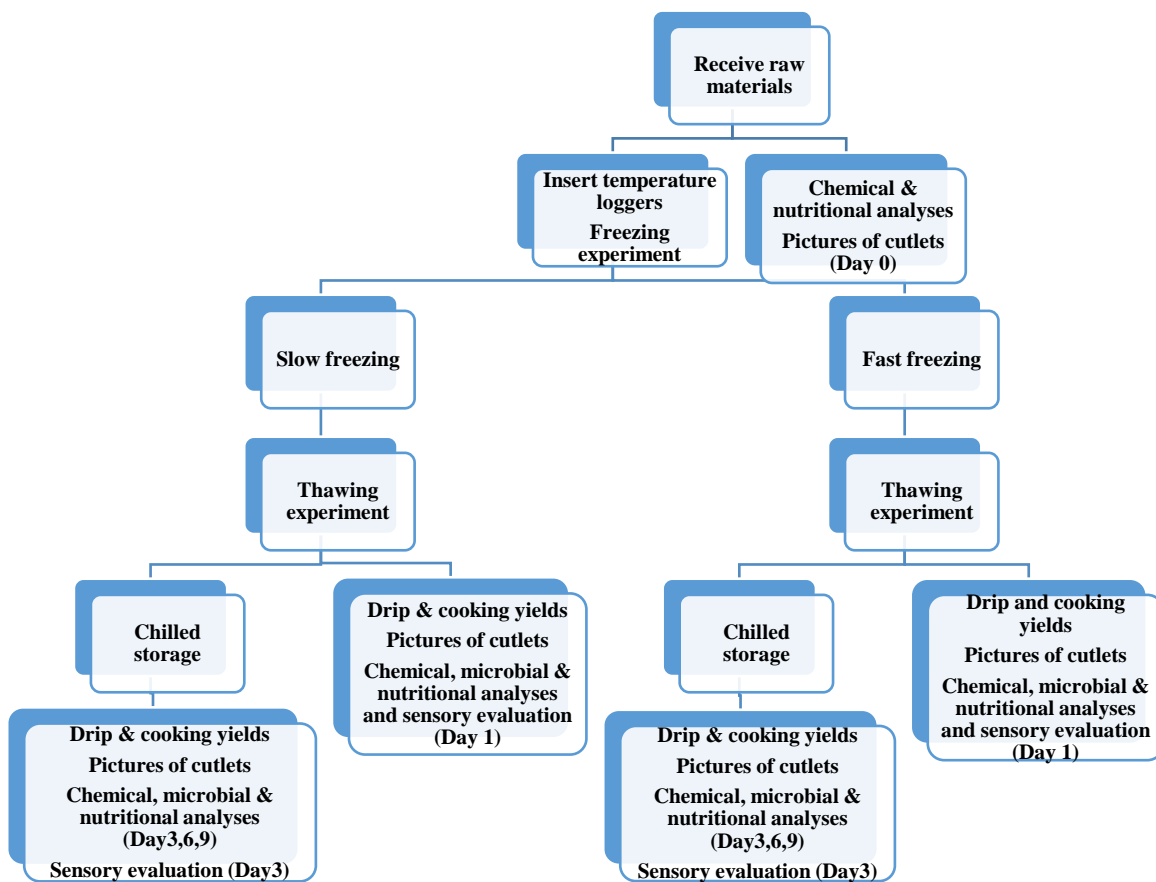


Figure 2: Flow chart illustrating the chronological sequence of sample collections intervals and corresponding analyses.



## 4 RESULTS

### 4.1 Chemical and basic nutritional analyses

The tabulated results and graphical trends of the total volatile bases-nitrogen, water, protein, fat and ash content analyses for fresh, thawed fast and slow frozen cod cutlets samples collected throughout the study are shown in Table 1 and Figures 3-5 below, where Day 0 is before freezing, Day 1-9 indicates the number of days after thawing.

Table 1: Results of TVB-N in mg of nitrogen per 100g and basic nutritional analyses of protein, fat, ash and water content expressed as percentages.

Sampling day	Number of samples	Protein (%)	Water (%)	Fat (%)	Ash (%)	TVB-N (mgN/100g)
<b>Day 0</b>	1	16.7	82.10	0.46	1.14	8.33
<b>Day 1</b>	(fast) 1	17.9	80.89	0.48	1.15	12.96
	(slow) 1	17.5	81.59	0.48	1.14	11.29
<b>Day 3</b>	(fast) 1		80.20			12.90
	(slow) 1		81.50			11.69
<b>Day 6</b>	(fast) 1		80.8			13.31
	(slow) 1		80.8			12.63
<b>Day 9</b>	(fast) 1	17.8	80.5	0.17	1.12	21.93
	(slow) 1	18.1	81.2	0.20	1.16	22.44

There are no significant differences in protein, fat and ash contents between the fishes that were fast and slow frozen as shown in Figure 3, where Day 0 is before freezing, Day 1 and 9 indicate number of days after thawing. These contents remained relatively constant over the freezing and chilled storage periods with no significant differences of the measured contents from before freezing (Day 0) to the final day of sampling (Day 9). Therefore, this indicates that a short frozen storage time (<5weeks) is not sufficient to observe changes in the protein content and other nutritional qualities of the fishery product.



Figure 3: Chart showing protein (green), fat (blue) and ash (yellow) content of the thawed fast and slow frozen cod cutlets for days sampled.

There is a slight decrease in the water content of both the fast and slow frozen cod fishes during the course of the study (Figure 4). There is a slightly higher decrease in water content for the fast frozen fishes than the slow frozen fishes. However, the difference between the two freezing methods is not statistically significant. At confidence level  $p < 0.05$ , it is observed that  $F (1.878)$  is less than  $F$  critical ( $5.317$ ). Freezing of whole cod fishes rapidly or slowly followed by ambient temperature thawing does not significantly influence the water content from its fresh state (Day 0) through to the final day in chilled storage (Day 9).

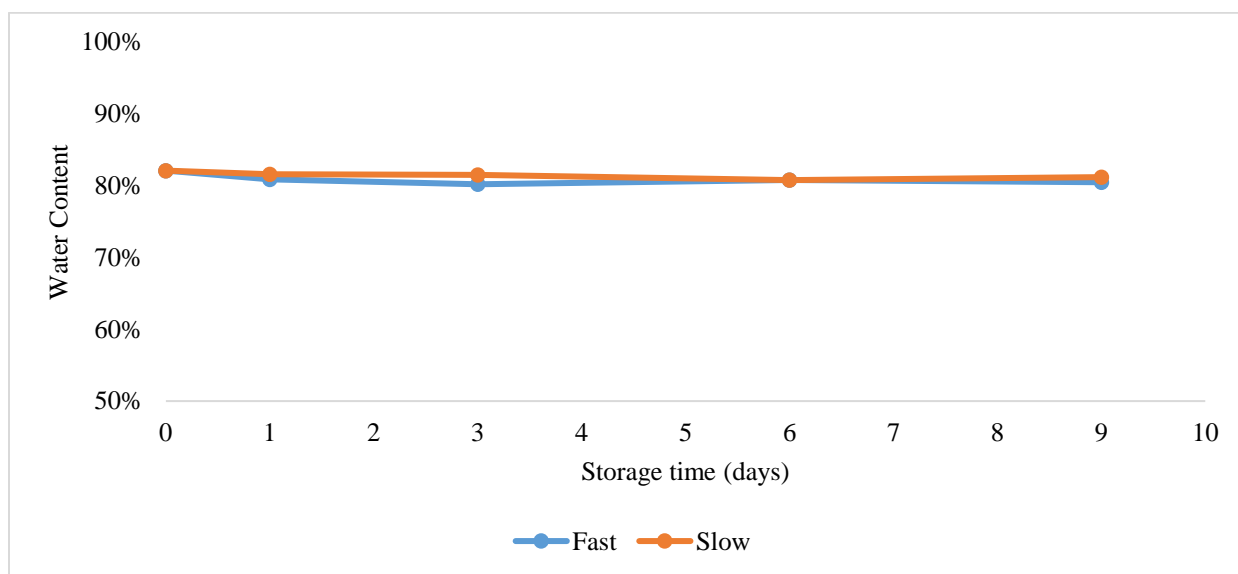


Figure 4: Results of percentage water content for fresh (Day 0) thawed, fast (blue) and slow (orange) frozen cod cutlets over a nine day period in chilled storage.

Generally, an increase in the TVB-N content of both the fast and slow frozen cod fishes was detected during the course of the study. In Figure 5, the TVB-N content for the fast frozen fishes is slightly higher than the slow frozen fishes. However, statistical test ( $p < 0.05$ ) showed that the difference between the two methods of freezing is not significant ( $F (0.034)$  is less than  $F$  critical (5.317)) for the days sampled.

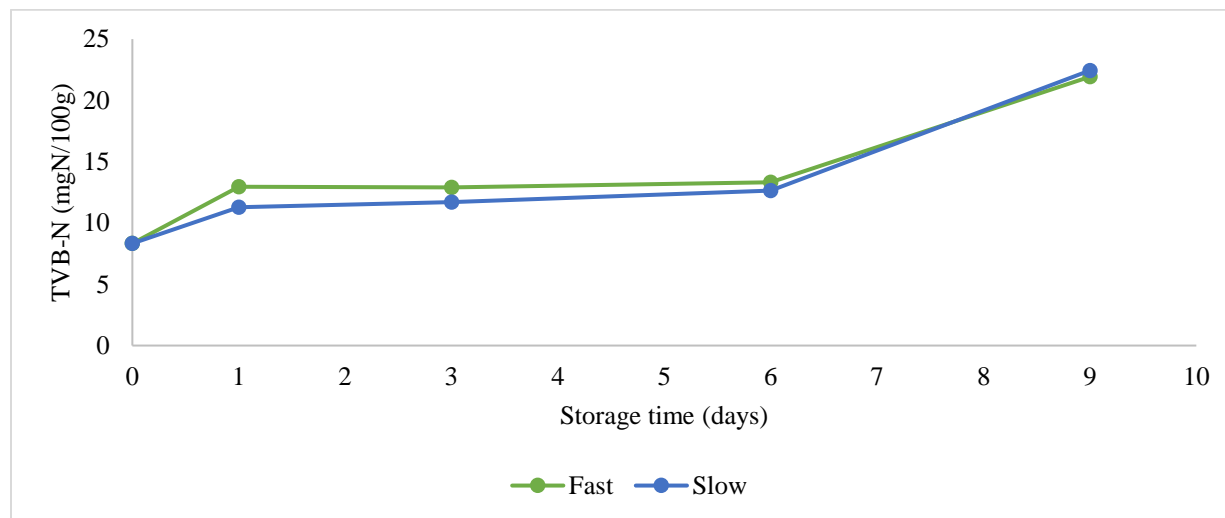


Figure 5: Results of TVB-N values for fresh (Day 0), thawed fast (green) and slow (blue) frozen cod cutlets over a ten day period in chilled storage.

## 4.2 Microbiological analysis

The tabulated results and graphical trends of the log total viable counts and hydrogen sulphide ( $H_2S$ ) producing bacteria (spoilage bacteria) counts for thawed fast and slow frozen cod cutlets samples collected throughout the study are shown in Table 2 and Figure 6 below. The correlation of TVB-N and the log of the  $H_2S$ -producing bacteria for the thawed fast and slow frozen cod cutlets is shown in Figure 7 and 8 respectively.

Table 2: Results of log total viable counts (TVC) and hydrogen sulphide ( $H_2S$ ) producing bacteria counts expressed as colony forming units per gram (CFU/g) for thawed fast and slow frozen cutlets over ten days in chilled storage.

Sampling day	Number of samples	TVC (Log CFU/g)	$H_2S$ (Log CFU/g)
Day 1	(fast) 1	3.61	2.00
	(slow) 1	3.72	2.30
Day 3	(fast) 1	5.57	3.30
	(slow) 1	5.54	4.85
Day 6	(fast) 1	7.20	5.85
	(slow) 1	7.18	5.70
Day 9	(fast) 1	8.00	7.49
	(slow) 1	8.04	7.90

There is a general increase in the TVC and H<sub>2</sub>S-producing bacteria counts for both the fast and slow frozen cod fishes, as shown in Figure 6. However, there is a slightly higher increase in spoilage bacteria counts for the slow frozen fishes than the fast frozen fishes. Statistical test ( $p < 0.05$ ) showed that there is no significant difference between the TVC of the thawed fast and slow frozen cutlets ( $F(0.0003)$  is less than  $F$  critical (5.987)) for the days sampled. Similarly, there is no significant differences between the H<sub>2</sub>S-producing bacteria counts of the thawed fast and slow frozen cutlets ( $F(0.096)$  is less than  $F$  critical (5.987)) for the days sampled. Therefore, indicating that the thawed fast and slow frozen cutlets kept in chilled storage are deteriorating at a similar rate.

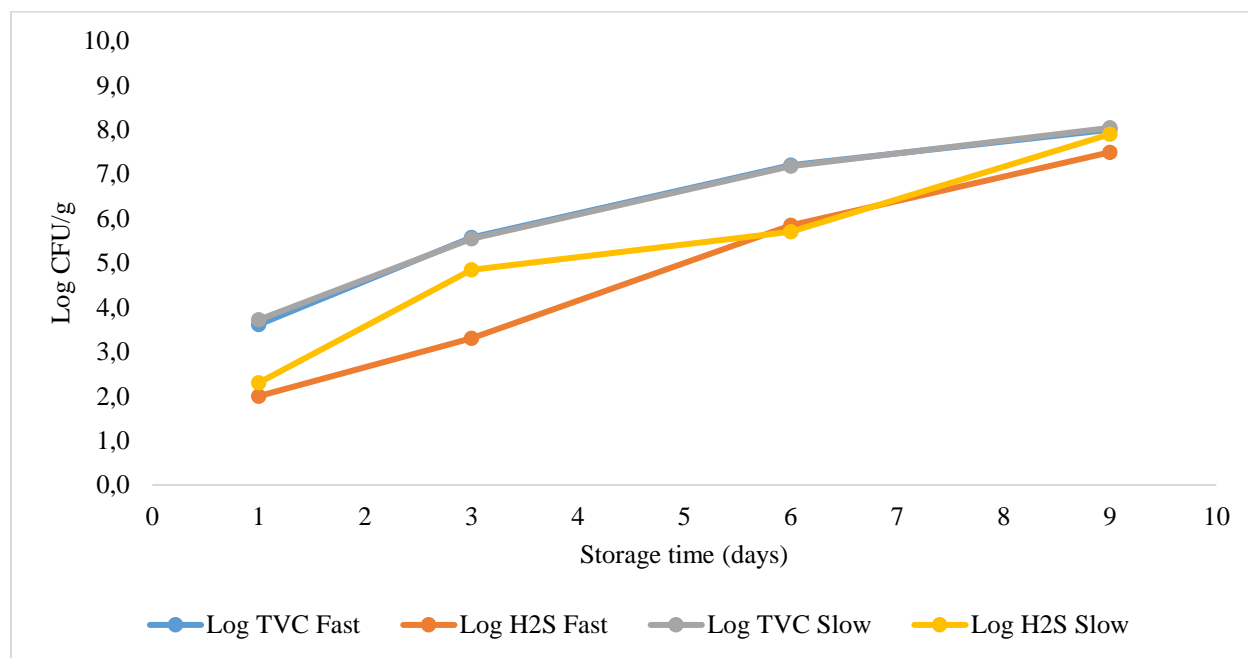


Figure 6: Trends of TVC & H<sub>2</sub>S counts over ten days in chilled storage for thawed, fast (blue and orange) and slow (grey and yellow) frozen cod cutlets expressed as the log of colony form units per gram of fish (CFU/g).

There are reasonable correlations between the TVB-N values and the log H<sub>2</sub>S producing bacteria for fast and slow frozen fishes as shown by the R-squared values displayed in Figure 7 and 8 respectively. Thus concluding that as spoilage bacteria counts are increasing likewise the TVB-N values, indicating a reasonable relationship. Although, higher TVB-N values were expected because of the high spoilage bacteria counts, since both are indications of the deterioration in product quality.

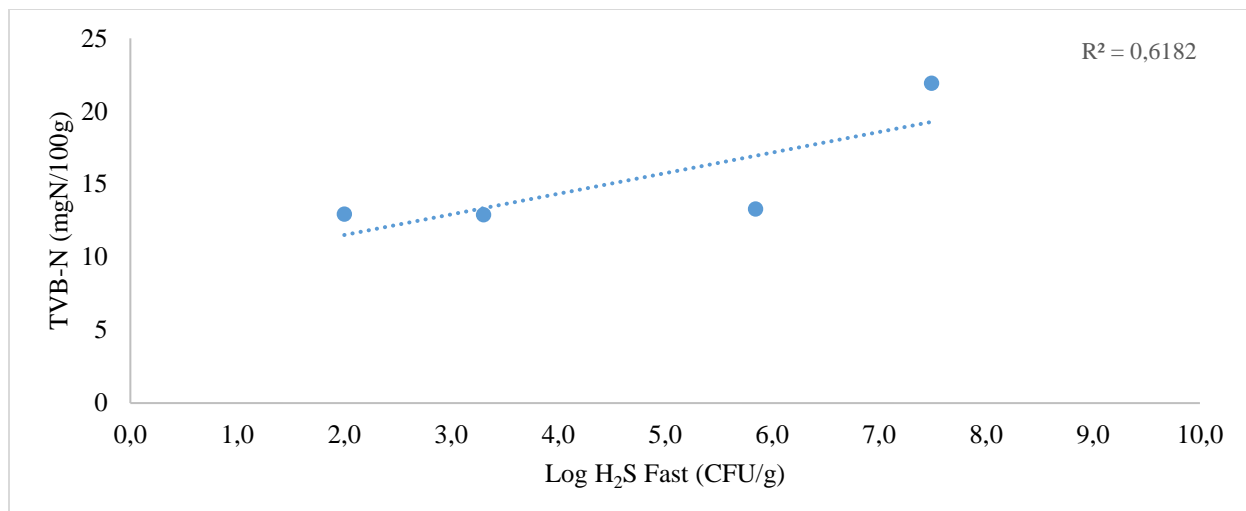


Figure 7: Correlation of TVB-N values in mg of nitrogen per 100g (y-axis) with log of H<sub>2</sub>S producing bacteria in colony forming units per gram (x-axis) for fast frozen cod.

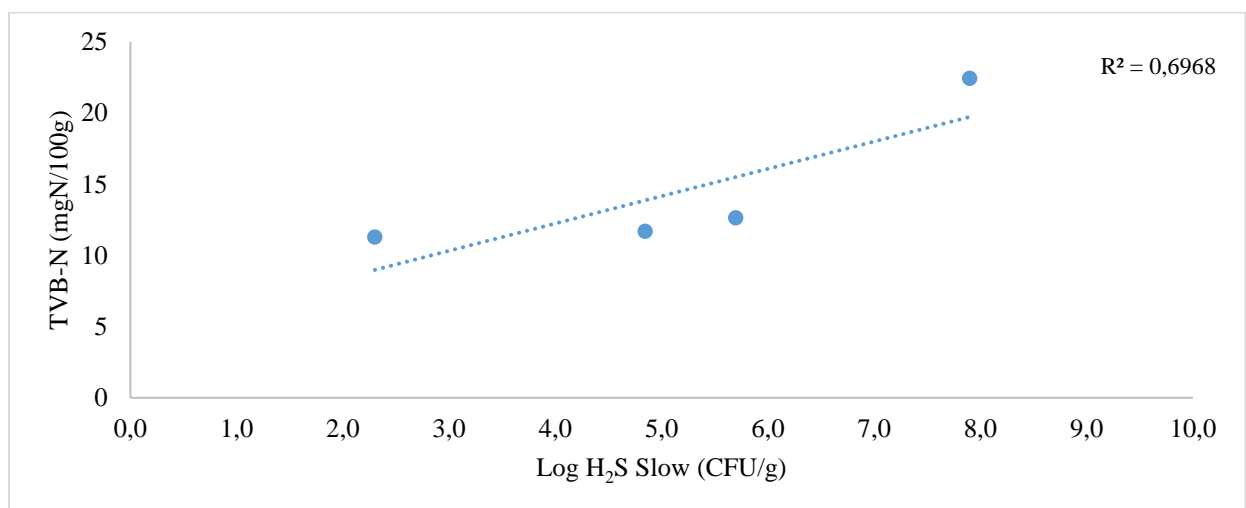


Figure 8: Correlation of TVB-N values in mg of nitrogen per 100g (y-axis) with log of H<sub>2</sub>S producing bacteria in colony forming units per gram (x-axis) for slow frozen cod.

### 4.3 Drip loss and cooking yield

The tabulated results and graphical trends of the drip losses and cooking yields for thawed fast and slow frozen cod cutlets throughout the study are shown in Table 3 and Figure 9 below as percentages.

Generally, there is an increase in drip losses and a decrease in cooking yields for both fast and slow frozen fishes in the duration of this study. Higher drip losses were observed for fast frozen fishes than for slow frozen fishes. However, at confidence level  $p < 0.05$ , the difference between

Table 3: Results of drip losses and cooking yields for thawed fast and slow frozen cod cutlets over ten days in chilled storage.

Sampling day	Number of samples	Drip loss (%)	Cooking yield (%)
<b>Day 1</b>	(fast) 1	3.2	14.6
	(slow) 1	3.7	12.6
<b>Day 3</b>	(fast) 1	8.3	7.5
	(slow) 1	6.4	7.3
<b>Day 6</b>	(fast) 1	8.2	6.9
	(slow) 1	7.5	5.2
<b>Day 9</b>	(fast) 1	8.2	5.0
	(slow) 1	7.8	4.2

the thawed fast and slow frozen fishes drip losses is not significantly different ( $F(0.159)$  is less than  $F$  critical ( $5.987$ )). Difference in cooking yields is not significant ( $p < 0.05$ ) between the freezing methods ( $F(0.074)$  is less than  $F$  critical ( $5.987$ )) for the days sampled. Therefore, these results indicate that freezing whole cod fast or slowly does not significantly influence its drip losses or cooking yields.

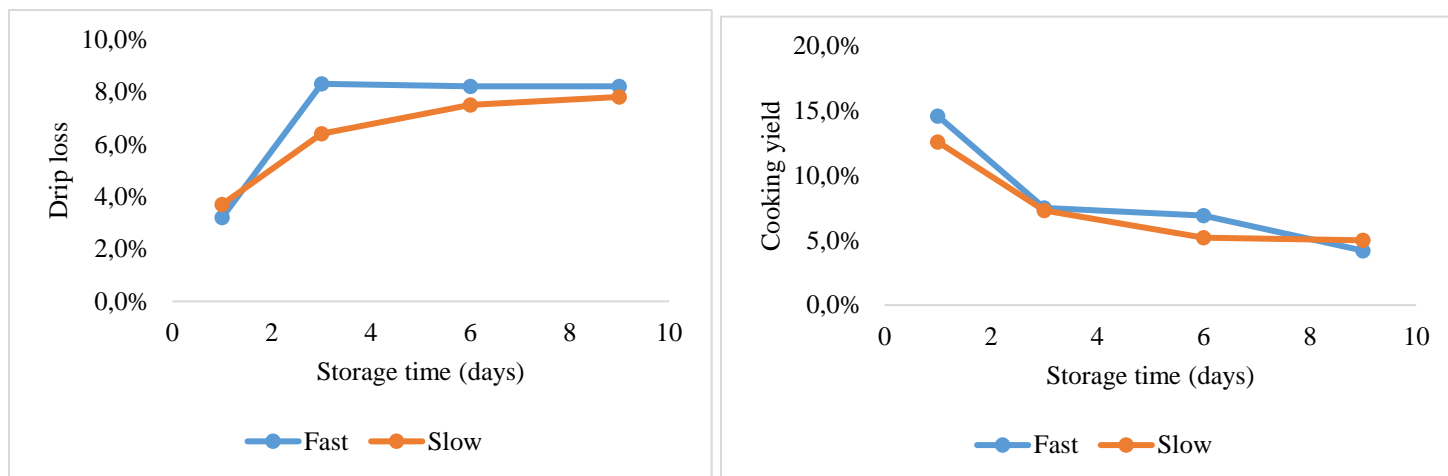


Figure 9: Trends over ten days in chilled storage of the drip losses (left) and cooking yields (right) for thawed, fast (blue) and slow (orange) frozen cod cutlets expressed as percentages.

There is a good correlation between the cooking yields and drip losses for both slow and fast frozen cod cutlets as shown by the R-squared values in Figure 10 and 11 respectively. This gives a good indication that as drip losses increases, the cooking yields decreases for cutlets of both slow and fast frozen cod fishes.

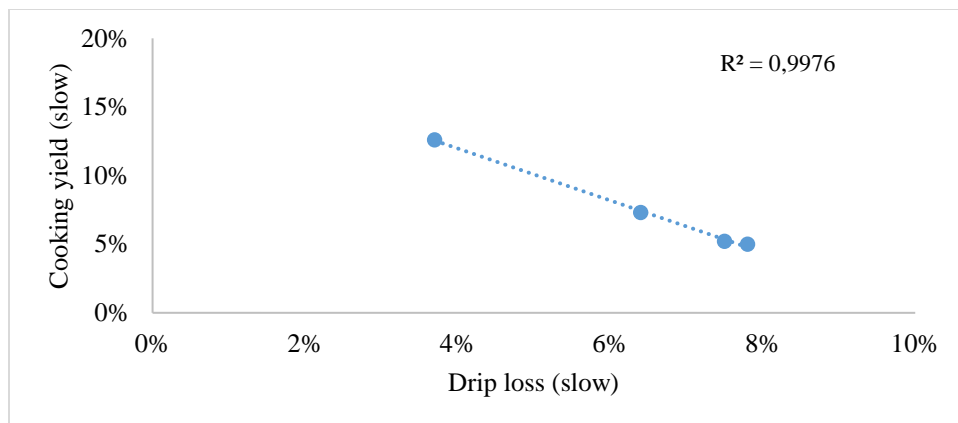


Figure 10: Correlation of cooking yields (y-axis) with drip losses (x-axis) for slow frozen cod cutlets expressed as percentages.

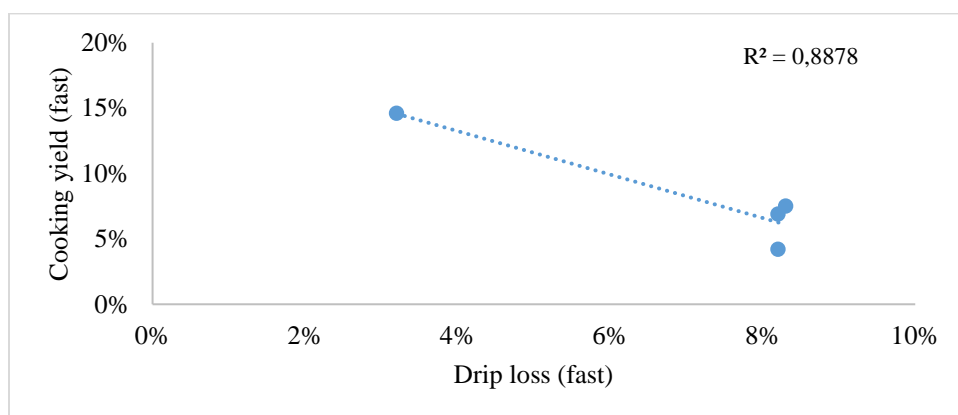


Figure 11: Correlation of cooking yields (y-axis) with drip losses (x-axis) for fast frozen cod cutlets expressed as percentages.

## 4.4 Sensory evaluation

### 4.4.1 Generic Descriptive Analysis (GDA)

The graphical trends of the GDA scores for odour, appearance, flavour and texture attributes given by panellist for thawed fast and slow frozen cod cutlets sampled on Day 1 (day thawed) and Day 3 (three days in chill storage) are shown below in Figures 12-15 respectively. The GDA scores ranged from 0 to 100, where 100 indicates a high detection of the specific attribute and 0 indicates no detection of the attribute.

The freshness odour attributes (sweet, shellfish, vanilla, potatoes) were reasonably detected among panellists for Day 1 of the thawed slow and fast frozen cutlets as indicated by the GDA scores in Figure 12. Whereas, the non-freshness attributes (cloth, TMA, sour, sulphur) were moderately detected on Day 3 for both thawed slow and frozen cutlets among panellists. Statistical test ( $p < 0.05$ ) showed that there is no significant difference between odour attributes of the fast and slow frozen cutlets ( $F(0.552)$  is less than  $F$  critical (2.946)) for the days sampled. Therefore,

panellist was unable to detect significant differences of the odour attributes between the slow and fast frozen cutlets for the sampled days.

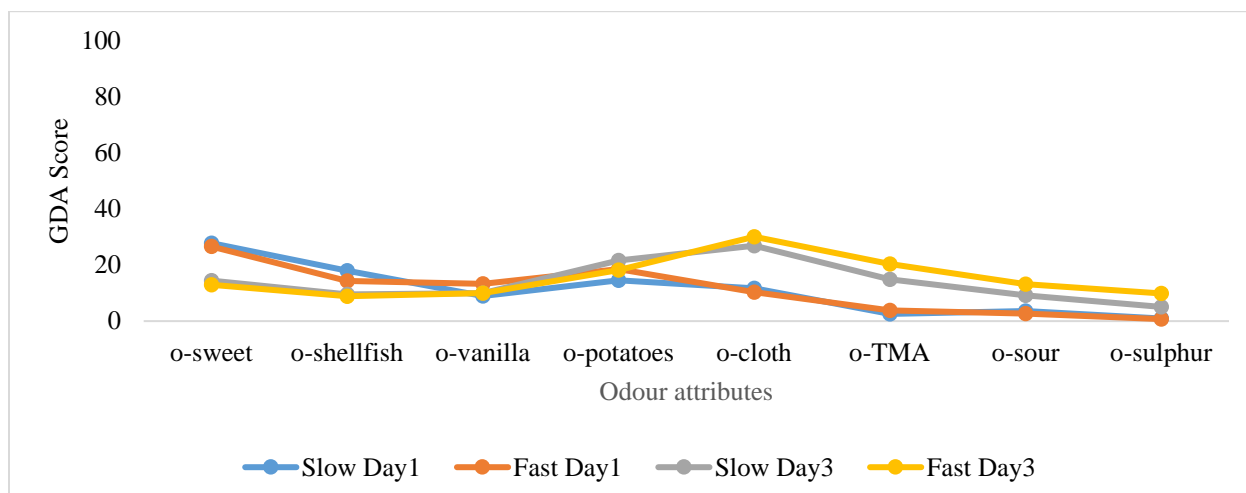


Figure 12: GDA scores of odour attributes for thawed fast (orange and yellow) and slow (blue and grey) frozen cod cutlets on Day 1 and Day 3.

The appearance attributes were moderately detected among panellists for thawed fast and slow frozen cod cutlets on both Day 1 and Day 3 as shown by GDA scores in Figure 13. However, the appearance attributes detection was higher for Day 3 than for Day 1 as shown by the score given for each (Figure 13). Statistical ( $p < 0.05$ ) showed that there is no significant difference between appearance attributes of the fast and slow frozen cutlets ( $F(0.862)$  is less than  $F$  critical (3.490)) for the days sampled. Therefore, panellist were unable to detect significant difference of the appearance attributes between the slow and fast frozen cutlets for the sampled days.

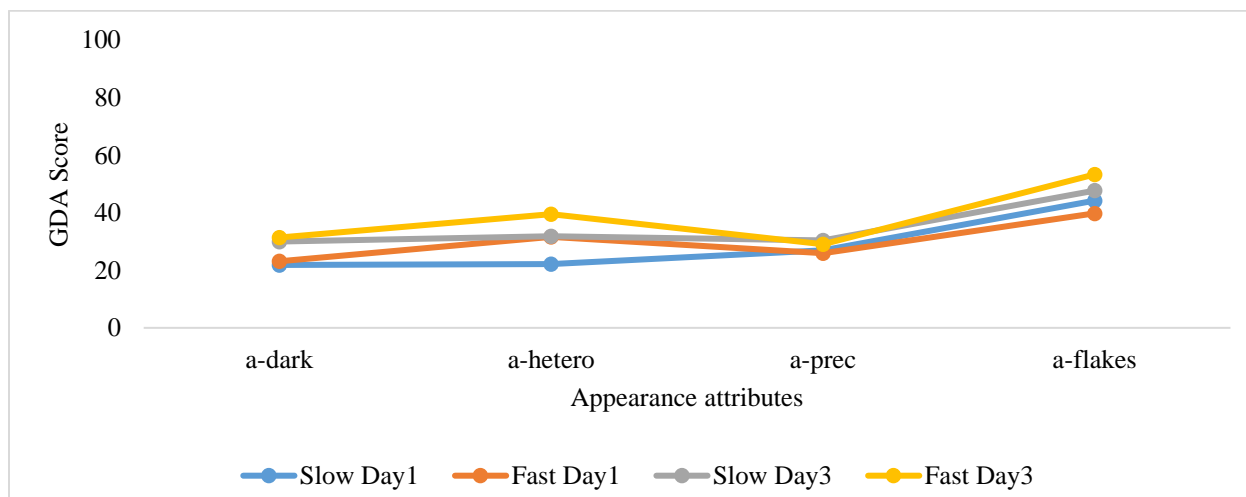


Figure 13: GDA scores of appearance attributes for thawed fast (orange and yellow) and slow (blue and grey) frozen cod cutlets on Day 1 and Day 3.



The freshness flavour attributes (salt, metallic, sweet) were moderately detected among panellists for Day 1 of the thawed slow and fast frozen cutlets as shown by the GDA scores in Figure 14. Whereas, the non-freshness attributes (pungent, sour, TMA, putrid) were reasonably detected on Day 3 for both thawed slow and frozen cutlets among panellists. Statistical test ( $p < 0.05$ ) showed that there is no significant difference between flavour attributes of the fast and slow frozen cutlets ( $F(0.216)$  is less than  $F$  critical (3.008)) for the days sampled. Therefore, panellist were unable to detect significant difference of the flavour attributes between the slow and fast frozen cutlets for the sampled days.

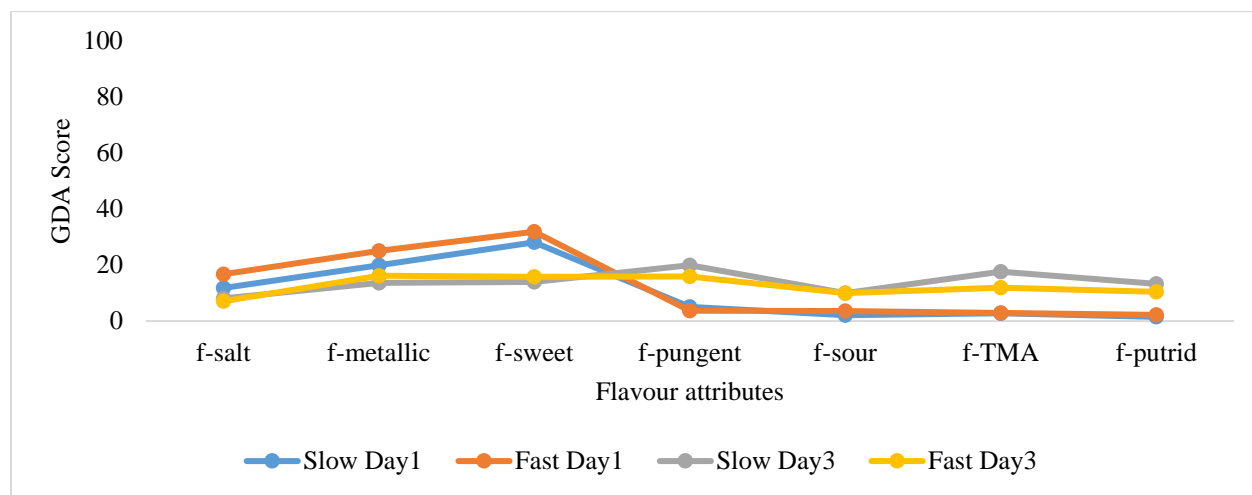


Figure 14: GDA scores of flavour attributes for thawed fast (orange and yellow) and slow (blue and grey) frozen cod cutlets on Day 1 and Day 3.

The freshness texture attributes (soft, juicy, tender) were highly detected among panellists for thawed fast and slow frozen cod cutlets on both Day 1 and Day 3 as shown by GDA scores (Figure 15). The non-freshness attributes (mushy, rubbery) were moderately detected among panellist for the thawed fast and slow frozen cutlets on Day 1 and Day 3 as shown by the scores in Figure 15. Statistical test ( $p < 0.05$ ) showed that there is no significant difference between the texture attributes of fast and slow frozen cutlets ( $F(0.130)$  is less than  $F$  critical (3.238)) for the days sampled. Therefore, panellist was unable to detect significant textural difference between the slow and fast frozen cutlets for the sampled days.

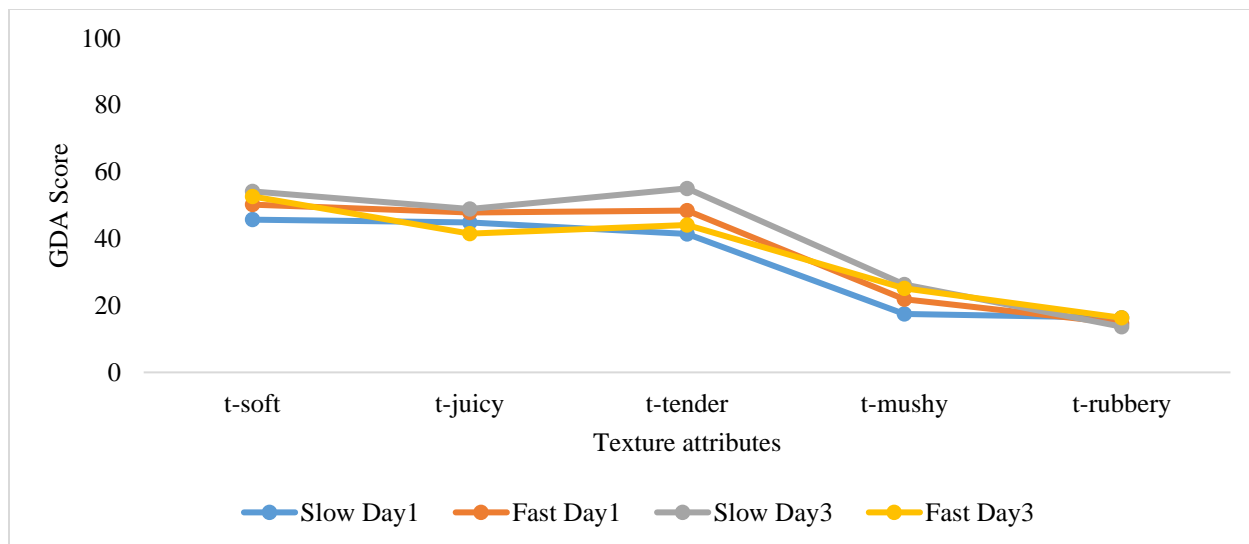


Figure 15: GDA scores of texture attributes for thawed fast (orange and yellow) and slow (blue and grey) frozen cod cutlets on Day 1 and Day 3.

#### 4.4.2 Torry freshness scheme

The tabulated results of the Torry freshness scores for thawed fast and slow frozen cod cutlets on Day 1 and Day 3 of this study are shown below. From the data shown, the panellists were not able to detect the freshness characteristics of the fish for both slow and fast frozen cutlets and considered the shelf life of the cutlets to have ended on Day 3 (three days in chilled storage).

Table 4: Torry freshness scores for thawed slow and fast frozen cod cutlets on Day 1 (day thawed) and Day 3 (three days in chilled storage).

	Slow	Fast	Inference
Day 1	7.6	7.5	A score of 7 indicates that the freshness characteristics of the fish product are no longer detected. The end of shelf life is reached at a score of 5.5
Day 3	4.5	4.4	

#### 4.5 Pictures of cutlets

The collage of pictures for fresh, thawed fast and slow frozen cod cutlets from Day 0 (fresh) through to Day 9 (final day in chilled storage) is shown in Figure 16 below. In the collage, the fish flesh appears fairly firm and not ruptured and the colour was characteristic of the species for Day 0 (fresh) cutlets. After freezing and thawing (Day 1), the fish flesh appears soft, gaping is visible in some cutlets and there are small colour changes for both fast and slow frozen cutlets.

After three days in chilled storage (Day 3), both fast and slow frozen fishes have reddish colour in the flesh visible especially around the bone, the fish flesh appears soft and gaping is visible in some cutlets. For both fast and slow frozen cutlets grey, yellow and brown shades are visible, the flesh appears soft and torn in the cutlets after six days in chilled storage (Day 6). The appearance and texture was unacceptable for the cutlets after nine days in chilled storage (Day 9) for both fast and slow frozen cutlets.

#### 4.6 Temperature profiles

Figures 17-21 below show the internal temperature records of the whole fast and slow frozen fishes and the respective cutlets that were kept under chilled storage conditions throughout this study, as well as the air temperatures. The point at which the internal temperature of the whole fishes reached the chosen freezing temperature of  $-18^{\circ}\text{C}$  is shown by the data label boxes in Figures 17-20, indicating the date and time this occurred. The fast frozen fishes' internal temperatures extended below  $-18^{\circ}\text{C}$  as shown in Figure 17 and 18, however, they were placed in cold storage after three hours in the air blast freezer, thus causing their internal temperatures to increase to  $-18^{\circ}\text{C}$ , the cold storage temperature.

The slow frozen fishes' internal temperatures attained  $-18^{\circ}\text{C}$  after, 14 hours and 30 minutes for the smallest fish and 16 hours and 30 minutes for the largest fish, been placed into the cold storage, as shown in Figures 19 and 20 data label boxes. Similarly, Figures 17-20, indicates the date, time and temperature at which the whole fishes could be considered fully thawed, that is, when the internal temperature of  $0^{\circ}\text{C}$  was reached (data label boxes in the positive temperature region). Figures 17-20 show that after three days in cold storage, the internal temperatures within all the fishes (slow and fast frozen) were one degree above the chosen freezing temperature of  $-18^{\circ}\text{C}$  while been kept in cold storage and at  $2^{\circ}\text{C}$  for the cutlets in chilled storage. Similarly, this trend is reflected in the air temperature records as shown in Figure 21.



Figure 16: Collage of pictures showing fresh (Day 0) cutlets at the top, thawed fast frozen (left) and thawed slow frozen (right) cutlets for Day 1 (second from top) through to Day 9 (bottom).

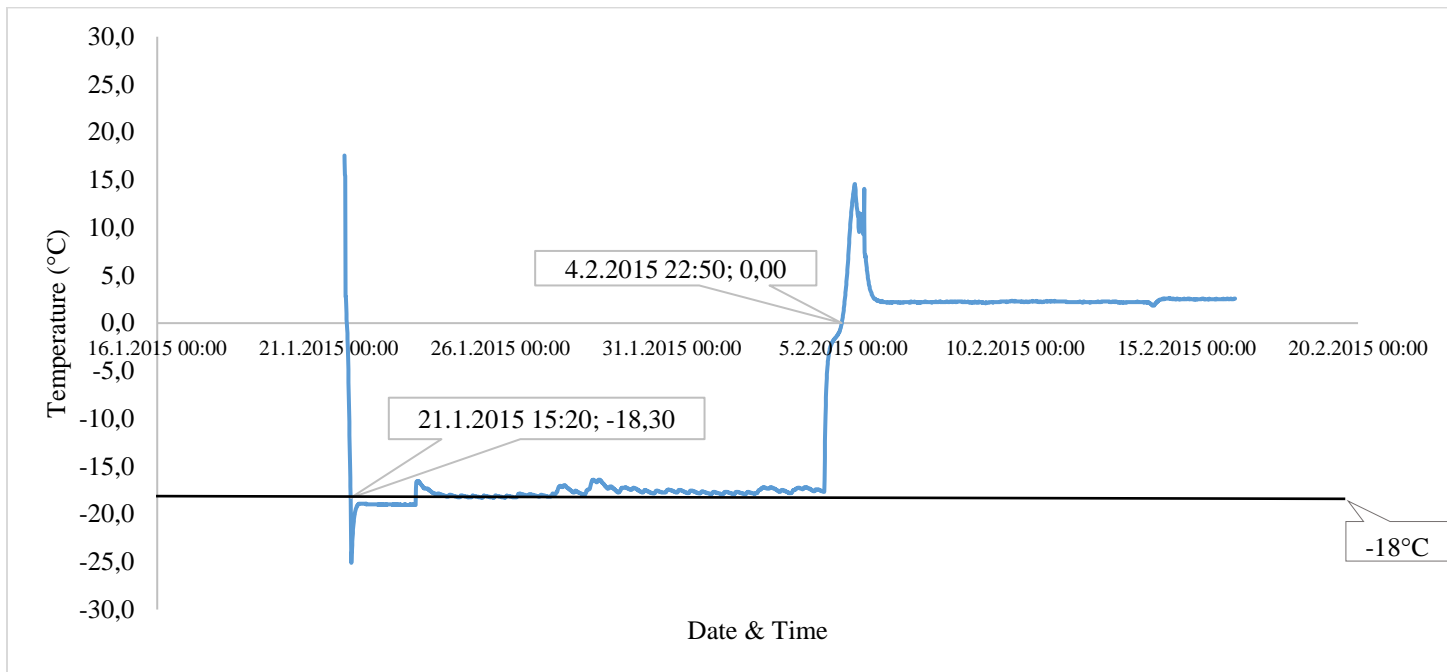


Figure 17: Internal temperature records of the smallest (4.412kg) whole fast frozen fish from freezing through cold storage and cutlets in chilled storage with data labels indicating the point of freezing (below 0°C) and thawing (above 0°C).

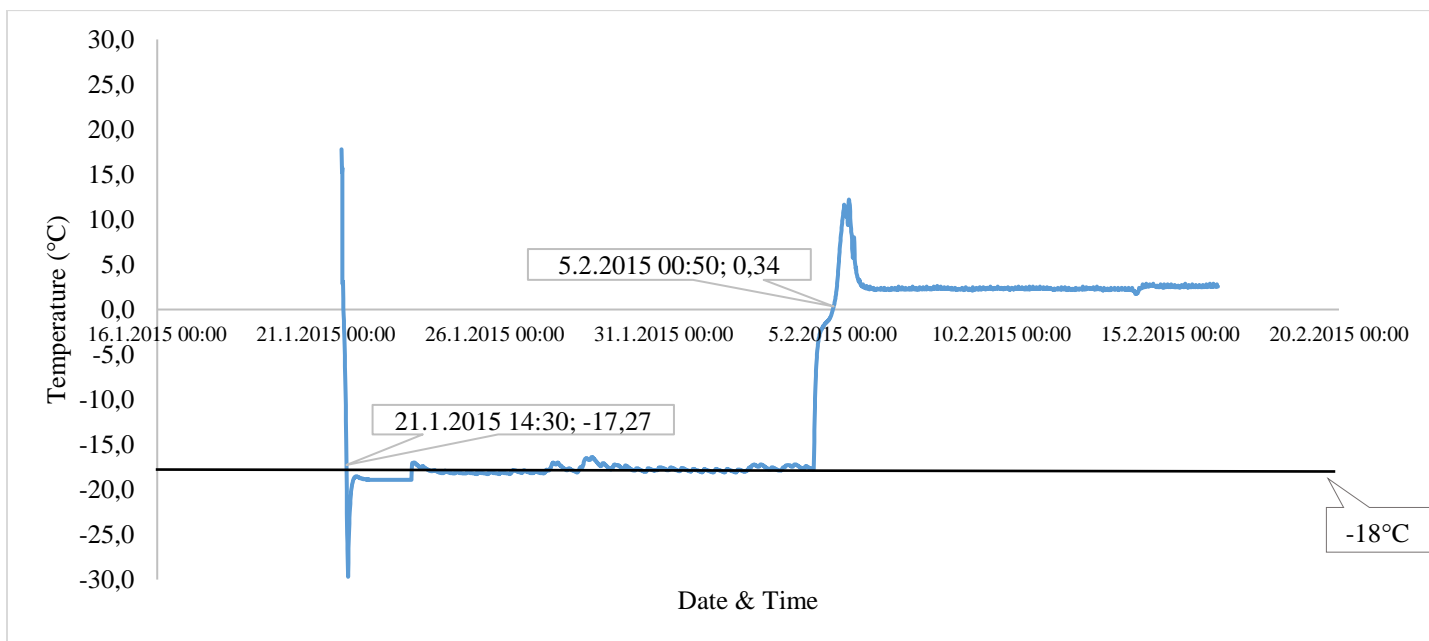


Figure 18: Internal temperature record of the largest (5.116kg) whole fast frozen fish from freezing through cold storage and cutlets in chilled storage with data labels indicating the point of freezing (below 0°C) and thawing (above 0°C).

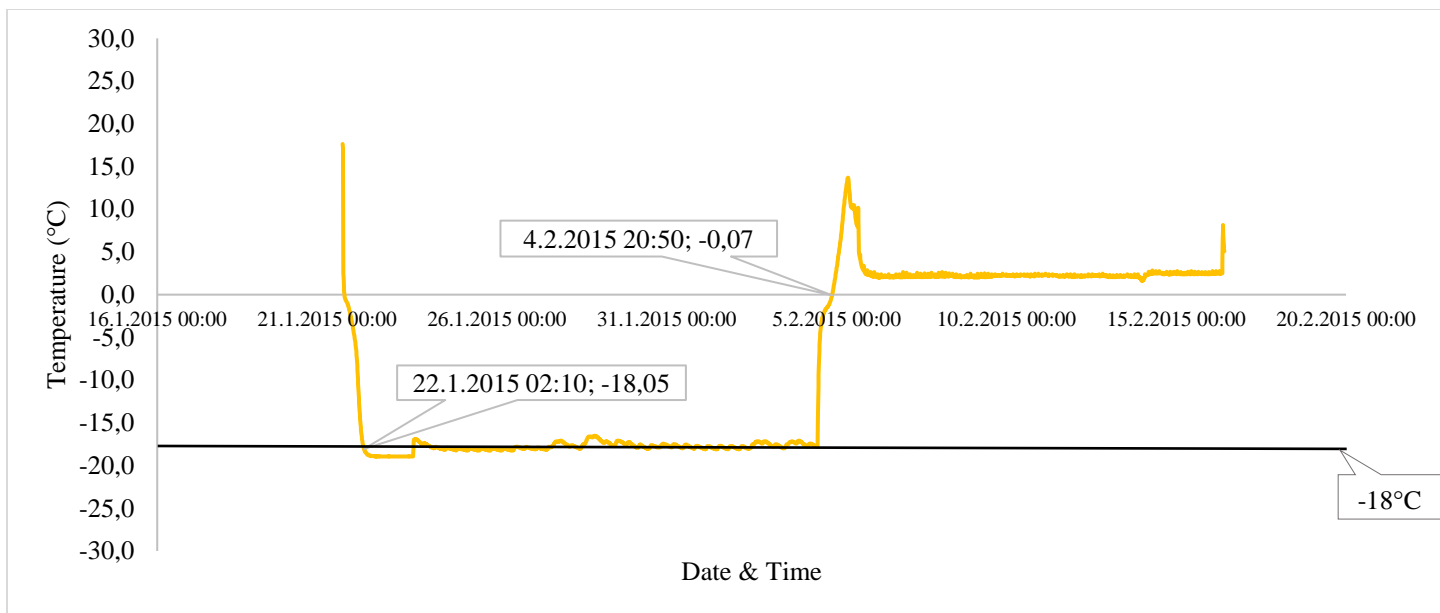


Figure 19: Internal temperature record of the smallest (4.719kg) whole slow frozen fish from freezing through cold storage and cutlets in chilled storage with data labels indicating the point of freezing (below 0°C) and thawing (above 0°C).

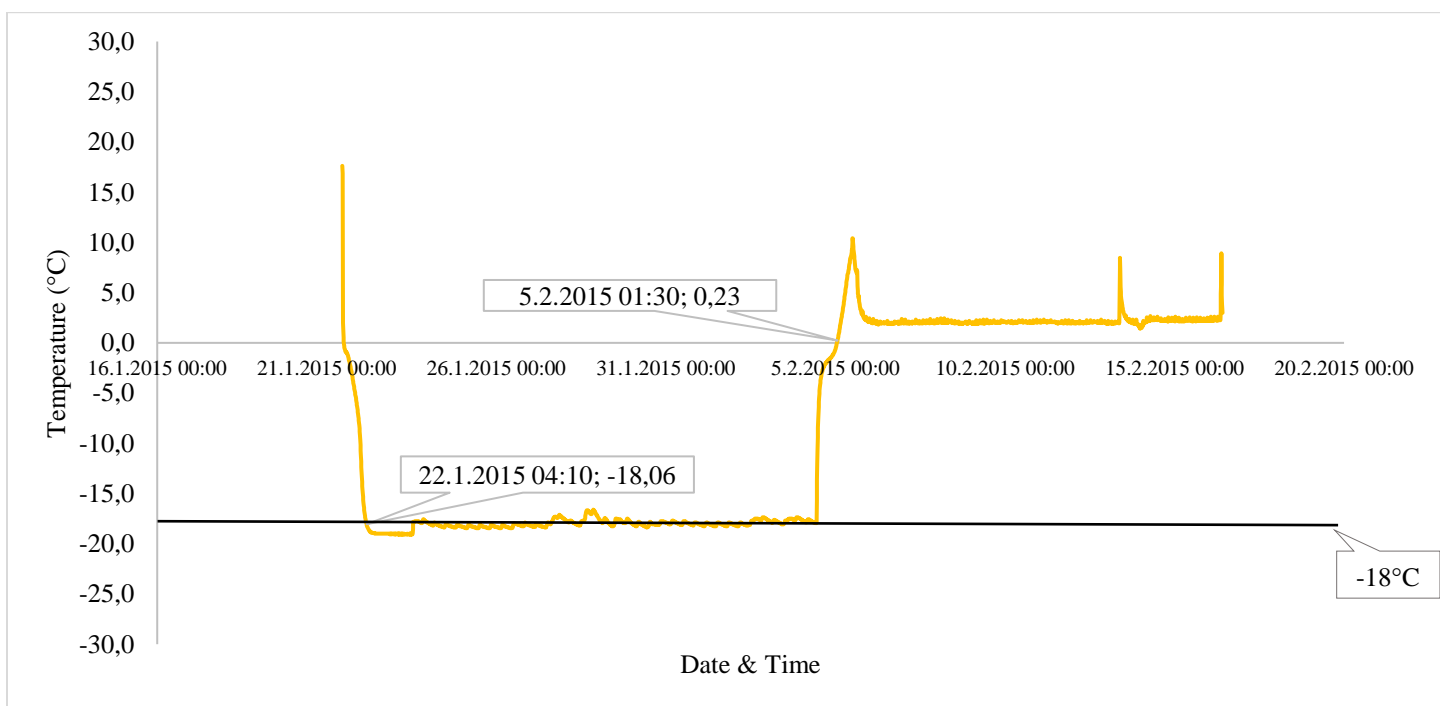


Figure 20: Internal temperature record of the largest (5.193kg) whole slow frozen fish from freezing through cold storage and cutlets in chilled storage with data labels indicating the point of freezing (below 0°C) and thawing (above 0°C).



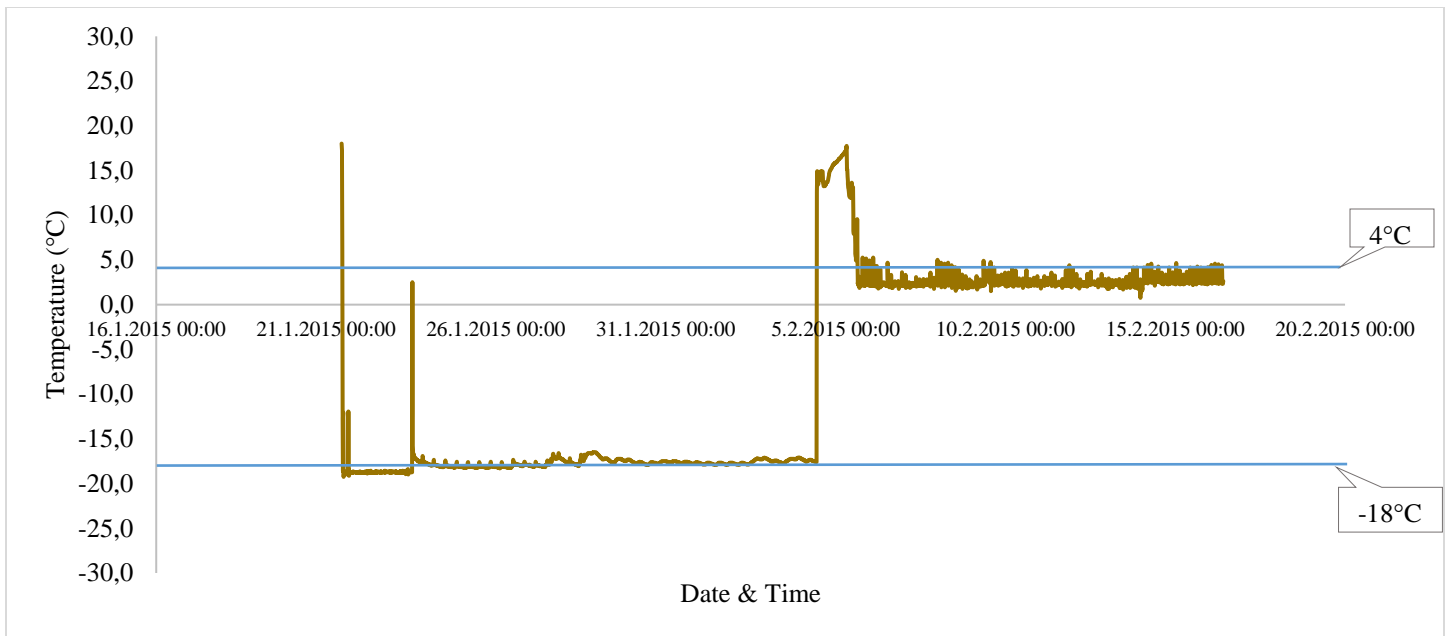


Figure 21: Air temperature record of the cold and chilled storage rooms for which the fishes were kept with labels indicating the freezing temperature (-18°C) and chilled storage temperature (4°C).

## 5 DISCUSSION

Throughout this study, chemical, microbiological and basic nutritional analyses and sensory evaluation did not indicate any significant differences between the rapid and slow freezing methods. The analyses of water content, protein, fat and ash content did not show any significant differences after freezing, thawing and several days in chilled storage for both fast and slow frozen fishes. Additionally, the data and statistical test ( $p < 0.05$ ) showed that there are no significant differences between the fishes that were fast and slow frozen. Perchance, this was attributed to the short frozen storage time of the fishes, although, the research of Martinsdottir & Magnusson (1995) have shown that these properties will decrease over time ( $\geq 5$  weeks) in frozen storage. Rapid (fast) freezing of fishery products which has a more rapid freezing rate than slow freezing should produce a better quality product once thawed than slow freezing (Arason, 2014). However, for products that would be held in cold storage for a short time ( $\leq 5$  weeks) this may not be applicable, hence, no significant difference between the freezing methods. Additionally, low temperatures below 15°C are preferred when thawing at ambient temperatures to prevent the development of microbial flora which causes spoilage of the product (Alizadeh *et al.*, 2007). Therefore, the high ambient temperatures (above 15°C as indicated in Figure 21) that were allowed during thawing could have caused the rapid proliferation of bacteria as well as the presence of the fish's blood, and thus, a shorten shelf life of the products kept in chilled storage. This in addition to the slow processing (cleaning and cutting into slices) prior to chilled storage of all the fishes once thawed, may have contributed to very little significant differences observed between the freezing methods. There was a slow development of total volatile bases of nitrogen (TVB-N) in the thawed fast and slow frozen cutlets for the duration of this study (Figure 5). The TVB-N values remained below the regulated limit of 35mgN/100g for species belonging to the *Gadidae* family (European Union Commission, 2005) for the fast and slow frozen cod fishes, although, spoilage was occurring as

indicated by microbial analyses [4.2](#) and sensory evaluations [4.4](#). The longer fishes have been kept frozen, the slower the TMA (trimethylamine) and TVB (total volatile bases) formation during chilled storage (Martinsdottir & Magnusson, 1995). Therefore, this could possibly be a reason for the slow development of TVB-N observed in the previously fast and slow frozen cutlets kept in chilled storage. During frozen storage, marine fishes which has TMAO (trimethylamine-N-oxide) naturally present in its muscle is broken down to DMA (dimethylamine) and FA (formaldehyde) by endogenous enzymes. However, this reaction is temperature dependant and can be inhibited if storage temperature is near -29°C. Therefore, at frozen storage temperature of -18°C the breakdown of TMAO by the enzymatic action of trimethylamine demethylase would occur in both fast and slow frozen fishes and thus, lead to a low detection of TVB-N observed throughout this study, since, FA would not be detected through this analysis for nitrogen based compounds.

Short term frozen storage ( $\leq 5$  weeks) has little effect on bacterial numbers (Martinsdottir & Magnusson, 1995). Thus, bacterial counts obtained from samples taken after thawing reflects bacterial numbers just prior to freezing, provide that the thawing processing was effectively controlled and did not allow for high temperatures which would aid in the proliferation of microbes. However, thawing was not effectively controlled in this study, hence, high initial bacterial counts for the two freezing methods which lead to rapid deterioration and shorten shelf life of the cutlets. The fair correlation of TVB-N values and the H<sub>2</sub>S-producing bacteria counts (Figure 7 and 8) gives a reasonable indication that as the spoilage bacteria counts increases so does the bases of nitrogen produced by bacterial reduction of TMAO.

Drip losses are inevitable after freezing and thawing of fishes whether slow or fast frozen, however, the quantity of losses should be controlled to maintain product quality. Additionally, excessive drip losses are considered to be mostly associated with the development of large intracellular ice crystals which is associated with slow freezing rates, fluctuating temperatures during frozen storage and long freezing times (Archer *et al.*, 2008). Therefore, the short frozen storage time and the minimal temperature fluctuations in frozen storage may have influenced the quantity of drip losses. The correlation charts (Figures 10 & 11) give a good indication of the importance of controlling drip losses, since it has a direct impact on the cooking yields and by extension the texture and juiciness of the fish. Drip loss may be up to 5% of the original product weight for properly frozen and cold stored whitefish, through it can be more if the thawing process is uncontrolled (Archer *et al.*, 2008). Therefore, the relatively high drip loss values (above 5%) obtained in this study could indicate that the thawing process was uncontrolled while being thawed at ambient temperatures.

According to sensory experts, the quality deterioration of fish is first characterized by the initial loss of the fresh fish odour and flavour (sweet, seaweedy) which is followed by the development of a neutral odour and flavour, leading to the detection of off odours and flavours. End of shelf life is usually determined when sensory attributes related to spoilage such as sour, pungent, TMA odour and/or flavour become evident (Lauzon *et al.*, 2010). As expected the fresh fish attributes were minimally detected among panellists (low GDA scores) for fast and slow frozen cutlets in the GDA evaluations (Figures 12-15). However, differences were detected between the storage days and the end of the cutlets shelf life could therefore be indicated as being after three days in chilled storage (Day 3).



It is important to maintain a stable temperature for the formation of small ice crystals, as thawing and refreezing as well as temperature fluctuations lead to formation of bigger ice crystals. In Figures 17-20, there are slight temperature fluctuations observed for the internal temperatures during the two weeks frozen storage of both the largest and smallest, fast and slow frozen fishes. The figures also show that the internal temperatures as well as the air temperature of the cold storage were above  $-18^{\circ}\text{C}$  for the frozen storage period, although, the fishes were initially frozen to  $-18^{\circ}\text{C}$ . These slight temperature fluctuations may have caused the development of intracellular ice crystals that were large enough to cause damage to the walls of individual cells for both fast and slow frozen fishes (Archer *et al.*, 2008), and thus, may have contributed to the slightly higher drip losses for the fast frozen fishes. Mørkøre & Lilleholt (2007) research of freezing fish at various temperatures for 10-11 days observed that there is a curvilinear relationship between freezing temperature and drip loss. The weight loss was twice the amount for fillets frozen at  $-10^{\circ}\text{C}$  compared to fillets frozen at  $-70^{\circ}\text{C}$ , which led to the conclusion that loss in liquid-holding capacity of the protein along with mechanical damage to cells by ice crystals are the main reasons for the higher amount of drip losses from fillets frozen at higher temperatures. Therefore, the relatively high freezing temperature of  $-18^{\circ}\text{C}$  may have also influenced the observed quantity of drip losses for both freezing methods in this study.

## 6 CONCLUSIONS

The comparison of previously fast frozen and slow frozen cod fishes for differences in quality changes over the period of ten days in chilled storage showed no significant differences between the two methods. Chemical changes in the fish flesh measured as TVB-N gradually increase over time, nevertheless, there was no observed significant differences between slow and fast frozen fishes. Similarly, microbial counts increased during chilled storage and showed no significant differences between the freezing methods. Sensory evaluations determined the shelf life of the product to have ended after three days in chilled storage for cutlets of both freezing methods. Therefore, whole fishes that would be slow or fast frozen then kept in cold storage for less than five weeks will show no significant differences in quality.

If this experiment is to be repeated, the following changes in the experimental process should be considered prior to executing it. Ensure that the chosen freezing and frozen storage temperature(s) are maintained throughout the duration of the study with temperature fluctuations  $\pm 0.5^{\circ}\text{C}$ . Reduce possible sources of microbial contamination that can shorten the shelf life of thawed product such as the presence of the fish's blood during freezing, thawing and chilled storage. Finally, thawing time and temperature should be better controlled and fishes should be processed quickly once thawing is completed then placed in chilled storage.

In application to SVG, the data obtained from this study could imply that the slow freezing method utilized there is sufficient for fishery products that would be kept for two weeks but less than five weeks in the cold storage. After which, the products can be thawed and kept in chilled storage for about three days. Similarly, if the thawing process is not effectively controlled and the fishes are not processed quickly after thawing is completed, there would be significant deterioration in the product quality. Although, the off-shore pelagic species caught throughout SVG has similar characteristics to cod, most of these species are known histamine producers. The microorganisms

are inactivated for histamine producing species during the process of freezing, however, the enzymes already present in the microorganisms will be active even at the sub-zero temperature, which may cause an increase in the histamine content (Lakshmisha *et al.*, 2008). Therefore, temperature fluctuations should be avoid when handling these histamine fish species and the fish freshness quality should be retained prior to freezing, hence, an acceptable quality product would be retained once thawed.

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## APPENDIX

Appendix 1: Generic Descriptive Analysis (GDA) grading score sheet.

Sensory attribute	Short name	Scale anchors	Description of attribute
<i>Odour</i>			
sweet	o-sweet	none   much	Sweet odour
shellfish, algae	o-shellfish	none   much	Shellfish, characteristic fresh odour
vanilla/warm milk	o-vanilla	none   much	Vanilla, sweet heated milk
boiled potatoes	o-potatoes	none   much	Reminds of whole warm boiled potatoes
dishcloth	o-cloth	none   much	Reminds of a dishcloth (damp cloth to clean kitchen table, left for 36 h)
TMA	o-TMA	none   much	TMA odour, reminds of dried salted fish, amine
spoilage sour	o-sour	none   much	Sour odour, sour milk, spoilage sour, acetic acid
sulphur	o-sulphur	none   much	Sulphur, matchstick
<i>Appearance</i>			
colour	a-dark	light   dark	Sample surface. Light; white colour, Dark; yellowish, brownish, grey
heterogeneous	a-hetero	homogenous  heterogeneous	Sample surface. Heterogeneous, discoloured, stains
white precipitation	a-prec	none   much	White precipitation on the sample surface
flakiness	a-flakes	none   much	The fish portion slides into flakes when pressed with the fork
<i>Flavour</i>			
salt	f-salt	none   much	Salty taste
metallic	f-metallic	none   much	Characteristic metallic flavour of fresh cod
sweet	f-sweet	none   much	Characteristic sweet flavour of very fresh (boiled cod)
pungent	f-pungent	none   much	Pungent flavour, bitter
spoilage sour	f-sour	none   much	Sour taste, spoilage sour
TMA	f-TMA	none   much	TMA flavour, reminds of dried salted fish, amine
putrid	f-putrid	none   much	Strength of putrid flavour
<i>Texture</i>			
soft	t-soft	firm   soft	Evaluate how firm or soft the fish is during the first bite
juicy	t-juicy	dry   juicy	Dry; draws juice from the mouth
tender	t-tender	tough   tender	Evaluated after chewing several times
mushy	t-mushy	none   much	Mushy texture
rubbery	t-rubbery	none   much	Rubbery texture, springy