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## **EFFECT OF COMBINED BLAST AND CONTACT (CBC) COOLING AND GUTTING ON THE QUALITY OF TILAPIA (*Oreochromis niloticus*) DURING CHILLED STORAGE**

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### **ABSTRACT**

A study was conducted to analyse the effect of the Combined Blast and Contact (CBC) cooling technique on the quality of gutted and ungutted tilapia during cold storage in terms of microbial, physicochemical and sensory attributes. The storage temperature was -1°C for the initial 6 days and after that 2°C for simulating sea freight and transportation and retail chain in Europe respectively. The storage time was 20 days. The CBC treated fish maintained a lower temperature compared to the untreated during the last days of the storage. Ungutted tilapia showed higher Total Viable Count (TVC), Specific Spoilage Organisms (SSO), pH, Total Volatile Base- Nitrogen (TVB-N) values and spoilage compared to gutted fish. Therefore, tilapia should be gutted during processing. The effect of CBC treatment and gutting were minor in shelf life determination. The shelf life of tilapia was 17 days on the basis of microbial and sensory analysis.

**Keywords:** Tilapia (*Oreochromis niloticus*), super chilling, combine blast and contact (CBC) cooling, gutting/ungutting, shelf life

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## 1 INTRODUCTION

Temperature is the single most important factor in keeping the quality of fish. After death, enzyme activities take place in the muscle of the fish and the pH increases. The activities of micro-organisms also increases with higher ambient temperature and higher pH (Huss 1995). The microbial activities are reduced with decreasing temperature and therefore, it is essential to cool the fish in order to maintain acceptable quality.

In Iceland, super chilling by combined blast and contact (CBC) cooling has developed in recent years and is commonly used in the fish processing factories. The cooling process is the combination of two stages, pre-cooling and CBC stage. In pre-cooling stage, the fish is immersed into liquid ice or ice slurry and within ten minutes the temperature is reduced to sub-zero level. Then the fish is transferred to CBC cooler, where the temperature of the fish is lowered quickly to around  $-1^{\circ}\text{C}$  by using both the advantages of contact cooling between the fish and the metal surface of the conveyor and cooling by blasting chilled air over the fish.

Iceland exports fish primarily to the European Union. The companies are targeting to export fishes to those countries. But high expense of air freight is one of the major impediment. Using sea freight is a possible solution. The major cities of Europe are 4-6 days away from Iceland by sea freight.

Bangladesh is a low lying country with numerous waterbodies covering an inland area of 4.575 million hectares and an Exclusive Economic Zone (EEZ) of 1, 66,000km<sup>2</sup>. These inland coastal and marine waters are the main sources of fish. The fisheries sector contributes 4.43% of the overall GDP 22.21% to agricultural GDP. Fish products supplements to about 60% of daily animal protein intake. Approximately 10% of the population are directly or indirectly dependent on the fisheries for their livelihood (DoF 2012).

Bangladesh is one of the world's leading fish producing countries with a total production of 3.26 million MT in the financial year 2011-12. About 55% of the fish is produced from aquaculture. The annual production of tilapia was estimated at 136,541 MT (System, 2013). In 2010-11, the production was approximately 104,968 MT. There is no separate data for tilapia in earlier years, but the trend shows an increasing production.

Tilapia was introduced in Bangladesh in 1954 for mosquito control. In the beginning, it was not successful as a commercial fish. It was then only used as an occasional source of fish and was called 'chicken fish' as they were easy to fish and being a prolific breeder. But in early 90's Genetically Improved Farmed Tilapia (GIFT) was introduced to Bangladesh and started to get acceptance as a commercial fish. At the start of this millennium, the introduction of monosex tilapia changed the whole scenario. Tilapia farming overtook pangas farming as it was more acceptable to the consumer and the market price relatively higher. Tilapia is harvested all the year around but peak harvesting season is from August to December (Ahmed, 2009). Most of the time, live fish is sold at the farm and is already dead when it reaches the market. During transportation from one city to another, quality loss of the fish is high mainly due to lack of processing and preservation technology.

Block ice is the most common type of ice used for keeping the quality of fish in Bangladesh. Traditional ice plants use sodium or calcium chloride brine as cooling medium. Water filled cans are submerged in cooling medium to produce ice. The freezing period is between 8 and 24 hours depending on the dimensions of the cans and the temperature of the brine. The block

weights from 12 to 150 kg. A common size of block ice is 2.5 x 1.5 x 1 feet weighing 70-80 kg (Alam 2007). No study has been done on superchilling in Bangladesh and no superchilling methods have been used in Bangladesh for tilapia.

Despite of having a huge possibility to access the world market, fish farmers are struggling to get even the lowest benefit. Due to an unstable domestic market, during the peak season the supply of fish is greater than the demand. Use of feed and other chemicals, medicines and farming inputs are common. Evidently due to the higher price of these ingredients the farmers get a lower profit margin. In the peak season the farmers struggle to make up their production costs. Some farmers feel exploited by the intermediaries, believing the prices they receive do not adequately reflect the final tilapia prices paid by the consumers. The possible solution is to export fish to the foreign countries. The world market for chilled tilapia is larger than for frozen tilapia and transportation by sea is cheaper than by air. Therefore research is needed to establish a guideline for keeping quality of tilapia under a chilled condition for extended period of time.

### 1.1 Objective

Sea freight is cheaper than the air freight. Any additional cost during production to consumer is added to the product cost. Therefore, sea freight can be a better option to export chilled fish if the storage life of the fish can be extended by improving the cooling technique. The goal of this project is to provide high quality fish to consumers with extended days of storage by improved cooling techniques for fresh farmed tilapia.

The objective is to analyse the effect of CBC cooling technique on the quality of gutted/ungutted tilapia during storage in terms of microbial, physio-chemical and sensory attributes.

## 2 STATE OF THE ART

### 2.1 Post mortem changes in fish

It is important to understand the post mortem changes in fish in order to apply better management in keeping the quality of the fish.

After the death of the fish the enzymatic activity starts to digest fish flesh. This enzymatic activity is called *autolysis*, which means self-digestion and the fish quality starts to decrease. The quality changes can easily be noticed and consist of changes in colour, odour or smell, taste, appearance and texture. After death, the fish goes through rigor-mortis or death stiffening due to the action of ATP breakdown. After rigor mortis the fish muscle becomes softer. The time of pre-rigor mortis and rigor mortis varies according to species. It also depends on many things like temperature, handling, size and physical condition of the fish (Huss 1995). Live specimens of the plaice *Paralichthys olivaceus* were spiked at the brain, stored at various temperatures ranging from 0-20°C and examined for changes in rigor tension and ATP degradation in the muscle. The ATP degradation rate was clearly slower at 5–15°C than at 0°C, resulting in retardation of rigor- mortis onset at the former temperatures. Lactic acid accumulation in the muscle correlated well with the decrease of ATP. The muscle showed full rigor when ATP completely disappeared and lactic acid attained the maximum plateau of 40–50 µmol/g (Iwamoto *et al.*, 1987).

The difference between acclimatization and storage temperature has a great influence on onset and progress of rigor. For difference between acclimatization and storage temperature of 0 and 5°C, the onset of rigor in carp was its maximum at 72 hour. But when the difference was 30°C, the onset of rigor was 24 hour. Maximal rigor index for 30°C and 0 & 5°C difference was almost 100% and only below 60% respectively. After transfer of carp from 14 to 30°C, it took 4 weeks to change the rigor-mortis progress to a typical 30°C pattern (Abe and Okuma 1991).

The muscle of the fish is free from bacteria just after death and bacterial fauna is found on fish skin, gills and intestine. According to Shewan (1962) the range in intestine and gills is between  $10^3$  to  $10^9$  cfu/g. But Liston (1980) stated the difference as  $10^2$  to  $10^7$  cfu/g/cm<sup>2</sup> in skin. Bacteria is temperature sensitive. Different species of bacteria are commonly grouped on the basis of temperature tolerance. Psychotropic or psychrophilic bacteria are able to grow and regenerate at a low temperature even close to 0°C but their optimal temperature is near 20°C (Morita 1975). Another group is the mesophilic bacteria which are a dominant tropical bacterial group. Prevalence of bacteria is less in low temperature. The dominant temperate region bacteria are the psychotropic gram negative bacteria (Huss 1995). Gram positive bacteria like Bacillus and Clostridium can be present in fish. Some of the other studies disagree with this idea and states that both the gram negative and positive bacteria can live anywhere (Gram *et al.* 1990).

Spoilage of fish is the result of microbiological growth and becomes evident as visible growth like moulds, pigmentation, slimy bacterial colony etc. There are direct relationship between the total number of microorganisms and degree of spoilage (Gram and Huss 1996).

At the beginning, the number of bacteria are low, which start to grow in favourable conditions. Some of the bacteria among these are capable of producing off-odours and off-flavours. The latter start growing very fast and at a point dominate to an absolute majority. Meanwhile, some of the chemical changes also evident and can be measured. This point can be identified by counting bacterial load and measuring the chemicals produced. This is the sensory and microbial rejection point for a fish due to spoilage (Gram and Huss 1996). The model described is presented in Figure 1 below.

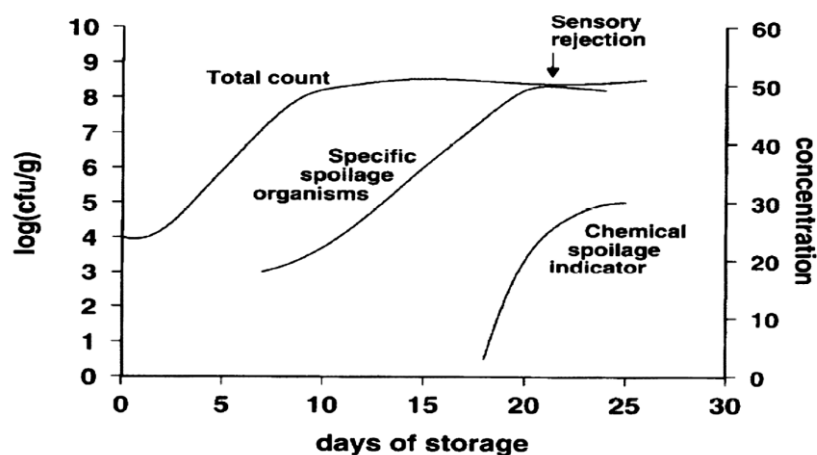


Figure 1. Model of changes in total count (TVC), specific spoilage organism (SSO) and chemical spoilage indicator during chilled storage of fish product (Gram and Huss 1996)

## 2.2 Super chilling and shelf life

The conventional chilling in the past was to reduce the fish temperature to around 4°C. This temperature may be enough to prevent the growth of microbes but not enough to preserve the fish for a longer period of time. For most of the species the initial freezing point in fish muscle is between -0.8° and -1.4°C (Sikorski 1990). At this temperature ice crystals start to form. Then, due to ice crystal formation and lower temperature, the microbial growth and survival is drastically reduced. This is how the idea of superchilling came about. Super chilling is also sometimes referred to as “partial freezing,” “deep chilling,” or “hard chilling.” Superchilling means reducing the temperature of fish uniformly to a point slightly below the melting ice, thereby extending the storage life of the fish. Contemporary practice of superchilling means reducing the temperature in fish to about -2.22°C, at which point half the water is frozen. But if the temperature is reduced to -2.78°C, the two thirds of the water frozen but slowly creating large ice crystal, which is actually slow freezing. This can cause cell damage and hence the temperature must be controlled carefully. By maintaining lower temperature to produce small ice crystal can reduce bacterial activity and spoilage resulting in a prolonged storage life (Waterman and Taylor 2001).

Superchilling increases storage life of fish. If fish is stored in ice the shelf life is 14 days, then the predicted shelf life at -1, -2 and -3°C would be 17, 22 and 29 days, respectively (Huss 1995). Similar result was observed in tilapia, when crushed ice was used to preserve it. It remained edible for about 15 days. When tilapia was superchilled to -1.11°C and -2.22°C under laboratory conditions the shelf life was extended to about 20 and 26 days respectively and cited in Figure 2 (Waterman and Taylor 2001).

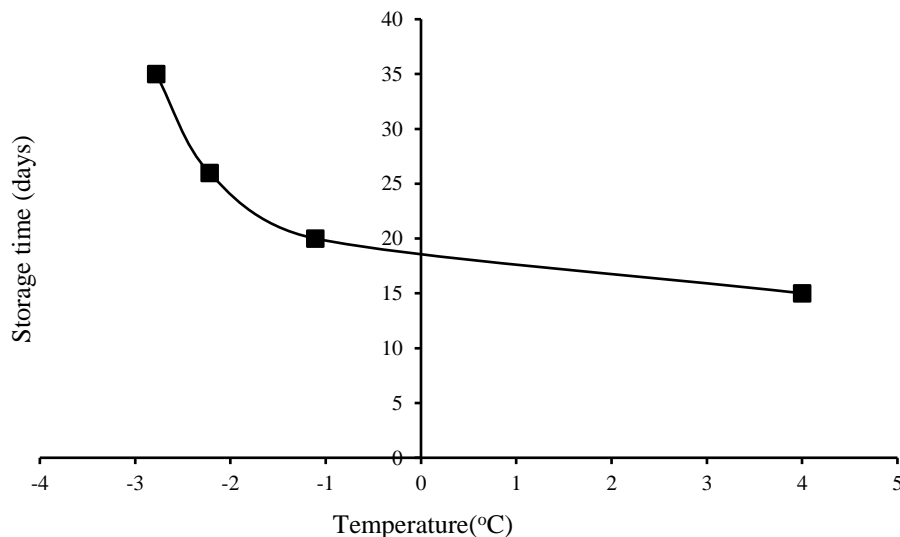


Figure 2. Storage life of Tilapia in different temperature (Waterman and Taylor 2001)

The ice crystal size is very important in superchilling fish. If the ice crystal size is large, then it can cause rupture of the cell. In -1.11°C and -2.22°C the ice crystal size is too small to create any cell rupture. If the temperature is reduced to -2.78°C, the shelf life may be as long as 35 days, but damage due to ice formation in slow freezing makes the fish unsuitable for filleting or smoking (Waterman and Taylor 2001).



However, the extension of storage time over conventional iced storage can be as much as 11 days under ideal conditions, and at least 6 days in commercial practice. That is why, the notion of superchilling has been always intended to increase shelf life of food during storage. So most of the studies have been focusing on improving this criteria (Wang *et al.*, 2008; Olafsdottir *et al.*, 2006b; Sivertsvik *et al.*, 2002; Arnþórsdóttir *et al.*, 2008). There is no difference in quality between chilled and superchilled Atlantic salmon after 11 days of post-mortem storage (Erikson *et al.*, 2011), which was in case of tilapia about 12 days in ice (Waterman and Taylor 2001). However, FAO guidelines recommend the narrow temperature range -2 to -2.5°C for industrial super chilling (Waterman and Taylor 2001).

### 2.2.1 CBC Cooling

CBC cooling is a technique where heat is extracted from fillets both by conduction through a teflon coated aluminium conveyor belt and by convection, where cold air is simultaneously blasted over the fillets. The CBC cooling machine was developed by Skaginn hf. Liquid cooling is a part of the CBC cooling process. It is performed before the CBC cooling to decrease the temperature of the fillets and slightly increase their salt content (Margeirsson *et al.*, 2010). CBC technique was used as a superchilling technique for fillet in most of the researches (Magnússon *et al.*, 2009, Purwaamidjaja 2010, Odoli *et al.*, 2013a, Margeirsson *et al.*, 2011, Olafsdottir *et al.* 2006a, Semwanga 2010, Arnþórsdóttir *et al.*, 2008). Very little research has been done on CBC effects on whole fish.

### 2.3 Gutting and bleeding

In most cases gutted fish tend to give a better fish quality than ungutted fish. Fish guts contain digestive enzymes and many bacteria. The latter will cause a violent autolysis post mortem, which may give rise to strong off-flavour especially in the belly area (Gilberg 1978).

Commonly, quality and storage life of fish decreases if they have not been gutted. On the other hand, gutting means exposing the belly area and cut surfaces to the air thereby rendering them more susceptible to oxidation and discoloration. Also flesh is exposed to microbial activity. In most cases, small and medium-sized fatty fish such as herring, sardines and mackerel are not eviscerated immediately after catch (Huss 1995). Exceptional result was found in gutted and ungutted aquaculture sea bass (*Dicentrarchus labrax*) stored in ice. Bacterial counts of whole ungutted sea bass were always higher than those obtained for gutted sea bass samples. But the shelf-life of the whole ungutted and gutted sea bass stored in ice as determined by the overall acceptability sensory scores and microbiological data was 13 and 8 days, respectively that was reverse to the bacterial load; although mesophilic count of gutted fish were higher than the ungutted fish. (Papadopoulos *et al.*, 2003)

Most of the North European countries compulsorily gut lean fish species. It is based on the assumption that the quality of these species suffers if they are not gutted. In the case of cod, it has been shown that not gutting causes a considerable quality loss and a reduction in the storage life of five or six days. After only two days from catch, discoloration of the belly area is visible and the raw fillet acquires an offensive cabbage odour. These odours are removed to some extent when they are boiled (Huss 1995).

Bleeding significantly reduced rancidity in minced trout whole muscle, minced mackerel light muscle, and intact mackerel dark muscle but not minced mackerel dark muscle stored at 2°C (Richards and Hultin 2002).

Time spent on board prior to bleeding or gutting showed greater effect on quality of cod rather than the Bleeding/gutting procedure. A controlled study was conducted under commercial conditions to assess the sensory quality of raw cod (dockside grade) stored in ice for approximately 24 hours. Prior to being iced, cod were bled and gutted using a two-step, a conventional (shallow cut) one-step, or a modified (deep cut) one-step procedure at each of four different times after the cod were brought on-board. The mean sensory quality was very dependent upon the treatment combination, but was more affected by the time on-board prior to bleeding/gutting than by the actual bleeding/gutting procedure. Any significant improvement in sensory quality by the two-step procedure only occurred when the cod were first brought on-board. The type of one-step procedure had little significant affect upon sensory quality (Botta *et al.*, 1986).

According to the citation given by Huss (1995), the volatile, foul-smelling compounds are mostly found in the gut and surrounding area whereas the amount of volatile acids and bases is relatively low in the fillet itself. These chemical parameters are, therefore, not useful for distinguishing between gutted and ungutted fish. Similar experiments with other cod-like species showed a more differentiated picture. In the case of haddock (*Melanogrammus aeglefinus*), whiting (*Merlangius merlangus*), saithe (*Pollachius virens*) and blue whiting (*Micromesistius poutassou*), it was observed that ungutted fish stored at 0°C suffered a quality loss compared with gutted fish. Some off-odours and off-flavours are detected, but ungutted haddock, whiting and saithe are still acceptable as raw material for frozen fillets after nearly one week on ice. Quite different results are obtained with South American hake (*Merluccius gayi*), where no difference is observed between gutted and ungutted fish (Huss 1995).

## 2.6 Methods to evaluate freshness

### 2.6.1 Sensory evaluation

Freshness, defined in terms of odour, flavour, texture and appearance, is a way to describe the overall quality of fish (Lougovois and Kyra 2005).

Sensory evaluation is the scientific discipline that evokes, measures, analyses and interprets human reactions to characteristics of food perceived through the senses of sight, smell, taste, touch and hearing. Sensory evaluation is an important method for the assessment of freshness and quality, and is commonly used in the fish sector and fish inspection services (Luten and Martinsdottir 1997). The method is quantitative, and numerical data are collected to establish relationship between product characteristics and human perception (Martinsdottir *et al.*, 2009). This method is frequently used in the industry and for fish inspection services (Luten and Martinsdottir 1997). In some of the countries of the world, it is also called organoleptic test and in most of the cases depends on the knowledge and experience of the assessor. Therefore, a fluctuation in result is quite normal in this situation. So standardization of methods are now a common trend to make it objective measurement, not the personal perception (Ólafsdóttir *et al.*, 1997).

The Quality Index Method (QIM) is one reliable way to measure the freshness of fish stored in ice (Martinsdóttir *et al.*, 2001). The idea of QIM method was originally developed by the Tasmanian Food Research Unit (CSIRO), which rely on the part of the body that are good indicator of fish freshness. This part is considered to be the sensory parameter and specific points are allocated for degree of changes in the parameters for the selected species or product (Sveinsdottir *et al.*, 2002, Martinsdóttir 2002).

In the QIM scheme, the lowest score or zero is given for each attributes for very fresh fish and higher score for deterioration of the fish. The addition of the scores of all the attributes are called Quality Index. As the scheme is based on the overall score, no excessive emphasis is given on a single attribute. A sample cannot be rejected on the basis of a single criterion and minor differences in results for any of the criteria do not unduly influence the total QIM score (Luten and Martinsdottir 1997).

QIM is developed on the basis of species and whole fish or fillet and raw or cooked. But the other factors which can influence the quality of fish is never been taken into account like-different storage conditions such as frozen-defrosted fish, storage in ice slurry, temperature abuse during storage, etc. Furthermore, novel technique like superchilling, MAP, vacuum packaging which extend the shelf- life of fish, alter the spoilage pattern (Martinsdóttir 2002).

QIM schemes have been developed for a number of fish species including fresh herring and cod (Jonsdottir 1992; Larsen *et al.*, 1992), Atlantic mackerel, horse mackerel and European sardine (Andrade *et al.*, 1997), red fish, brill, dab, haddock, pollock, sole, turbot and shrimp (Luten 2000; Martinsdottir *et al.*, 2001), gilthead seabream (Huidobro *et al.*, 2000), frozen cod fillets (Warm *et al.*, 1998) and fresh cod fillets (Bonilla *et al.*, 2007) and arctic charr (Odoli *et al.*, 2008). Some QIM schemes have developed on the same species but different for the culture condition like cultured and wild sea bass (Alasalvar *et al.*, 2001)

There is no QIM scheme developed for whole tilapia. The only standardized method developed by Odoli *et al.*, (2013a) is on the fillet of tilapia.

### 2.3.2 *Physical and chemical analysis*

#### Total volatile basic nitrogen (TVB-N)

Total volatile basic nitrogen (TVB-N) is a general term which includes the measurement of trimethylamine (TMA), dimethylamine (DMA), ammonia and other volatile basic nitrogenous compounds. These compounds are associated with seafood spoilage and TVB-N is one of the most widely used measurements of seafood quality. But according to Anthoni *et al.*, (1990), there are some tropical freshwater fishes which are abundant in TMAO. During spoilage of fish TMAO is reduced to TMA which is noticeable during sensory test. TMA can be produced in fresh water fishes also in other way. Sometimes amino acid is degraded and TMA produce that characteristic spoilage odour. TVB-N analysis does not reflect spoilage in earlier stage but it can indicate advanced spoilage of fish (Huss 1995). The concentration of TVB-N in freshly caught fish is typically between 5 and 20 mg N/100 g muscle, whereas levels of 30– 35 mg N/100 g in a muscle is generally regarded as the limit of acceptability for ice-stored cold water fish (Huss *et al.*, 1988; Connell 1995). According to Adoga *et al.*, (2010), the rejection value of TVB-N was 38.75 mgN/100g. However, Odoli *et al.*, (2013b) suggested that TVB-N analysis alone was not good indicator of tilapia spoilage.

## pH measurement

It is common to measure the pH of the muscle tissue for deterioration (Howgate 2009). After death of the fish, autolytic action takes place, which produce lactic acid in fish. As a result of this pH value decreases. After rigor during storage pH value increase due to the production of basic compounds like ammonia mainly derived from microbial action by fish spoilage bacteria (Boskou & Debevere, 2000; Ruiz-Capillas & Moral, 2001).

## Colour measurement

Kiessling *et al.*, (2004) reported that colour can define the quality of fish where other physical test like texture and gaping cannot define it. Robb *et al.*, (2000 ) stated that electro-stimulation of 1.5 kg rainbow trout (*Oncorhynchus mykiss*) immediately after slaughter did not only result in short times to rigor, but the flesh colour was affected and suggested that the changes in colour with slaughter was due to changes in muscle structure. Therefore, colour change can be used to define the quality of fish.

There are three systems of describing colour. The earliest one is called the CIE system and was developed in 1931. The CIE system is based on the imaginary positive primaries X, Y, and Z; which are transformed from real red, green and blue trichromatic primaries which may contain negative values. In CIE, colour is located by (Y, x, y), where, Y= luminous reflectance of transmittance (containing the entire light stimulus) and chromatically co-ordinates. This system is not visually uniform. In order to resolve this issue, the Hunter system was developed in 1958. In the Hunter system, colour is more uniform than CIE and is defined by (L, a, b); where, L= relates to lightness and a, b means red/green and yellow/blue hues (MacDougall, 2002). The combination of these primaries is able to define colour.

### 2.3.3 Microbiological methods

#### Total viable counts (TVC)

In a post mortem fish, spoilage is the most important natural consequences that affect the quality to rejection level. The foundation of spoilage is created by autolysis by releasing nutrients and the micro-organism takes the opportunity, invade into the muscle and spoil the fish. According to Murray and Shewan (1979), only a very limited number of bacteria found to invade the flesh during iced storage.

There are various methods for microbial assessment. Total Viable Count (TVC) is one of the most widely practiced methods. The term is also called Total Aerobic Count (TAC) and Standard Plate Count (SPC), meaning the total number of bacteria that are capable of forming visible colonies on a culture media at a given temperature (Huss 1995). There are two types of bacteria, one of the group is involved in fish spoilage. This group is seldom a good indicator of the sensorial quality or expected shelf life of the product (Huss *et al.*, 1974). TVC does not reflect the count of spoilage bacteria in food. In ice-stored Nile perch, the total count was  $10^9$  cfu/g for days before the fish was rejected (Gram *et al.*, 1989) and in lightly preserved fish products high counts prevail for a long time before rejection. At the point of sensory rejection, the TVC in fish products are typically  $10^7$ - $10^8$  cfu/g. In case of tilapia the sensory rejection

point was found to be log 7 cfu/g (Odoli *et al.*, 2013a). However, standards, guidelines and specifications often use much lower TVC as indices of acceptability (Ólafsdóttir *et al.* 1997).

### Specific spoilage organisms (SSO)

Spoilage reactions in food are complicated and dynamic as the spoilage reactions and micro-organisms may change as a function of product characteristics and storage conditions (Dalgaard *et al.*, 2002). Spoilage rate is dependent of the activity of the spoilage bacteria (Ronsivalli and Charm 1975). As mentioned earlier, not all of the micro-organism are involved in the spoilage of fish. The micro-organisms involved in the spoilage are called Specific Spoilage Organisms (SSO). These bacteria can produce fowl H<sub>2</sub>S odour which indicates spoilage of the fish. The qualitative ability to produce off-odours (spoilage potential) and the quantitative ability to produce spoilage metabolites (spoilage activity) are essential in the identification of an SSO (Gram and Dalgaard 2002). Gram *et al.*, (1987) used different peptone-rich substrates containing ferric citrate and found that H<sub>2</sub>S-producing bacteria such as *Shewanella putrefaciens* are producing black colonies. Spoilage by *Vibrionaceae* family can also be detected using iron agar. The sensory rejection point for tilapia was found to be log 6 cfu/g (Odoli *et al.*, 2013a).

### 3 MATERIALS AND METHODS

#### 3.1 Collection of fish

The experimental fish was Tilapia (*Oreochromis nilotica*). Samples were collected from a fish farm in the south of Iceland and transported to the slaughter house nearby. The number of fish was 130, weighing  $953.50 \pm 93.13$ g on average. From these, 10 fish were packed separately in a Styrofoam box as reference sample and 60 fish were left whole and 60 fish were gutted. The fish was transported to the processing plant and treated with Combined Blast and Contact cooler (CBC) at  $-8^{\circ}\text{C}$  for 10 minutes. The samples were packed in Styrofoam (EPS) boxes and cooling mats were put inside the boxes and transported to Matis.

#### 3.2 Experimental design

The experiment had 4 treatments, gutted with CBC treated, ungutted with CBC treated, gutted without CBC and ungutted without CBC. Among the 130 fish, 10 were separately packed as stated earlier. Rest of the fishes, were packed in EPS/ Styrofoam boxes with false bottom. All the EPS boxes were stored in to the cooler in Matis. The temperature was set to a  $-1^{\circ}\text{C}$  up to day 6 and day 7 to the end of the experiment to a  $2^{\circ}\text{C}$  simulating the sea freight temperature and transportation and storage in retail chain temperature. There was one reference point and five sampling points. The sampling points were day 6, 10, 14, 17 and 20. Selection of fish was done randomly. For better management of the project, short code were used for the treatment names. The arrangement of treatments are shown on Table 1 and a layout of experimental design is presented in Figure 3.

Table 1. Arrangement of treatments

Treatment Name	Code	No. of fish	No. of boxes
Gutted with CBC	CG	30 fish	5
Ungutted with CBC	CU	30 fish	5
Gutted without CBC	AG	30 fish	5
Ungutted without CBC	AU	30 fish	5

#### 3.3 Analysis of sample

A randomly picked reference sample was transported to the laboratory for analysis. Insulated boxes were kept in in the cooler at a temperature ranging from  $-1$  to  $2^{\circ}\text{C}$ . On each sampling day, 1 box from each treatment were randomly selected and required number of fish were collected from each box for sensory, physical, chemical and microbial analysis. Sampling were done after 6, 10, 14, 17 and 20 days.

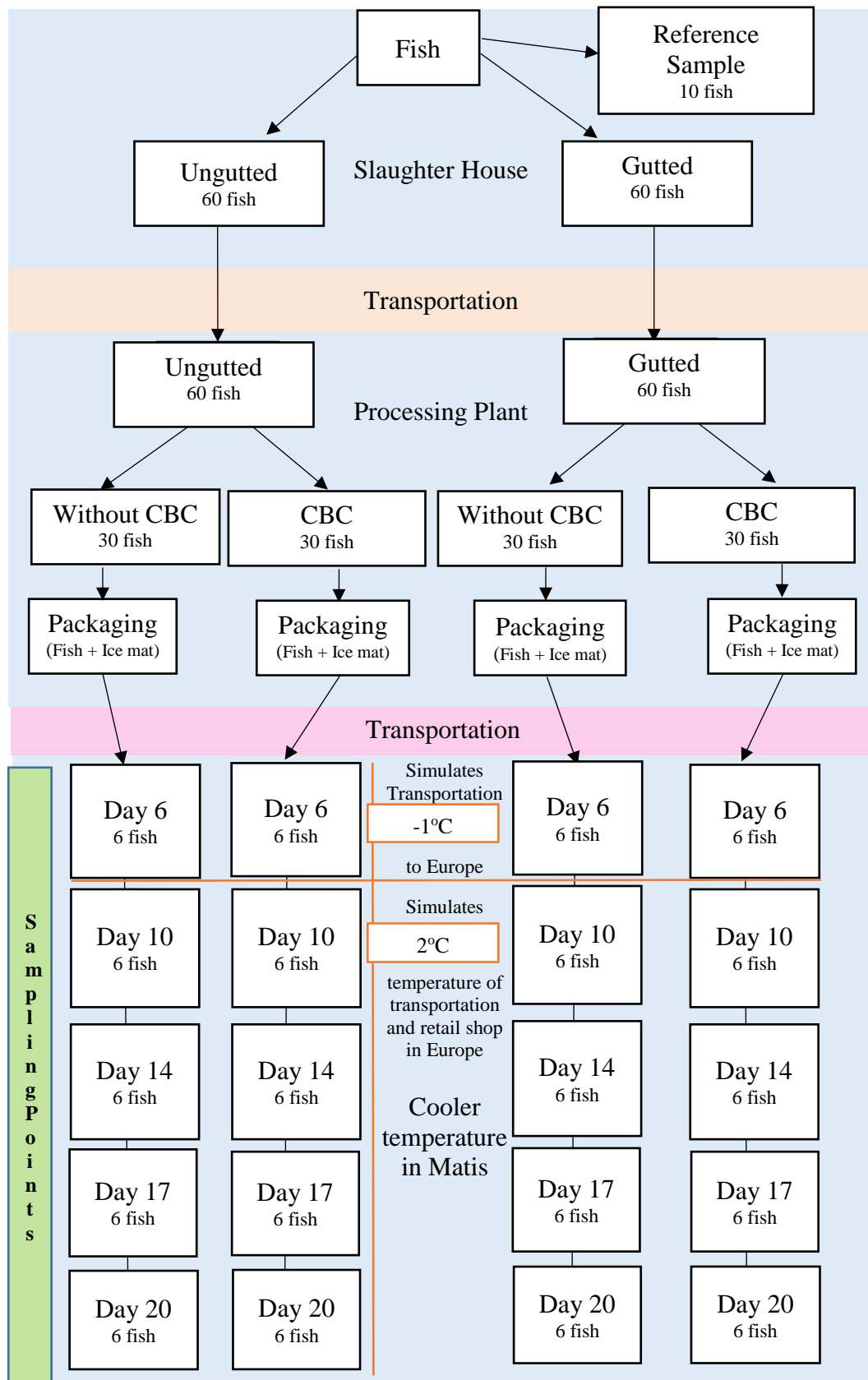


Figure 3. Experimental Design

### 3.4 Temperature logging

Temperature loggers were used during the storage to monitor the temperature condition of the fish. One logger was placed inside every box to monitor the ambient temperature, and another logger was placed inside the muscle of one fish (Figure 3). A couple of loggers were set in the cooler to monitor the temperature condition inside. Temperature recordings were at 10 minutes interval. Thus, two types of logger were used, the iButton temperature loggers, type DS1922L (Figure 4) and TidbiT v2 Water Temperature Data Logger - UTBI-001 (Figure 5).

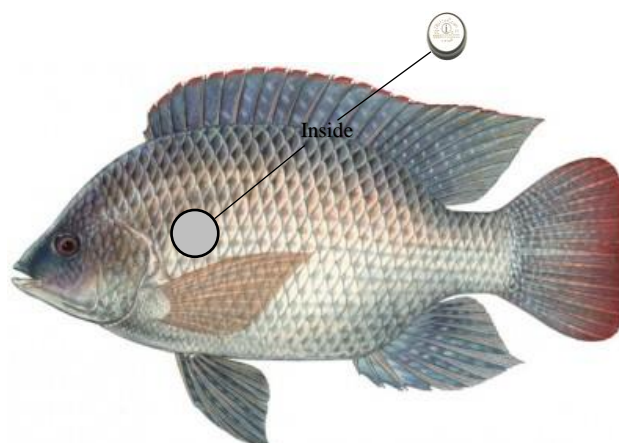


Figure 4. Position of temperature logger on the fish

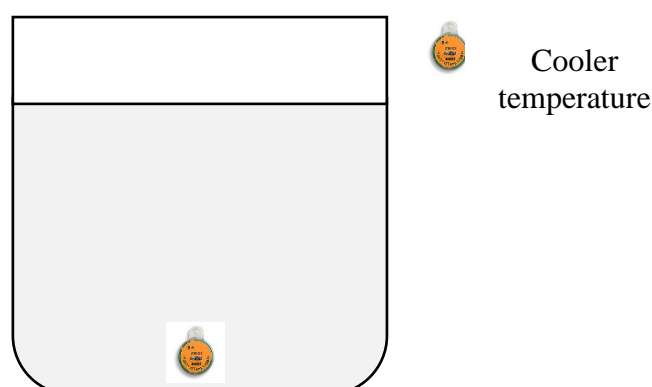


Figure 5. Placement of the water-temperature logger

### 3.5 Sensory evaluation

To estimate the freshness of tilapia, the Quality Index Method (QIM) developed by Odoli *et al.* (2013a) was followed during the sensory evaluation. A panel of 6 to 7 members trained according to International Standards, ISO 1993 were used. For each sampling day, three tilapia fillets from each sample group were coded with a three-digit random numbers and placed on a white clean table at room temperature, under white fluorescent light.

There are six sensory attributes in the scheme; namely- colour of the lateral line, colour of loin, colour of belly flap, mucus, texture and odour of tilapia. Total QIM score is thirteen. The rejection of the fish, according to the scheme, determined by the total score at the rejection point. The rejection point as determined by Odoli *et al.*, (2013), however on the basis of



microbial counts in terms of TVC  $10^7$ cfu/g and SSO  $10^7$  cfu/g was however 6.5. The QIM scheme followed is described in Table 2.

Table 2. QIM method for tilapia fillet

Fish part	Quality parameter	Description	Score
Skin side	Colour *	Dark red, red brown	0
		Red brown, lighter colour	1
		Light brown	2
Flesh	Colour, loin	light, beige, trace of red or bluish	0
		A little darker colour, a little brownish or greyish	1
		Greyish, brownish, yellowish	2
	Colour, flap	bluish, transparent	0
		light, milky colour	1
		Greyish or brownish	2
	Mucus	No mucus, mat texture	0
		A little shiny, trace of mucus	1
		Milky or greenish mucus	2
	Texture	Firm	0
		Rather soft	1
		soft	2
Odour	Fresh, neutral	0	
	Seaweed, marine, grass	1	
	Sour milk, silage	2	
	Acetic, putrid	3	
Quality index (0-13)			

\* Stripes at the middle of the loin (lateral line)

### 3.6 Microbial measurements

Total viable psychrotrophic counts (TVC) and counts on H<sub>2</sub>S producing bacteria were done by following a modified procedure originally developed by Gram *et al.* (1987). Duplicate samples were analysed for each treatment on each day of sampling. Each sample consisted of muscles taken from different part of the tilapia. First the skin was disinfected with 70% isopropanol and then aseptically cut away and the underlying muscle collected and minced. Total of 20 g was weighed and mixed with 180 g of diluent and blended in a Stomacher® Lab Blender 400 (Seward, UK) for 1 min to obtain 1/10 dilution. Serial 10-fold dilution were done as needed in 9ml cooled MRD. The diluted samples were cultured in iron agar using spread plate technique and incubated at 17°C for 5 days. Total Viable psychrotrophic Counts (TVC) and counts on H<sub>2</sub>S producing bacteria (Black colonies) were done for identification of spoilage.

### 3.7 Physical measurement

#### 3.7.1 Colour

The intensity of the flesh colour was measured with a Minolta CR-400 Chromameter (Minolta camera Co., Ltd; Osaka, Japan) in Hunter Lab system (Hunter, 1958). The instrument records

the L value, lightness on the scale of 0 to 100 from black to white; a value, (+) red or (-) green b value, (+) yellow or (-) blue (MacDougall, 2002).

Measurements were taken in selected points for lateral line maintaining the same direction (Figure 6) simulating colour attributes in Quality Index Method (QIM) scheme. Measurements were also taken for the bone-side at the loin and the belly flap maintaining the number and direction shown in Figure 6. Measurements were always taken in the direction from head to tail.



Figure 6. Colour measurement points for lateral line and bone-side fillet

### 3.7.2 Cooking yield

Cooking yield is defined as the amount of liquid lost during cooking. Cooking yield was determined by steam cooking the fillets at 95°C to 100°C for 8 min in a Convostar oven (Convotherm, Elektrogeräte GmbH, Eglfing, Germany). After the cooking, the fillets were cooled down to room temperature for 15 min before weighing for cooking yield determination. The yield after cooking (%) was calculated as the weight of the cooked fillets in contrast with the weight of raw fillets.

The cooking yield (CY) was calculated by the formula:

$$CY = \frac{W_{\text{cooked}}}{W_{\text{raw}}} \times 100 \quad (\%)$$

Where,

$W_{\text{cooked}}$  is the weight of cooked sample

$W_{\text{raw}}$  is the weight of sample before cooking

### 3.8 Chemical measurements

#### 3.8.1 Total Volatile Bases (TVB-N)

Minced samples were extracted, distilled and followed by the titration method according to Helrich (1990). TVB-N was performed through direct distillation into boric acid using a Kjeldahl-type distillator (Struer TVN distillator) (Malle and Poumeyrol 1989). The acid was titrated with diluted H<sub>2</sub>SO<sub>4</sub> solution and normality was recorded for calculation. The TVB-N content was expressed in mgN/100 g tilapia tissue. Samples were analysed in duplicate and results were presented as an average. The TVB-N content was calculated by the following formula:

$$\frac{14\text{mg/mol} \times a \times b \times 300}{25\text{ml}} \quad (\text{mgN}/100\text{g})$$

Where,

a = ml of sulphuric acid;

b = normality of sulphuric acid.

#### 3.8.2 pH

The pH was measured by mixing 5 g of mince with 5 mL of deionised water. The pH meter was previously calibrated using the buffer solutions of pH 7.00±0.01 and 4.00±0.01 (25°C) (Radiometer Analytical A/s, Bagsvaerd, Denmark).

### 3.9 Data analysis

Data analysis was done by following standard statistical methods using Microsoft Office Excel 2013 and an add-in for Excel, XLSTAT. For data analysis, two-way ANOVA was used, which was further analysed by using Tukey (HSD) test using XLSTAT. Significance of differences was defined at the 5% level ( $p < 0.05$ ).

## 4 RESULTS

### 4.1 Temperature results

#### 4.1.1 Cold chain

A cold chain was maintained throughout the study. Fish were kept in a cold environment maintained from the catch until it perished. Initially ice was used for temporary preservation of fish and also transportation. The temperature were below 0°C after catch, in the slaughter house and during transportation.

At Matis, samples were put in a cooler at -1 °C, for the first 6 days after slaughter, and on day 7 the samples were put at 2°C to simulate the transportation to the markets in Europe. The temperature of the CBC treated fish was lower compared to the untreated fish, as shown in Figure 7.

In the case of gutted without CBC, the initial fish core temperature of 0°C to subzero temperature a day after storage and temperature rize again after 8 days of storage. Fish core temperature in the other treatments were lower.

For CBC upto day 11, fish core temperature did not change with the changing cooler temperature. But without CBC from day 7 the temperature in the cooler increased, as well as the core temperature of fish in all the treatments, as shown in Figure 7.

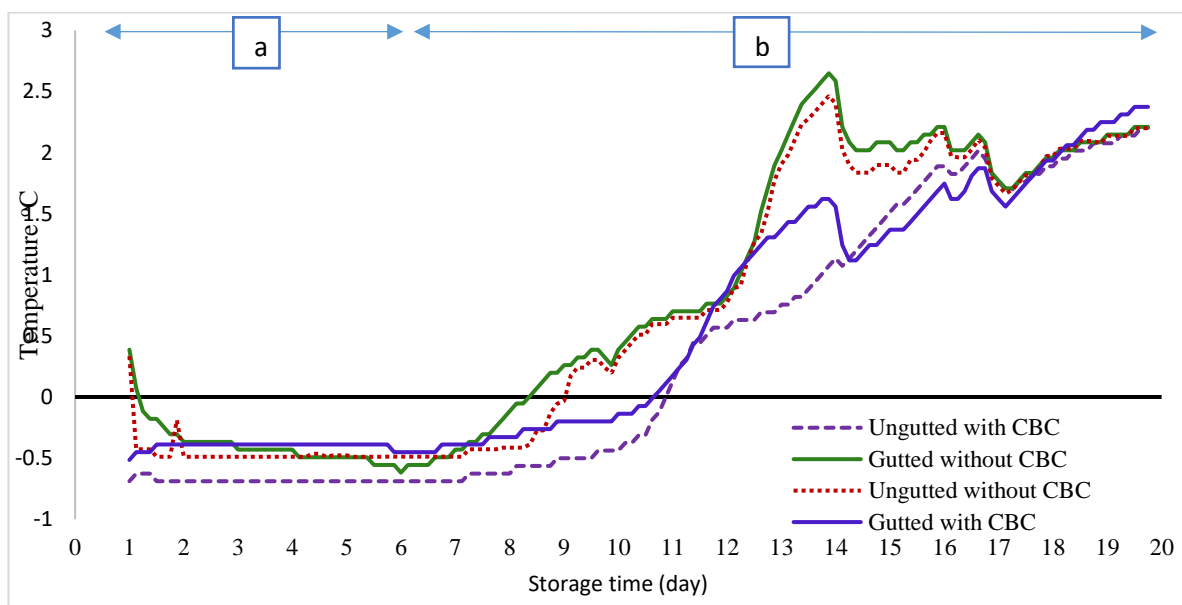


Figure 7. Influence of storage temperature on different treatments

The fish core temperature was lower due to CBC treatments. Temperature profile of fish, box and cooler is shown in Figure 8. The cooler temperature directly influenced the box temperature. But as box was made of styrofoam, which is a low heat conductor. So temperature was never very close to cooler temperature. The normal process is that fish release heat when preserved

in colder temperature. But fish core temperature was lower due to the CBC effect. Therefore, the treatments maintained an opposite trend during the experimental period.

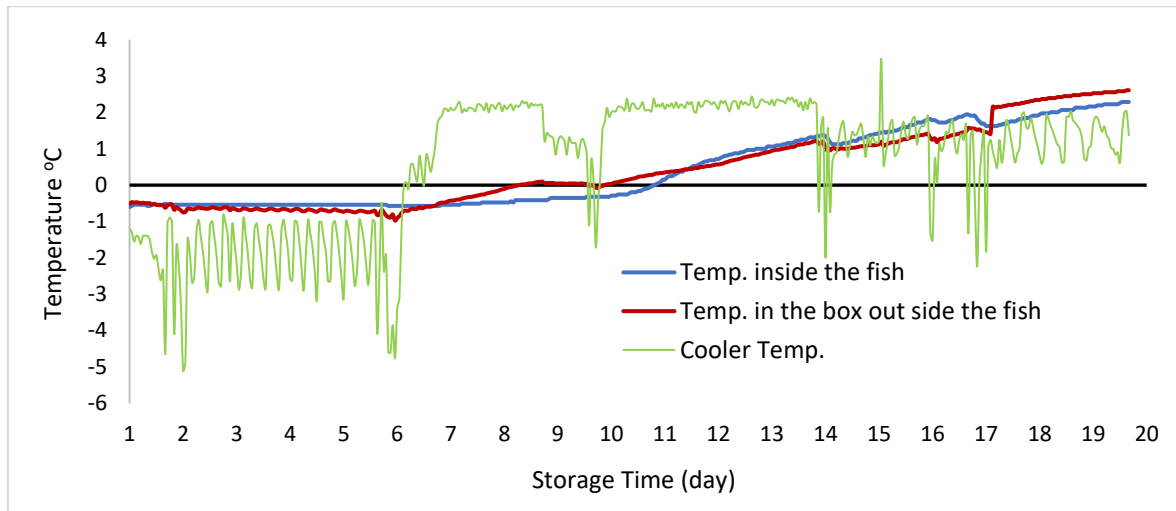


Figure 8. Temperature profile in fish treated with CBC during storage

Temperature profile of fish, box and cooler is shown in Figure 9. The cooler temperature directly influenced the box temperature but less effective due to low conductivity of styrofoam boxes. So temperature into the box was never very close to cooler temperature. The initial fish core temperature was higher than 0°C. After storage at first, the temperature inside the box went down and influenced fish core temperature. Then the fish core temperature also went down. The normal process is that fish release heat when preserved in cold temperature. Therefore the same trend was maintained during the experimental period.

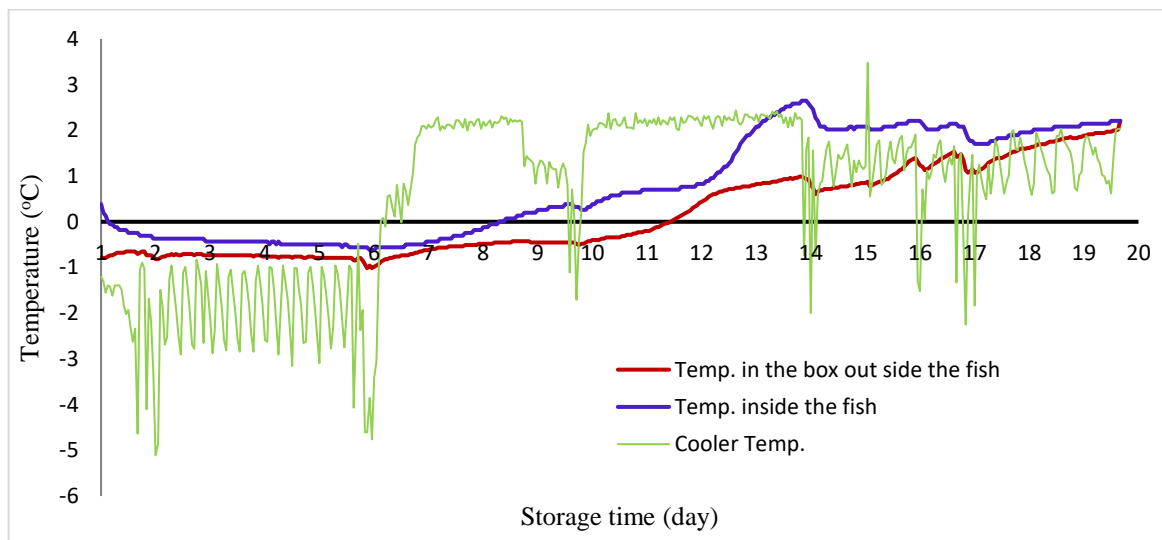


Figure 9. Temperature profile in fish without CBC during storage

Initially there was a clear distinction in fish core temperature between CBC treated and untreated treatments on day 1 as CBC treated fish core temperature was around  $-0.5^{\circ}\text{C}$  and without CBC was around  $0^{\circ}\text{C}$  and getting down. Fish core temperature become equal in both CBC treated and untreated treatments on day 3. This temperature condition continued up to the

time when cooler temperature was reset to 2°C on day 6. On day 8, the core temperature of CBC treated and untreated was readily differentiable. The CBC untreated temperature was increased by the influence of increased temperature of the cooler but temperature was lower than the treatments without CBC treated. Effect of CBC on core temperature of fish shown in Figure 10.

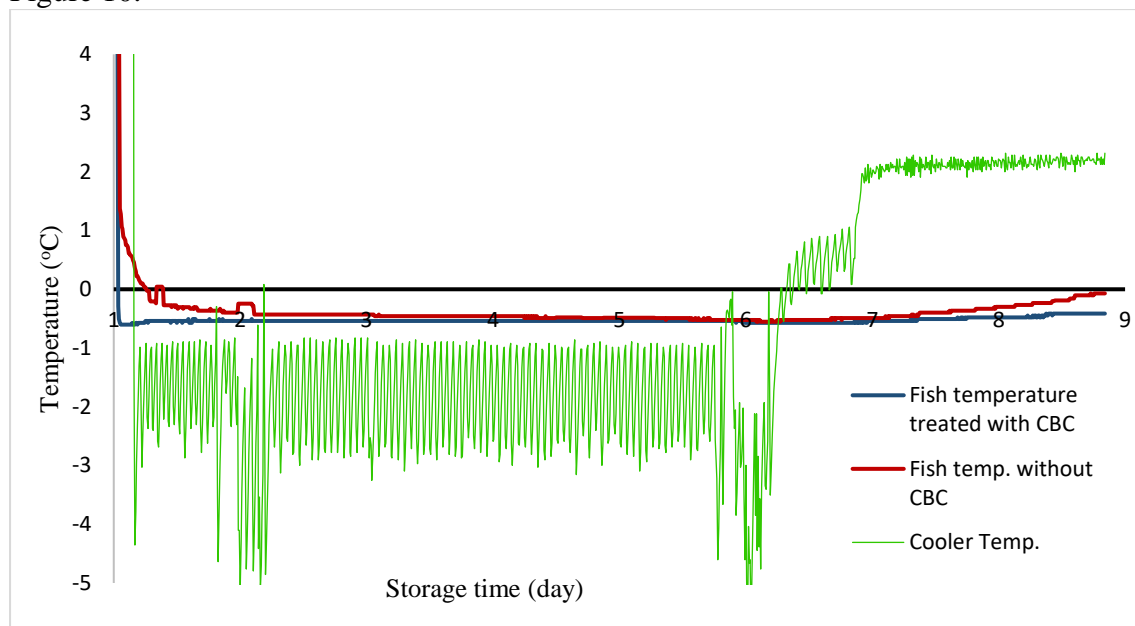


Figure 10. Difference in core temperature of CBC treated and un treated fish

## 4.2 Sensory Evaluation

### 4.2.1 QIM score

The results from the sensory evaluation of fillets with the QIM scheme showed significant differences ( $p < 0.0001$ ) day 17 and day 20 compared to other sampling days (Figure 11). The graph shows the similar pattern of score increase as described by Bonilla *et al.*, (2007) and Odoli *et al.*, (2013a). At the day 17, all the groups passed that rejection point, especially gutted with CBC scored the highest point, which indicates the quality deterioration of fish. Which might due to the combined effect of CBC forming ice crystals in skin initially very good but after ice crystal melt down it becomes susceptible to micro-organisms and due to the gutting of fish expose the body cavity to the environment. However, the difference is not significant.

The samples were still not rejected by the panellists. On day 20, every panellist was certain about the spoilage and rejected all the samples.

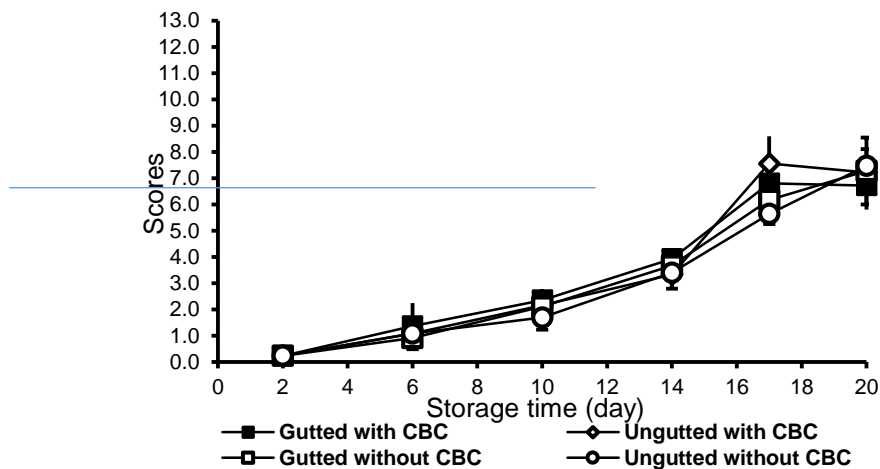


Figure 11. QIM Score (Mean ± SD) for different treatments at storage.

The lateral line is one of the best sensory attributes indicating sensory spoilage (Odoli, et al., 2013a). The normal colour of the lateral line is bright to darkish red colour, which tend to fade up and become light red and ultimately become darkish brown. Significant difference was found between gutted fish treated with CBC, compared to unguttred without CBC. No significant difference was found in treatments on the same day. There was no significant difference between the days other than between day 20 and day 17. The result indicates that tilapia sample was degrading in course of time irrespective to treatments. The changes in lateral colour is shown in Figure 12.

Loin colour for different sampling days is shown in Figure 143. No significant difference was found between the treatments on the same day. But significant difference were found between day 20 treatments with other sampling days except day 17. Day 17 also significantly differs from all treatments of day 2, 6, 10 and unguttred without CBC on day 14.

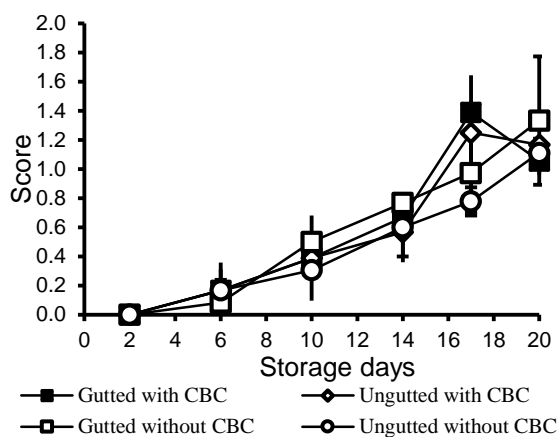


Figure 12. Lateral line colour (Mean ± SD) for the treatments on sampling days

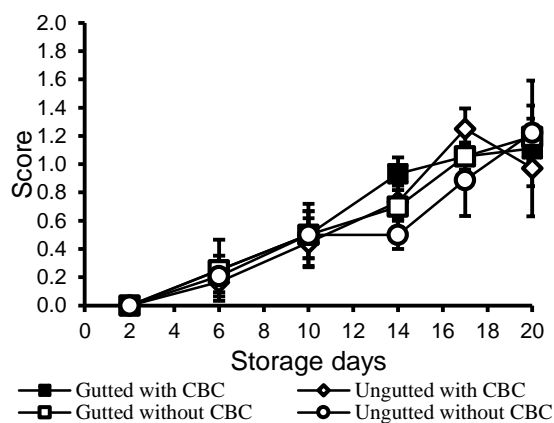


Figure 13. Loin colour (Mean ± SD) for the treatments on sampling days

Belly flap colour for different sampling days is shown in Figure 14. Belly flap colour decreased over time except in day 10, which might be due to the lean margin of difference made the panellist in a 50-50 situation. This indicates that, there is a very little change in between day 2 to day 10. Significant variation was found between day 17 and 20 in treatments compared with earlier days except gutted with CBC on day 6.

Mucus level in different sampling days is shown in Figure 15. The result for this parameter is not consistent. Because the mucus that was described in QIM scheme was about the tilapia fillet stored in zero or sub-zero condition. But in this experiment, whole fish was stored and filleted on the sampling day. So there was simply a thin layer of slime over the fillet more or less similar to detect any difference.

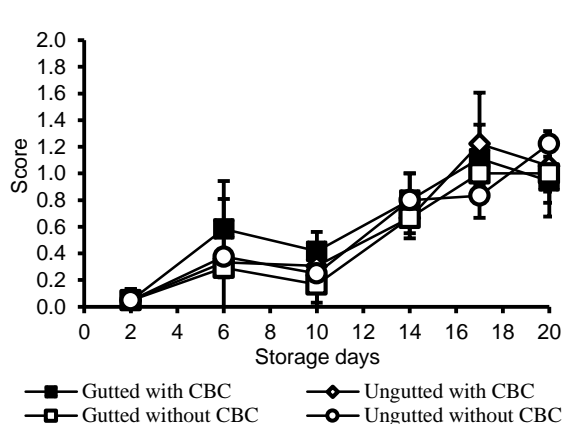


Figure 14. Belly flap colour (Mean  $\pm$  SD) for the treatments on sampling days

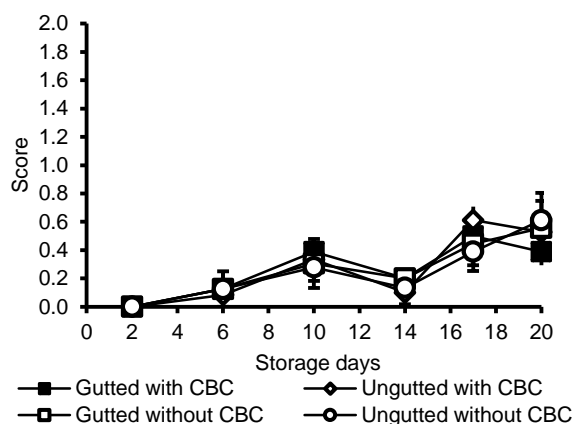


Figure. 15 Mucus (Mean  $\pm$  SD) for the treatments on sampling days

The texture score for the treatments on different sampling days is shown in Figure 16. The fish muscle became firmer during the storage time. No significant difference was found between the treatments during the storage time. Significant variation was only found between earlier sampling points and final sampling points, which indicates slow progress towards deterioration.

For odour of fish, no significant difference was found between the treatments on the same day. But significant differences were found between day 20 treatments with other sampling days except day 17. Similarly, significant differences were found between day 17 treatments with other sampling days except day 20. The graph, as shown in Figure 17, also indicates that there was almost no change in development of odour up to day 10. Then odour started to develop exponentially and on day 17 unguttled fish with CBC treatment reached the same level like day 20. For overall treatments, day 20 showed the highest score for odour and indicated the spoilage of fish.



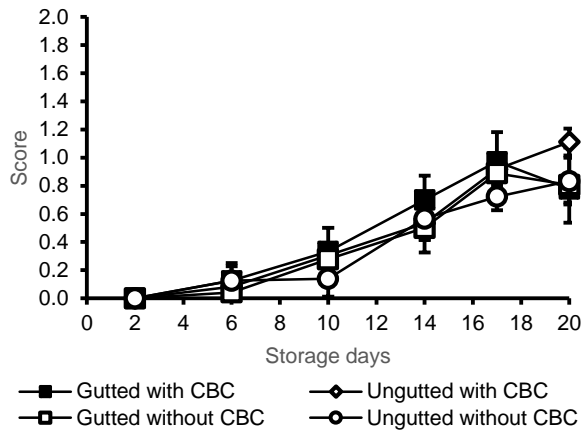


Figure 16. Texture score (Mean ± SD) for the treatments on sampling days

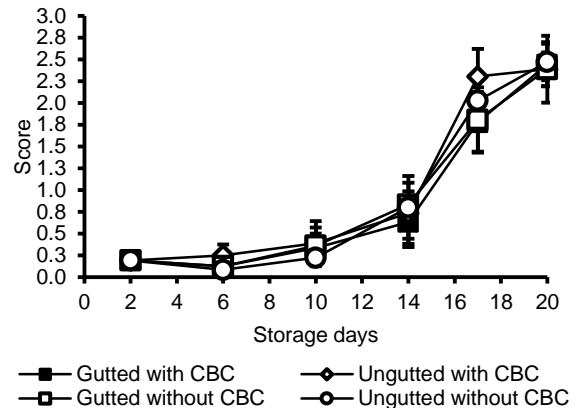


Figure 17. Odour score (Mean ± SD) in the treatments on sampling days

### 4.3 Physical parameters

#### 4.3.1 Colour

Colour measurements of lateral line were taken to make contrast with sensory result. At the start of the trial the fillets were medium bright and a combination of red and yellow, which indicates that the fillet might be red brown in colour. But as time progresses, the fillet colour became darkish and little increase in abundance of red and yellow pigments that suggest that the fillet became dark brown. No significant difference ( $p > 0.05$ ) was found in lightness or darkness either for the treatments or for the sampling days. For red/green colour, significant difference was only found in gutted without CBC on day 17 with ungutted CBC on day 10 and 20. Significant difference was found in yellow and blueness between ungutted CBC on day 14 and gutted with/ without CBC on day 20. The colour changes for lightness, red/ green and yellow/ blue are shown in Figures 18, 19, and 20.

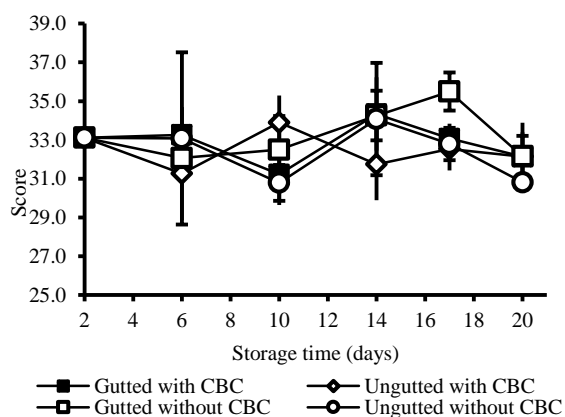


Figure 18. Light intensity in lateral line (L) (Mean ± SD)

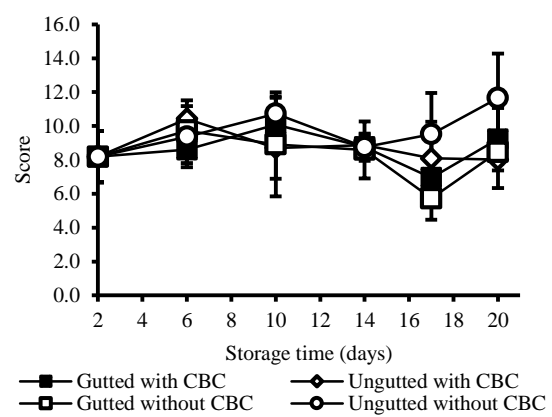


Figure 19. Red/greenness in lateral line (a) (Mean ± SD)

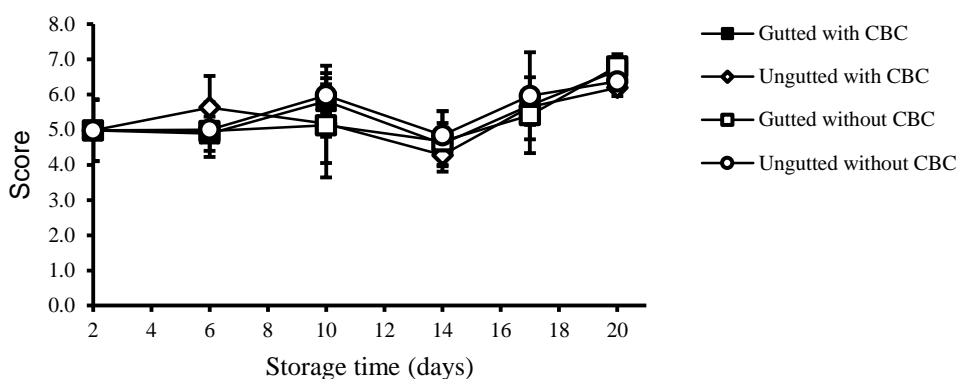


Figure 20. Blue/yellowness (Mean ± SD) in lateral line (b)

The bone-side colour changes in terms of lightness, red/ green and yellow/ blue are shown in Figures 21, 22, and 23. At the start of the trial the fillets were medium bright and a combination of green and light yellow, which indicated that the fillet might be whitish in colour and absence of redness was evident. But as time progressed, the fillet colour became a little bit darkish, with a small decrease in greenness and yellow pigments which indicates that the fillet became pale in colour. No significant difference was found in lightness or darkness either for the treatments or for the sampling days except between guttured with CBC on day 14 and unguttured with/without CBC on day 20 and guttured without CBC on day 10. No significant difference was found for the treatments or for the sampling days for red/green and yellow/blue colour.

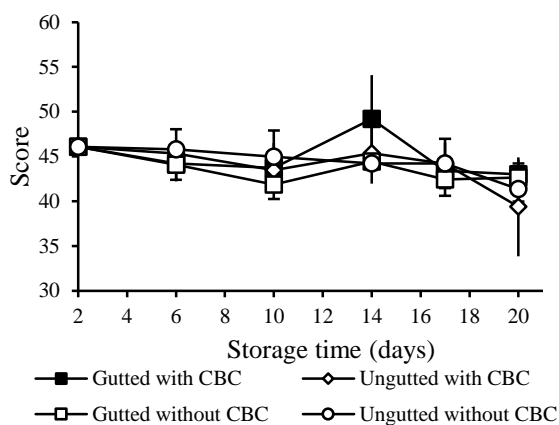


Figure 21. Light intensity (Mean ± SD) in bone side fillet (L)

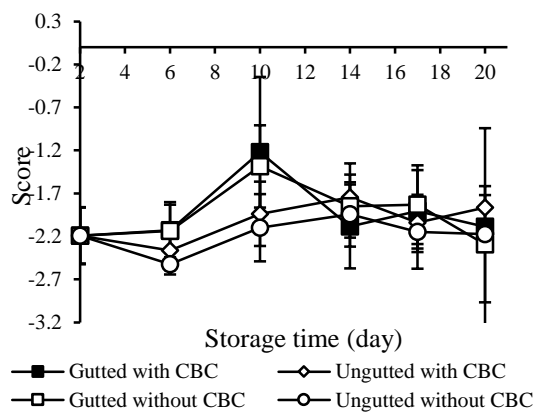


Figure 22. Red/greenness (Mean ± SD) in bone side fillet (a)

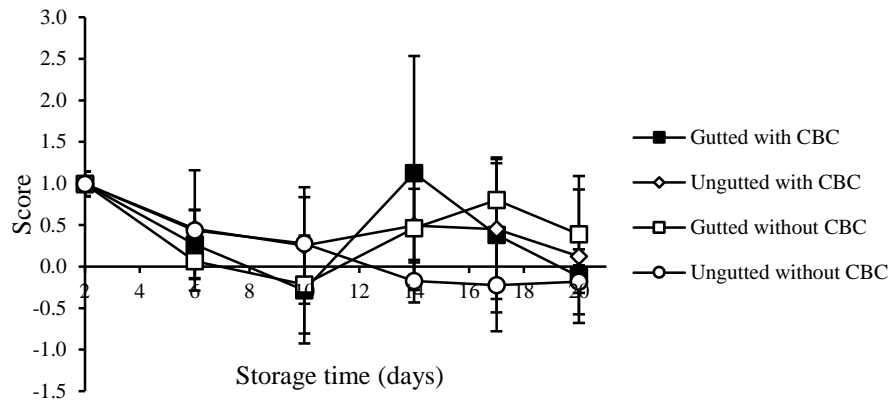


Figure 23. Blue/yellowness (Mean  $\pm$  SD) of bone side fillet (b)

#### 4.3.2 Cooking yield

Cooking yield of tilapia of every group are shown in Figure 24. On day 6, cooking yield of CBC untreated tilapia was higher than CBC treated tilapia. Similar result were found on day 10. Which might be due to the initial frozen outer layer of the fish due to CBC treatment reduced drip loss and during cooking higher temperature melt down ice crystal resulted in less cooking yield. However, the difference was not significant. But after that no significant variation was found, although it maintained a slow increase rate up to the end of the storage life. No significant variation was also found from the beginning to the end days.

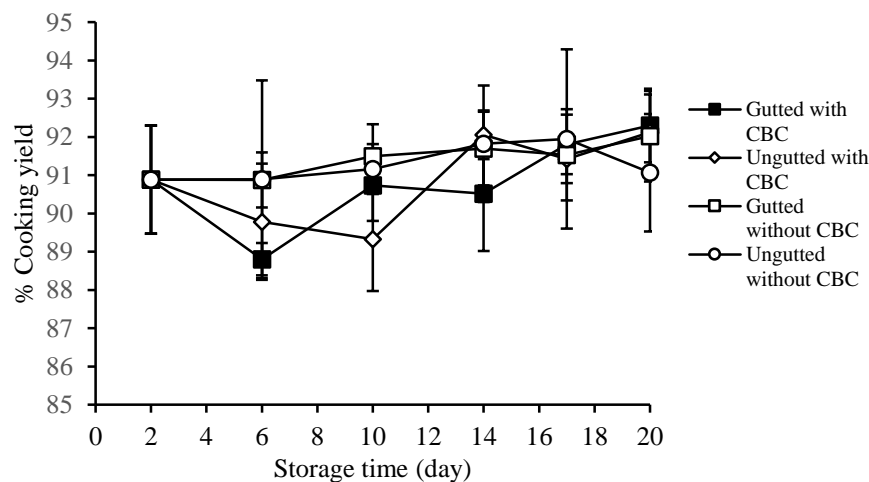


Figure 24. Cooking yield (Mean  $\pm$  SD) in different treatment in sampling days

## 4.4 Chemical assessment

### 4.4.1 Total Volatile Base- Nitrogen (TVB-N)

Results from Total Volatile Base- Nitrogen (TVB-N) content analysed are illustrated in Figure 25. No significant variation found in terms of day and treatment up to day 14. No significant difference was found between the treatments in the same day. But significant difference was

found between day 20 ungutted without CBC with day 14 treatments and day 6 ungutted with/without CBC treated. No significant difference was observed in case of day 6.

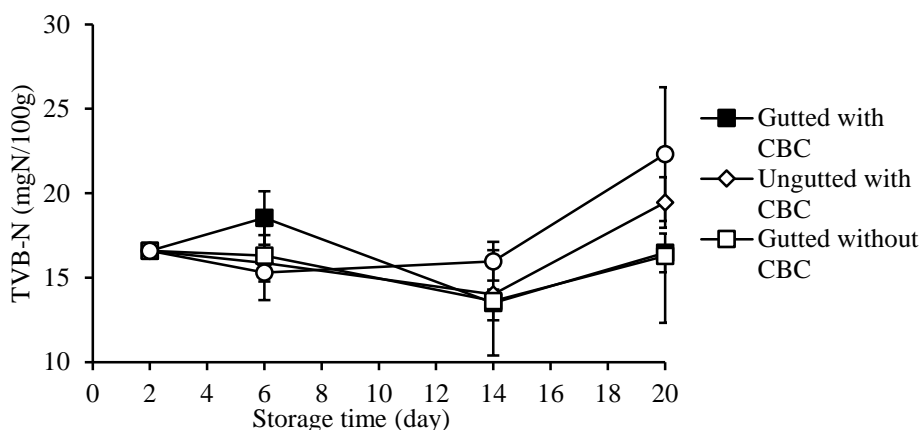


Figure 25. TVB-N (Mean  $\pm$  SD) in the treatments in different sampling days

#### 4.4.2 pH measurement

The results of pH measurement are shown in Figure 26. The gutted CBC treatment tend to show lower in pH value but not significantly. Significant difference was found between ungutted treated with CBC treatment on day 6 and gutted/ungutted CBC treated treatments on day 17 and gutted without CBC on day 20. No significant variation was found in other cases in terms of comparison of days and treatments. But there was an increasing tendency in pH found in the treatments. In tilapia, after death stored in 0-2°C, rigor finishes within 26.5 hours (Huss, 1995). The fish were on the day 2 of storage during the first sampling. So result always showed some increasing tendency.

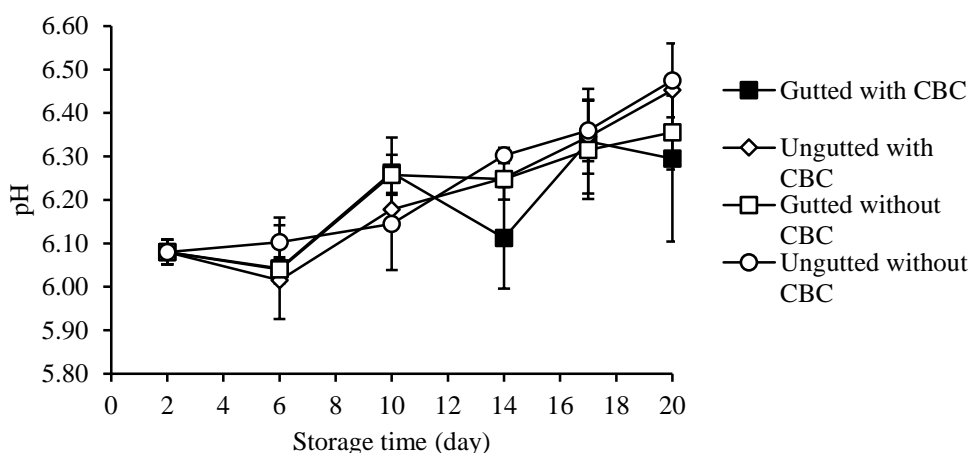


Figure 26. Changes in pH (Mean  $\pm$  SD) in different treatments on sampling days

## 4.5 Microbial

### 4.5.1 Total Viable Count (TVC)

Changes in TVC is shown in Figure 27. The initial microbial load in terms of TVC was log 3, which remained unchanged up to day 6. No significant difference was found up to day 6. After change of temperature from  $-1^{\circ}\text{C}$  to  $2^{\circ}\text{C}$  the scenario changed drastically. On sampling day 10, 14 and 17 TVC crossed log 4-5, 5-6 and around 6 respectively. On Day 20, the ungutted treatments (both with/without CBC) found with relatively higher TVC, though there was no significant variation between the treatments. However, after day 6 TVC result showed significant difference for all the sampling days; which indicated a rapid growth of psychotropic bacteria.

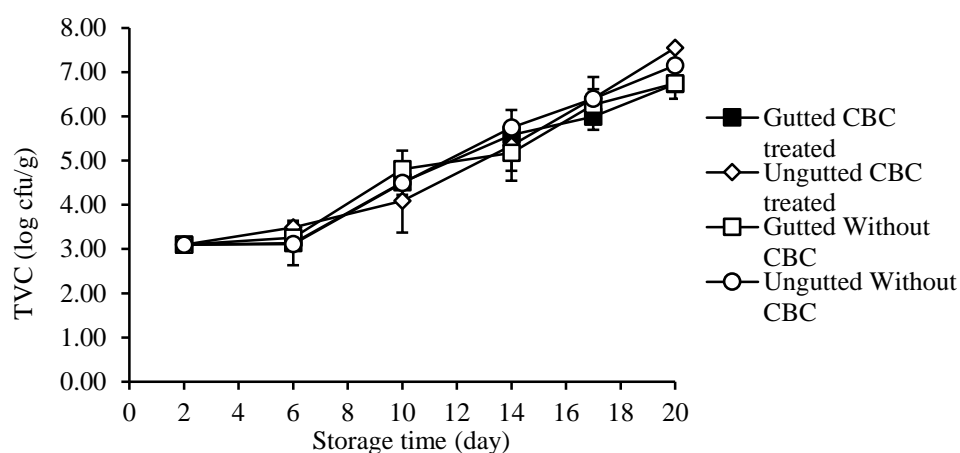


Figure 27. Microbial load in terms of Total Viable Count (TVC),  $\log \text{cfu.g}^{-1}$  (Mean  $\pm$  SD) in the treatments on sampling days

### 4.5.2 Specific Spoilage Organisms (SSO)

The initial sampling day showed absence of Specific Spoilage Organism (SSO). On day 6, ungutted CBC treated treatment showed presence of spoilage bacteria but was absent in the other treatments. From that day on, the temperature was increased from  $-1$  to  $2^{\circ}\text{C}$ . The growth of  $\text{H}_2\text{S}$  producing bacteria started to develop and in the next sampling day (day 10) for all the treatments. The bacterial growth was rapid until it reached day 20, when it was rejected in the sensory method. No significant variation was found between treatments on the same sampling days except for day 6 ungutted CBC treated one. No significant difference was found between gutted fish treated with CBC and all the treatments from day 10 onwards. Hence SSO growth was slowest in this treatment, which indicates the gutted with CBC treated can provide little better storage than the other treatments. Changes in Specific Spoilage Organism is shown in Figure 28.

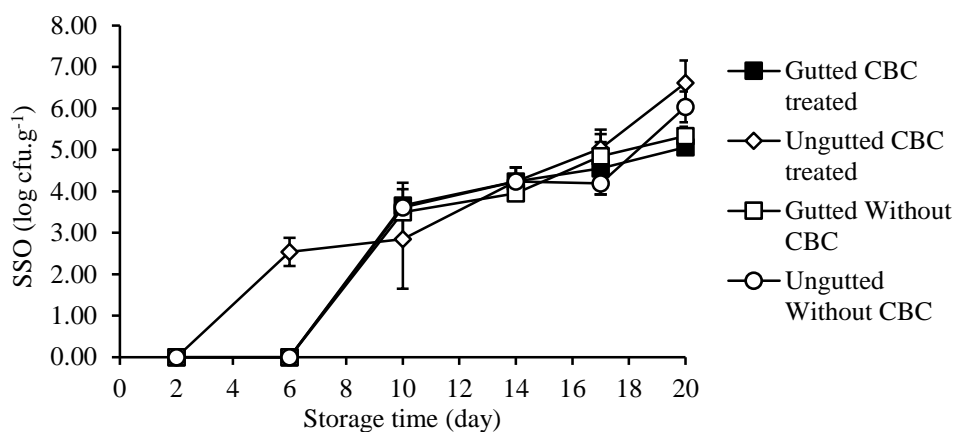


Figure 28. Specific Spoilage Organisms (SSO) load in log cfu.g<sup>-1</sup> (Mean  $\pm$  SD) in different treatments

## 5 DISCUSSION

### 5.1 Temperature effect

The cooler temperature was maintained at  $-1^{\circ}\text{C}$  up to day 6 simulating sea freight. Then the temperature was increased to  $2^{\circ}\text{C}$  to simulate the transportation and retail chain in Europe. The temperature in the cooler fluctuated around sub-zero temperatures up to day 6, after that the cooler temperature fluctuated around  $1-2^{\circ}\text{C}$ .

### 5.2 CBC effect

The fish core temperature, in case of CBC treated fish, was very close to the cooler temperature but temperature inside the box was higher than both cooler and fish. This might be due to the low conductivity of EPS/Styrofoam boxes (Margeirsson *et al.*, 2011) and fish core temperature was already sub-zero due to the CBC treatment. The temperature for the treatments without CBC, on the other hand, showed a different trend. The fish core temperature was initially higher than  $0^{\circ}\text{C}$ . The box temperature decreased slowly but fish core temperature decreased even more slowly. This indicates that heat was transferred from fish core to the box to the cooler.

The result for the CBC and without CBC treatments indicated that the core temperature was always lower in earlier stated treatments than the latter. Similar result was recorded by Magnusson *et al.* (2009), where different combination of conditions like precooling with plate ice, liquid ice and no cooling; then treated with or without CBC were used; and all the treatments stored in  $-1^{\circ}\text{C}$  or under real temperature simulation at Matís. The temperature was always cooler in CBC treated groups. The result was also similar to Purwaamidjaja (2010), which found that CBC treated fillets tend to have lower increase in temperature in an increased storage temperature.

A great deal of research on the CBC superchilling technique technique (Magnússon *et al.*, 2009; Purwaamidjaja 2010; Odoli *et al.*, 2013; Margeirsson *et al.*, 2011; Olafsdottir *et al.*,

2006a; Semwanga 2010; Arnþórsdóttir *et al.*, 2008) almost nothing no research exists on the effect of CBC on whole fish.

For determination of freshness of tilapia, the indicative tests are sensory and microbiological tests (Odoli *et al.*, 2013). There was little difference found between CBC treated and untreated on the same sampling day both for microbial test and for sensory attributes. This might be because after CBC treatment, the fish core temperature was decreased to sub-zero level. Both CBC treated and untreated fish were stored in the same cooler in the same sub-zero temperature to simulate sea freight transportation. On day 3, the core temperature of all the treatments became similar. Therefore, the temperature advantage gained by CBC treatment could not influence the overall quality of fish. Similar results were reported by Ólafsdóttir *et al.*, (2012), which indicated that the superchilled processing for whole cod was not important if the storage condition is similar. But some difference can be found if the temperature in the storage condition fluctuates.

Bjarnason (2012) stated that CBC was applied for 30 minute and stored for one hour showed that the fish flesh did not reach initial freezing. Hence, he assumed that a lower temperature or a longer chilling period needs to be applied for an around 2.5 kg whole fish. Sikorski (1990) recorded that the initial freezing point of fish is  $-0.8$  to  $-1.4^{\circ}\text{C}$ , but Johnston *et al.*, (1994) reported that the initial freezing point for fish is  $-1^{\circ}\text{C}$ . Kaale *et al.*, (2011) stated that in superchilling, the temperature of a food product is lowered to  $1-2^{\circ}\text{C}$  below the initial freezing point. Waterman and Taylor (2001), specified the temperature to a narrow range of  $-2$  to  $-2.5^{\circ}\text{C}$ . The temperature of CBC treated fish in this experiment were around  $-0.5^{\circ}\text{C}$ . The scale present in tilapia skin is a heat transfer barrier. Therefore, may be only the skin reached initial freezing as the muscle temperature recorded at around  $-0.5^{\circ}\text{C}$ .

### 5.3 Effect of gutting

The decision of a processor to gut fish or not is sometimes a tricky one. When a fish is gutted, then the enzymes which are the precursors of spoilage are removed. On the other hand, the fish undergoes some handling stress and some of the areas are exposed to micro-organisms when it is gutted (Huss, 1995). In the current study, no significant change in was observed between day 2 and day 6 sampling in terms of TVC, SSO, pH and TVB-N and sensory. Which might be due to the sub-zero temperature reduced the enzymatic and bacterial activity. But after 6 days, when the temperature was reset, it went from super -chilled to chilled storage and bacteria grow slowly in chilled conditions (Hobbs, 1982). On day 10, a steady increase in TVC and SSO was observed, while pH was also increased but slowly. At the day 20, the fish TVC value for ungutted with/without CBC was around log 7, while the gutted treatment was still just over log 6. But statistically the difference was not significant. In case of SSO, growth was very fast from day 6 to day 10, then the growth became slower but towards the end of the experiment the result of TVC and SSO was closer that indicates the dominance of  $\text{H}_2\text{S}$  producing bacteria in fish. This bacteria was responsible for producing putrid odour that induced a sensory rejection. However SSO count was higher in case of ungutted fish treated with/without CBC. On the other hand the TVB-N value was more or less similar to the earlier results on day 14 and in day 20, the result for the ungutted fish showed both for CBC treated & without CBC are  $22.32 \pm 3.96$  &  $19.46 \pm 1.49$  mgM/100g respectively; whereas gutted fish showed low score, although the result was not statistically significantly different. The pH was increasing up to day 20. Therefore, spoilage was taking place inside fish muscle and the byproduct of basic nature produced inside increased the pH of fish.

## 5.4 pH

pH is commonly used to measure of fish deterioration (Howgate, 2009). It is an indicator of the environment and indicates the suitability of the post mortem fish for enzymatic and bacterial activity. After death of fish, autolytic action takes place, which produce lactic acid in the fish. As a result of this pH value decreases. After rigor during storage, pH value increases due to the production of basic compounds like ammonia mainly derived from microbial action by fish spoilage bacteria (Boskou and Debevere, 2000; Ruiz-Capillas and Moral, 2001). The starting pH of the treatments was ( $6.08 \pm 0.03$ ), which was lower and indicates that the fish had just passed the rigor stage. In tilapia, after death stored in 0-2°C, rigor finishes within 26.5 hours (Huss, 1995), the fish in this case had passed already 2 days in a lower pH. Therefore it can be assumed that the pH measurement started at the earlier post rigor stage. At day 6, the pH was more or less similar for all the treatments that however indicates that the environment was the same for all the treatments. The result was supported by the microbial growth, where the production of Specific Spoilage Organisms was more or less static. When the temperature was reset, the pH started to increase again and that increase was evident up to the end of the experiment, which indicates the presence of basic compounds produced by the micro-organisms.

## 5.5 Shelf life of fish

Temperature plays a vital role in maintaining the freshness of fish. For the first 6 days of this experiment, temperature was set at sub-zero level (-1°C). At that time, the quality of fish was about the same as fresh caught fish. On day 6, three out of four treatment showed absence of spoilage bacteria in the fish muscle, fourth had a small SSO load. The QIM score was near about 0, on that day. But when the temperature was increased to around 1-2°C, then bacteria inactive at sub-zero temperature started to grow rapidly. The bacterial load in all the treatments increased exponentially. The H<sub>2</sub>S producing spoilage bacteria increased from 0 to log 3 within 4 days. At day 20, the TVC and SSO load was very close. The rejection point as described by the ICMSF (1986), Gram *et al.* (1987) and Odoli *et al.*, (2013a) is 10<sup>7</sup> cfu/g for TVC and 10<sup>6</sup> cfu/g for SSO. On day 20, the result showed that all the samples are around these points irrespective to treatments. The difference between the treatments were minor. Gram and Huss (1996) reveal a relational model on bacterial load, specific spoilage bacteria and TVB-N. The difference between TVC and SSO was found to be narrow in day 20 as mentioned by the model, which is the indicator of spoilage or end of shell life. But TVB-N was not found to show the similarities with the model. According to Cyprian (2013b) TVB-N value in tilapia is a good indicator of spoilage. Ólafsdóttir *et al.*, (2012) found minor differences in values of pH, sensory and bacterial growth between the superchilled and non-superchilled whole fish groups. At day 17, all the groups crossed rejection line, especially Guttled with CBC scored the highest point, which indicates the quality deterioration of fish. This might due to the combined effect of CBC forming ice crystals in skin which was initially very good for storage but after ice crystals melt down skin became susceptible to micro-organisms and due to the gutting of fish expose the body cavity to the environment. However, the difference is not significant. Based on the result, it can be assumed that the sensory rejection point is 17 days. Odoli *et al.*, (2013b) revealed a shelf life of 13–15 days for air-packaged fillets during storage at 1°C and 20 days at -1°C based on the result of sensory analysis of cooked samples. The changes in total count (TVC), specific spoilage organism (SSO) and chemical spoilage indicator during chilled storage of fish product are shown in Figure 29 below.



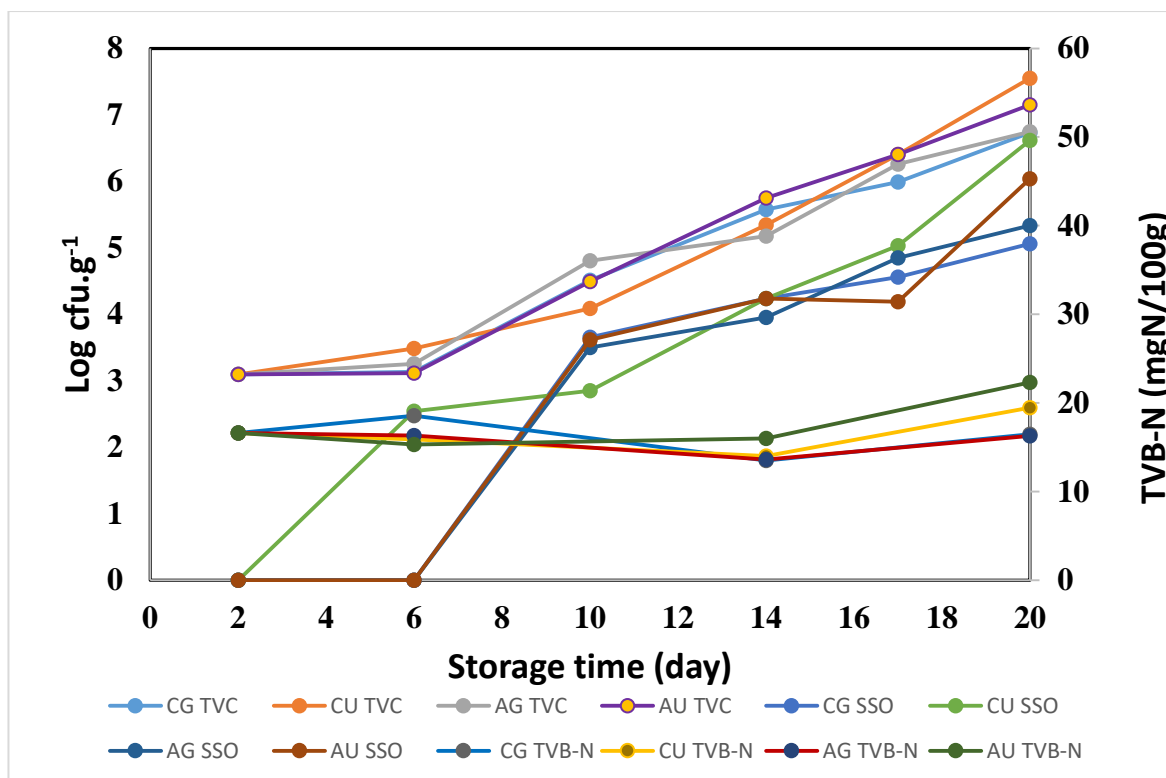


Figure 29. Changes in total count (TVC), specific spoilage organism (SSO) and chemical spoilage indicator during chilled storage of fish product.

## 5.6 Retail purchase decision

### 5.6.1 Cooking yield

The study of cooking yield showed that the cooking yield had minor increasing tendency. Cooking yield is related to total yield. Total yield consists of yield (opposite to drip loss) and cook yield. The changes in total yield could be affected by decomposition of fish muscle. The reason for fish spoilage is the combined effect enzymatic activity and bacterial growth. Another possible cause is the ice crystal formation due to super chilling and storage temperature. According to Martino *et al.*, (1998) and Bahuaud *et al.*, (2008), with time muscle degrades and more water comes out which results in increased fluid loss and may result in less free water to release in cooking yield. Bao *et al.*, (2007) compared cooking yield of arctic charr fillet stored with or without ice packs in superchill, dry ice and chilled storage found no significant difference between them, i.e. neither within the treatments nor on the sampling days.

### 5.6.2 Sensory analysis

At the beginning of the storage, on days 2 and 6, the sensory score was very low. The lateral line colour was bright red. This was supported by the result found in colour test, where the combination of L, a, b indicates similar colour.

On day 20, the colour of lateral line was like darkish brown. Similar result was found in colour test, where it showed reduction in light, increase in redness and increased yellowness indicates

a darkish brown colour. According to Odoli *et al.*, (2013b) colour is a major factor in influencing retail purchase decision. The fillet side colour, however did not show characteristic difference perhaps due to the number of measurement points were 2 in the loin and 2 in the belly flap; and belly flap was diversely coloured.

The most important attribute in retail decision is odour. On day 17, the odour average score was near to highest score. But on day 20, the odour was very strong putrid and recognizable to all the panellists. Some of the attributes like mucus was still in good condition and microbial load was also in the limits, but due to colour and odour the sensory score crossed rejection point.

## 6 CONCLUSIONS AND RECOMMENDATIONS

For the simulation of sea freight, the quality of tilapia in terms of sensory, pH, microbial, chemical and colour attributes was similar to the fresh caught fish. But when the temperature was increased the fish quality was degrading very quickly. So for transportation and retail chain either the temperature could be decreased or cooling effect could be increased during storage.

Evidently, the period at which the temperature was set at  $-1^{\circ}\text{C}$  in the cooler. The fish core temperature in CBC treated fish were around  $-0.5^{\circ}\text{C}$  and without CBC was around  $0^{\circ}\text{C}$  at the starting and reduced to  $-0.5^{\circ}\text{C}$  on day 3 to day 6. The core temperature of CBC treated was colder than without CBC throughout the experiment indicating the cooling efficiency of CBC. However, the advantage gained by CBC treatment was neutralized by storage in  $-1^{\circ}\text{C}$  for 6 days. Furthermore, fish core temperature for CBC treated fish was not below initial freezing. Therefore, the fish is not superchilled. Only the skin may be superchilled. A lower temperature or increased cooling time or both should be used for CBC treatments in case of whole fish. Further research is needed on this ground.

Higher microbial load, SSO count, pH and TVB-N was found in ungutted fish. The gut content was spoiled at the later stage of the experiment, which was obnoxious during filleting, meaning that it would also be the same for the consumers. Therefore it would be better to gut the fish during processing.

Based on the sensory and microbial results, the shelf life of tilapia was about 17 days during the simulation study for the treatments.

CBC can be used as a superchilling technique, but treatment time should be longer or temperature should be lower or it can be combination of both.

There is no sensory method for checking the freshness of the whole tilapia. Only validated method developed was only for the fillets. There a QIM method should be developed for assessing freshness of tilapia.

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APPENDIX

**Appendix A: Lateral line colour of different treatments in the sampling days**

	Gutted with CBC	Ungutted with CBC	Gutted without CBC	Ungutted without CBC
Day 2				
	Reference			
Day 6				
Day 10				
Day 14				



### Appendix B: Results (Mean $\pm$ SD) for different tests of the treatments in the sampling points

Storage time	Treatment	QIM score	TVC	SSO (H <sub>2</sub> S producing bacteria)	Cooking yield	pH
Reference	Ungutted without CBC	0.22 $\pm$ 0.10	3.09 $\pm$ 0.13	0.00 $\pm$ 0.00	90.89 $\pm$ 1.41	6.08 $\pm$ 0.03
Day 6	Gutted with CBC	1.14 $\pm$ 0.43	3.13 $\pm$ 0.50	0.00 $\pm$ 0.00	88.80 $\pm$ 0.42	6.04 $\pm$ 0.12
	Ungutted with CBC	1.00 $\pm$ 0.57	3.49 $\pm$ 0.10	2.54 $\pm$ 0.34	89.78 $\pm$ 1.52	6.02 $\pm$ 0.01
	Gutted without CBC	0.95 $\pm$ 0.41	3.26 $\pm$ 0.25	0.00 $\pm$ 0.00	90.87 $\pm$ 0.72	6.04 $\pm$ 0.03
	Ungutted without CBC	0.76 $\pm$ 0.22	3.11 $\pm$ 0.00	0.00 $\pm$ 0.00	90.89 $\pm$ 2.58	6.10 $\pm$ 0.04
Day 10	Gutted with CBC	2.39 $\pm$ 0.35	4.51 $\pm$ 0.28	3.66 $\pm$ 0.06	90.74 $\pm$ 0.93	6.26 $\pm$ 0.08
	Ungutted with CBC	2.28 $\pm$ 0.35	4.09 $\pm$ 0.72	2.85 $\pm$ 1.20	89.33 $\pm$ 1.36	6.18 $\pm$ 0.04
	Gutted without CBC	2.22 $\pm$ 0.10	4.80 $\pm$ 0.42	3.50 $\pm$ 0.71	91.49 $\pm$ 0.84	6.26 $\pm$ 0.05
	Ungutted without CBC	1.83 $\pm$ 0.60	4.49 $\pm$ 0.33	3.62 $\pm$ 0.12	91.16 $\pm$ 0.65	6.15 $\pm$ 0.11
Day 14	Gutted with CBC	4.03 $\pm$ 0.40	5.58 $\pm$ 0.18	4.24 $\pm$ 0.34	90.52 $\pm$ 1.50	6.11 $\pm$ 0.12
	Ungutted with CBC	3.63 $\pm$ 0.35	5.34 $\pm$ 0.80	4.24 $\pm$ 0.34	92.06 $\pm$ 0.63	6.25 $\pm$ 0.05
	Gutted without CBC	3.40 $\pm$ 0.61	5.17 $\pm$ 0.41	3.95 $\pm$ 0.07	91.70 $\pm$ 0.96	6.25 $\pm$ 0.00
	Ungutted without CBC	3.47 $\pm$ 0.45	5.75 $\pm$ 0.10	4.24 $\pm$ 0.34	91.83 $\pm$ 1.52	6.30 $\pm$ 0.02
Day 17	Gutted with CBC	6.89 $\pm$ 0.92	5.99 $\pm$ 0.30	4.56 $\pm$ 0.63	91.80 $\pm$ 0.78	6.34 $\pm$ 0.12
	Ungutted with CBC	6.22 $\pm$ 0.95	6.40 $\pm$ 0.21	5.03 $\pm$ 0.35	91.42 $\pm$ 0.62	6.35 $\pm$ 0.08
	Gutted without CBC	7.67 $\pm$ 1.15	6.26 $\pm$ 0.06	4.85 $\pm$ 0.64	91.53 $\pm$ 1.19	6.32 $\pm$ 0.11
	Ungutted without CBC	5.72 $\pm$ 0.38	6.40 $\pm$ 0.49	4.19 $\pm$ 0.27	91.95 $\pm$ 2.34	6.36 $\pm$ 0.07
Day 20	Gutted with CBC	6.61 $\pm$ 0.89	6.73 $\pm$ 0.12	5.06 $\pm$ 0.03	92.30 $\pm$ 0.96	6.30 $\pm$ 0.19
	Ungutted with CBC	7.11 $\pm$ 1.37	7.55 $\pm$ 0.03	6.62 $\pm$ 0.54	92.13 $\pm$ 0.99	6.45 $\pm$ 0.00
	Gutted without CBC	7.11 $\pm$ 0.17	6.75 $\pm$ 0.35	5.33 $\pm$ 0.22	92.02 $\pm$ 1.18	6.36 $\pm$ 0.08
	Ungutted without CBC	7.31 $\pm$ 0.60	7.15 $\pm$ 0.40	6.04 $\pm$ 0.37	91.07 $\pm$ 1.54	6.48 $\pm$ 0.08

**Appendix C: Scores of the attributes (Mean  $\pm$  SD) of QIM in different treatments on the sampling days.**

		Lateral line	Loin	Belly flap	Mucus	Texture	Odour
Day 2	Reference	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.05 $\pm$ 0.08	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.19 $\pm$ 0.08
Day 6	Gutted with CBC	0.17 $\pm$ 0.14	0.25 $\pm$ 0.22	0.58 $\pm$ 0.36	0.13 $\pm$ 0.13	0.13 $\pm$ 0.13	0.13 $\pm$ 0.13
	Ungutted with CBC	0.17 $\pm$ 0.19	0.17 $\pm$ 0.07	0.33 $\pm$ 0.07	0.08 $\pm$ 0.07	0.08 $\pm$ 0.14	0.25 $\pm$ 0.13
	Gutted without CBC	0.08 $\pm$ 0.07	0.25 $\pm$ 0.00	0.29 $\pm$ 0.07	0.13 $\pm$ 0.13	0.04 $\pm$ 0.07	0.13 $\pm$ 0.13
	Ungutted without CBC	0.17 $\pm$ 0.07	0.21 $\pm$ 0.14	0.38 $\pm$ 0.43	0.13 $\pm$ 0.00	0.13 $\pm$ 0.00	0.08 $\pm$ 0.14
Day 10	Gutted with CBC	0.39 $\pm$ 0.29	0.50 $\pm$ 0.17	0.42 $\pm$ 0.14	0.39 $\pm$ 0.10	0.33 $\pm$ 0.17	0.33 $\pm$ 0.17
	Ungutted with CBC	0.39 $\pm$ 0.13	0.44 $\pm$ 0.17	0.31 $\pm$ 0.13	0.33 $\pm$ 0.00	0.31 $\pm$ 0.05	0.39 $\pm$ 0.25
	Gutted without CBC	0.50 $\pm$ 0.00	0.50 $\pm$ 0.22	0.17 $\pm$ 0.17	0.31 $\pm$ 0.17	0.28 $\pm$ 0.10	0.36 $\pm$ 0.21
	Ungutted without CBC	0.31 $\pm$ 0.05	0.50 $\pm$ 0.17	0.25 $\pm$ 0.22	0.28 $\pm$ 0.10	0.14 $\pm$ 0.13	0.22 $\pm$ 0.10
Day 14	Gutted with CBC	0.67 $\pm$ 0.06	0.93 $\pm$ 0.12	0.80 $\pm$ 0.20	0.20 $\pm$ 0.00	0.70 $\pm$ 0.17	0.63 $\pm$ 0.29
	Ungutted with CBC	0.57 $\pm$ 0.21	0.73 $\pm$ 0.12	0.67 $\pm$ 0.15	0.10 $\pm$ 0.10	0.53 $\pm$ 0.12	0.73 $\pm$ 0.35
	Gutted without CBC	0.77 $\pm$ 0.06	0.70 $\pm$ 0.17	0.67 $\pm$ 0.12	0.20 $\pm$ 0.00	0.50 $\pm$ 0.17	0.83 $\pm$ 0.15
	Ungutted without CBC	0.60 $\pm$ 0.20	0.50 $\pm$ 0.10	0.80 $\pm$ 0.20	0.13 $\pm$ 0.12	0.57 $\pm$ 0.15	0.80 $\pm$ 0.36
Day 17	Gutted with CBC	1.39 $\pm$ 0.25	1.06 $\pm$ 0.10	1.11 $\pm$ 0.25	0.50 $\pm$ 0.17	0.97 $\pm$ 0.21	1.78 $\pm$ 0.34
	Ungutted with CBC	1.25 $\pm$ 0.22	1.25 $\pm$ 0.14	1.22 $\pm$ 0.38	0.61 $\pm$ 0.10	0.92 $\pm$ 0.08	2.31 $\pm$ 0.32
	Gutted without CBC	0.97 $\pm$ 0.27	1.06 $\pm$ 0.10	1.00 $\pm$ 0.08	0.44 $\pm$ 0.19	0.89 $\pm$ 0.10	1.81 $\pm$ 0.38
	Ungutted without CBC	0.78 $\pm$ 0.10	0.89 $\pm$ 0.25	0.83 $\pm$ 0.17	0.39 $\pm$ 0.10	0.72 $\pm$ 0.10	2.03 $\pm$ 0.10
Day 20	Gutted with CBC	1.06 $\pm$ 0.10	1.11 $\pm$ 0.48	0.94 $\pm$ 0.27	0.39 $\pm$ 0.10	0.78 $\pm$ 0.10	2.44 $\pm$ 0.25
	Ungutted with CBC	1.17 $\pm$ 0.00	0.97 $\pm$ 0.13	1.06 $\pm$ 0.19	0.53 $\pm$ 0.05	1.11 $\pm$ 0.10	2.39 $\pm$ 0.19
	Gutted without CBC	1.33 $\pm$ 0.44	1.19 $\pm$ 0.13	1.00 $\pm$ 0.22	0.56 $\pm$ 0.19	0.81 $\pm$ 0.27	2.39 $\pm$ 0.38
	Ungutted without CBC	1.11 $\pm$ 0.10	1.22 $\pm$ 0.19	1.22 $\pm$ 0.10	0.61 $\pm$ 0.19	0.83 $\pm$ 0.17	2.47 $\pm$ 0.21

**Appendix D: Colour measurement results for the treatments in the sampling days by lightness (L), redness/greenness, yellowness/ blueness (Mean  $\pm$  SD).**

Day	Treatments	Lateral line colour			Bone-side fillet colour		
		L	a	b	L	a	b
Day 2	Reference	33.12 $\pm$ 0.37	8.19 $\pm$ 1.51	4.98 $\pm$ 0.87	46.08 $\pm$ 0.82	-2.19 $\pm$ 0.33	1.00 $\pm$ 0.15
Day 6	Gutted with CBC	33.27 $\pm$ 1.44	8.60 $\pm$ 0.79	4.89 $\pm$ 0.49	44.20 $\pm$ 1.99	-2.13 $\pm$ 0.33	0.27 $\pm$ 0.42
	Ungutted with CBC	31.27 $\pm$ 2.42	10.42 $\pm$ 1.10	5.63 $\pm$ 0.90	45.33 $\pm$ 1.22	-2.37 $\pm$ 0.28	0.46 $\pm$ 0.22
	Gutted without CBC	32.07 $\pm$ 0.76	9.72 $\pm$ 0.64	4.96 $\pm$ 0.23	44.07 $\pm$ 1.69	-2.14 $\pm$ 0.30	0.06 $\pm$ 0.20
	Ungutted without CBC	33.08 $\pm$ 4.44	9.36 $\pm$ 1.80	5.00 $\pm$ 0.78	45.80 $\pm$ 2.24	-2.53 $\pm$ 0.07	0.43 $\pm$ 0.72
Day 10	Gutted with CBC	31.23 $\pm$ 1.58	10.08 $\pm$ 1.59	5.81 $\pm$ 1.01	43.71 $\pm$ 2.79	-1.22 $\pm$ 0.88	-0.29 $\pm$ 0.64
	Ungutted with CBC	33.90 $\pm$ 1.39	8.71 $\pm$ 1.81	5.17 $\pm$ 1.12	43.48 $\pm$ 1.12	-1.94 $\pm$ 0.37	0.25 $\pm$ 0.70
	Gutted without CBC	32.50 $\pm$ 1.73	8.92 $\pm$ 3.07	5.13 $\pm$ 1.48	41.85 $\pm$ 1.58	-1.39 $\pm$ 0.48	-0.22 $\pm$ 0.59
	Ungutted without CBC	30.79 $\pm$ 0.93	10.73 $\pm$ 1.02	5.98 $\pm$ 0.48	44.96 $\pm$ 2.94	-2.10 $\pm$ 0.39	0.28 $\pm$ 0.56
Day 14	Gutted with CBC	34.34 $\pm$ 1.89	8.76 $\pm$ 0.57	4.60 $\pm$ 0.60	49.20 $\pm$ 4.89	-2.09 $\pm$ 0.49	1.13 $\pm$ 1.41
	Ungutted with CBC	31.75 $\pm$ 1.85	8.87 $\pm$ 0.64	4.28 $\pm$ 0.31	45.37 $\pm$ 3.39	-1.75 $\pm$ 0.39	0.49 $\pm$ 0.44
	Gutted without CBC	34.26 $\pm$ 1.28	8.59 $\pm$ 1.68	4.67 $\pm$ 0.86	44.44 $\pm$ 0.94	-1.85 $\pm$ 0.37	0.46 $\pm$ 0.67
	Ungutted without CBC	34.07 $\pm$ 2.90	8.75 $\pm$ 0.80	4.85 $\pm$ 0.68	44.21 $\pm$ 0.54	-1.94 $\pm$ 0.37	-0.18 $\pm$ 0.26
Day 17	Gutted with CBC	33.08 $\pm$ 0.76	6.95 $\pm$ 0.27	5.69 $\pm$ 0.29	43.39 $\pm$ 1.59	-1.91 $\pm$ 0.48	0.38 $\pm$ 0.93
	Ungutted with CBC	32.54 $\pm$ 1.11	8.09 $\pm$ 2.16	5.65 $\pm$ 0.51	44.17 $\pm$ 2.37	-2.04 $\pm$ 0.30	0.45 $\pm$ 0.84
	Gutted without CBC	35.49 $\pm$ 0.98	5.73 $\pm$ 1.26	5.41 $\pm$ 1.08	42.44 $\pm$ 1.83	-1.83 $\pm$ 0.46	0.80 $\pm$ 0.45
	Ungutted without CBC	32.79 $\pm$ 0.84	9.52 $\pm$ 2.42	5.96 $\pm$ 1.24	44.23 $\pm$ 2.76	-2.15 $\pm$ 0.43	-0.23 $\pm$ 0.55
Day 20	Gutted with CBC	32.13 $\pm$ 1.26	9.22 $\pm$ 1.84	6.69 $\pm$ 0.28	43.02 $\pm$ 1.62	-2.09 $\pm$ 1.15	-0.12 $\pm$ 0.13
	Ungutted with CBC	32.14 $\pm$ 1.75	8.02 $\pm$ 1.69	6.20 $\pm$ 0.24	39.38 $\pm$ 5.52	-1.87 $\pm$ 0.15	0.12 $\pm$ 0.80
	Gutted without CBC	32.16 $\pm$ 1.05	8.47 $\pm$ 0.72	6.80 $\pm$ 0.35	42.65 $\pm$ 1.59	-2.29 $\pm$ 0.68	0.39 $\pm$ 0.70
	Ungutted without CBC	30.82 $\pm$ 0.35	11.65 $\pm$ 2.63	6.37 $\pm$ 0.27	41.36 $\pm$ 1.40	-2.17 $\pm$ 0.07	-0.18 $\pm$ 0.39