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P.O. Box 1390, Skulagata 4
120 Reykjavik, Iceland

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EFFECT OF DIFFERENT BLEEDING CONDITIONS ON THE COLOUR TONE OF FRESH, FROZEN AND SALTED FILLETS OF ATLANTIC COD (*Gadus morhua)*

EUNICE NUERKIE TETTEH
Ministry of Food and Agriculture, Fisheries Commission,
Marine Fisheries Research Division,
P. O. Box BT62, Tema, Ghana,
eunicomgh@yahoo.com

Supervisor:
Jónas R. Viðarsson,
jonas.r.vidarsson@matis.is
Matis

ABSTRACT

Effects of different bleeding treatments on the colour of fresh, frozen and salted cod fillets were examined. Three bleeding methods were used for the fresh fillets i.e. bleeding, gutting and washing immediately after capture (A), bleeding, gutting and cleaning a whole day after capture (B) and bleeding upon capture, but gutting and cleaning the day after (C). The fish that was bled, gutted and cleaned immediately after capture was significantly whiter than fish in the other two groups. Despite not being as white as group A, group C turned out to be whither than group B. Seven different bleeding treatments were used for the frozen and salted fillets, where experiments were done with variable time, temperature and water-throughput in the bleeding tank. Results from the frozen fillets showed that bleeding the fish for 15 minutes in seawater returned the whitest fillets, but by increasing water-throughput or not allowing the fish to bleed in a bleeding tank at all returned the darkest fillets. The salted fillets however did not return the same results as fish that was only allowed a short time in the bleeding tank yielded the whitest fillets and fish that was bled in a bleeding tank with increased water-throughput yielded the darkest fillets. The results from the experiment are therefore inconclusive, as different processing methods return different results.

Key words: bleeding, colour, heme iron, whiteness, Atlantic cod

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1 INTRODUCTION

In the past few decades fish for human consumption has increased considerably, while fish for non-food purposes has remained relatively stable. Of the world fish production 81% was used for human consumption in 2008, of which 49% was fresh or live fish, 25% frozen and the remaining part was processed in some other way (FAO, 2010). Since the 1990s the world production of fresh seafood has doubled, while the ratio of other production methods (frozen, canning, cured) has been decreasing.

Along with this increasing emphasis on fresh deliveries, today's consumers have become particular about the nature and quality of the fish they consume. The colour and odour of the raw fish flesh are the most important indicators by which consumers use to evaluate the freshness and quality of the fish (Pearson and Dutson 1994). Producers of seafood are therefore now more careful with the quality of the end product, as considerable price premiums can be demanded for a high quality product.

The colour tone of fish flesh depends amongst other things on the myoglobin and haemoglobin content in the muscles. High content of these blood components have a negative impact on the appearance and quality of the fish. For this reason, most producers want to get their fish drained of as much blood as possible immediately upon capture.

Bleeding is usually quicker and more effective when carried out at a relatively low temperature and when the fish are still alive (Smith and Hardy 2001). It is good practice with some fish to bleed them prior to gutting. On the other hand, in some fisheries, bleeding and gutting is done at the same time. In both cases, the fish will bleed better if they are freshly caught. For this purpose fishermen should try to bring fish on-board alive. If bleeding and gutting is done on dead fish, the fillets from such fish will have a pronounced discolouration compared to the appearance of properly bled fish.

During the past few years increasing interests have been awarded to refining bleeding techniques on-board industrial vessels. This particularly applies to whitefish fisheries in the North East Atlantic, such as cod and haddock fisheries in Iceland and Norway. In some other parts of the world the importance of proper bleeding has not been met with the same enthusiasm. In Ghana for example, most of the catch is landed without being bled. The bleeding of the catch may occur at a later time or not at all. This procedure tends to affect the appearance, odour and quality of the fish.

Bleeding of fish is of high importance and should be a common practice in all fisheries. This especially applies to the tuna vessels in Ghana, because the blood of tuna struggling on a hook attains high organic waste (lactic acid) content and the temperature of the blood can rise up to 35°C (Blanc *et al.* 2005). Bleeding removes the organic waste and helps to cool the fish's body. The fish can therefore be refrigerated more quickly and will have a better quality flesh.

The aim of this project is to examine different bleeding conditions of Atlantic cod and to study its effect on the colour tone of fresh, frozen and salted fillets. The results will then be used to determine the best method of adopting proper bleeding techniques in the Ghanaian fishing sector.

2 THE GHANA FISHERIES SECTOR

Ghana is located on the west coast of Africa (Figure 1). It has a population of 24 million and total land area of 238,537 km^2 with a coastline that is approximately 550 km long. The narrow continental shelf is 24,300 km^2 ranging in depths from 75-120 m (MOFA 2004). This area supports the fishing activities of the artisanal and semi-industrial fishing fleet.

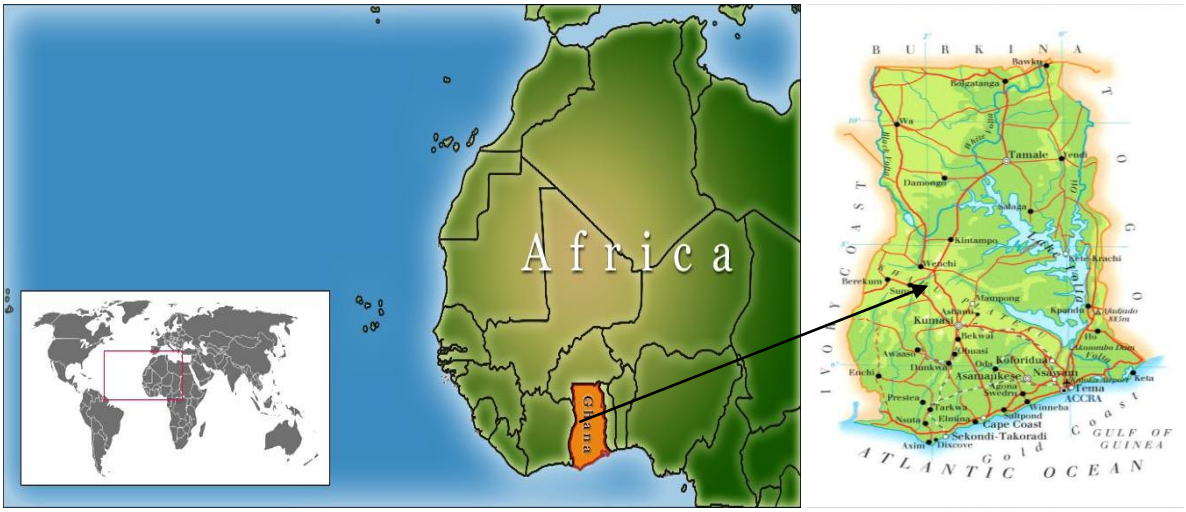

Figure 1: Global location of Ghana.

The Ghanaian fisheries sector is comprised of three main groups, each targeting a different fishery. These are the freshwater fisheries located on Lake Volta and other inland waters, marine fisheries and aquaculture. Fisheries account for 3% of the GDP in Ghana and about two million people derive their livelihood from the fishing sector (FC 2009). The most important use of fish in Ghana is for domestic consumption where fish forms approximately 60% of the animal protein in the diet of Ghanaians. The export market is made up primarily of tuna catches.

2.1 Marine fisheries

The marine sector is further divided into artisanal, semi-industrial/inshore and industrial fisheries. Artisanal fishing is conducted with the use of canoes, many of which are motorised. The fishing gear used in this sector includes purse seines, beach seines, cast nets, draft gill nets and hook and line. This fishery contributes between 70% – 80% of the marine fish landed and includes mainly mackerel, sardinellas and anchovy species (MOFA 2004). This industry operates from some 304 landing sites in 189 fishing villages along the Ghanaian coastline.

The semi-industrial fleet consists of locally built wooden vessels measuring between 8 and 37 meters in length and employs the use of trawls or purse seines. There has been a decrease in the number of vessels in the semi-industrial fleet because of the decline in target species and a high maintenance and operation cost (MOFA 2004).

The industrial fleet comprises vessels that are foreign-built trawlers, shrimpers, tuna pole and line vessels, and purse seiners. The Fisheries Act 625 of 2002 makes provisions for a joint

venture enterprise in this sector. This is evident in the tuna fishery where the Ghanaian nationals own 50% shares in all vessels.

The main small pelagic species targeted by the Ghanaian fishing fleet includes the families of Clupeidae (Sardinellas), Scombridae (chub-mackerel) and Engraulidae (anchovy) and Thunnidae (tunas). The most important demersal species are of the families Sparidae, Mullidae, Serranidae and Penaeida among others.

The artisanal fleet has by far the highest production every year, even though volumes fluctuate considerably between years (Figure 2) (MFRD 2010). The next highest marine production is the one done by the tuna vessels where most of their catches are exported. Almost all catches from the artisanal fleet are used locally, with just a small percentage being exported to the neighbouring countries. The inshore (semi-industrial) fleet accounts for a minor portion of the marine fish production and most of the catch from these vessels are used domestically.

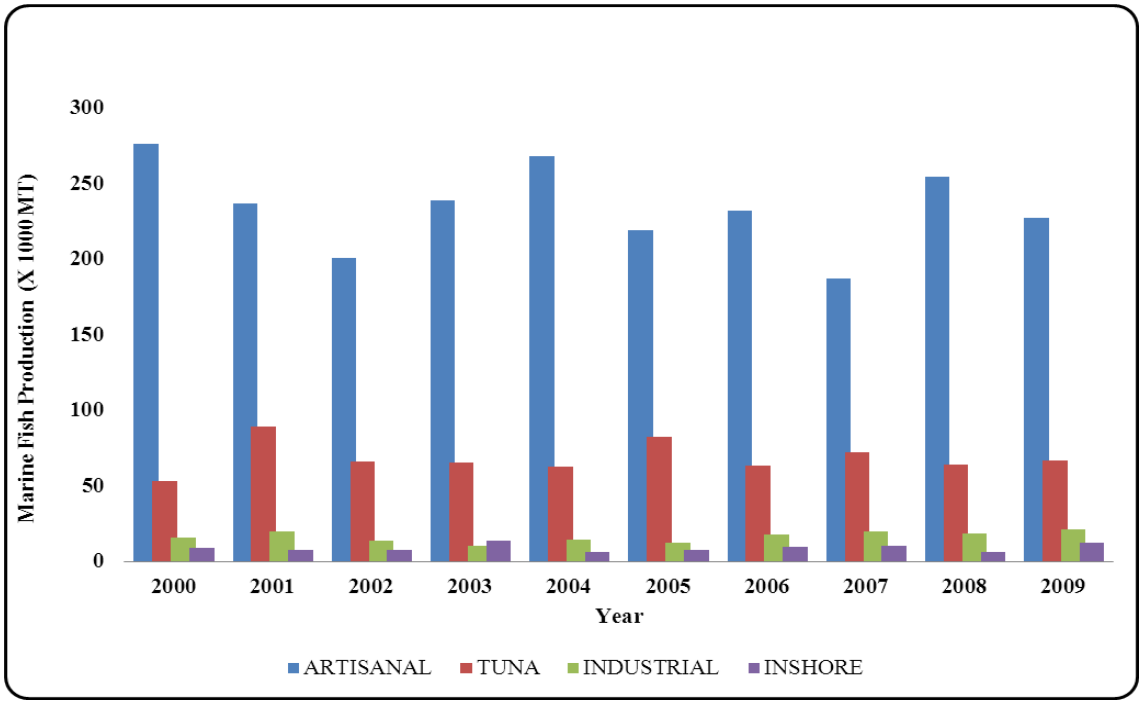

Figure 2: Ghanaian Marine Fish Production from 2000 – 2009 according to fleet type.

Tuna represents the majority of the seafood export value in Ghana (MOFA 2004). The commercial tuna fisheries of the East Atlantic and for that matter the Ghanaian tuna fishery are based on the harvests of bigeye (*Thunnus obesus*), skipjack (*Katsuwonus pelamis*), and yellowfin (*Thunnus albacares*). In Ghana, bait-boats and purse seiners commercially harvest these species and the total landed volume is between 50,000 MT and 90,000 MT (Figure 3) (MFRD 2010). The majority of the tuna is canned and exported, but part is sold locally (MOFA 2004).

Figure 3: Annual total tuna catch and its distribution (exported or sold locally) of Ghanaian tuna vessels 2000-2009.

Bait-boats are the main exploiters of tuna in Ghanaian waters, using live anchovy (*Engraulis encrasicolus*) as the main bait for their operation with young sardinellas occasionally used. In addition to the use of bait to attract tuna, about 3000 bamboo rafts are used as fish aggregating devices. These are known to attract more juvenile fish (MFRD 1999 and 2000).

Two companies account for the mainstay of the tuna landed in Ghana. They are Pioneer Food Cannery (PFC) owned by Heinz Europe which is able to process 175-200 tons per day, and Ghana Agro-Foods Company Limited (GAFCO) which is able to processes 7- 10 tons per day (MFRD 2000). Both canneries are located in Tema. Fish below minimum size of 1.5 kg is not sold to the canneries, but goes to the local market, which is then sold usually smoked using traditional ovens with firewood. The products from the canneries are primarily exported to Europe.

2.2 Freshwater and lagoon fisheries

Lake Volta provides about 90 % of the total inland fisheries production (MOFA 2004). The fishery on the lake is solely made up of artisanal fishery where the main gear types are cast nets, gillnets, hook and lines and traps. Tilapias and *catfish* species account for about 70% of the catch in the lake.

There are over 50 lagoons of various sizes along the coast of Ghana. These lagoons play an important role in preserving biodiversity and fish stocks in the coastal waters, as some species (e.g. jack mackerel, snappers, groupers) spend a part of their life cycle in the lagoons. The most abundant species of fish in the lagoons are the tilapias (e.g. *Tilapia zilli*) and marine fishes e.g. channel flounder (*Scyacium micrurum*).

2.3 Aquaculture

Aquaculture is a fairly new but rapidly developing sector in Ghana. There are about 1000 fish farmers with over 2000 ponds currently involved in aquaculture, but data collection in this sector is not very precise. This is due to the fact that most commercial operators have consistently refused to provide data on their production to enable estimation of national fish production figures (FC 2009). Semi-intensive and extensive cultures are practiced with the main species of fish cultured being tilapia (*Oreochromis niloticus*) and catfish (*Clarias gariepimnus*).

The fisheries sector in Ghana is important in providing employment to a fairly large part of the population and fish is an important source of animal protein for the whole nation. Considerable effort has been made to make sure that Ghanaian seafood products are of good quality, so the people working in the sector earn more and those consuming the products also have better quality for what they buy to consume. Quality of fish depends on the handling along the value chain, it starts from the moment the fish is caught and ends when the consumer gets the fish that he/she is satisfied with. Every step in the process of handling has an effect on the final product and this means that every step taken in the handling process has to be done carefully.

The steps involved in fish handling on-board vessels in Ghana differ from one type of vessel to another. Those in the artisanal sector normally do not spend a long time at sea and generally take little or no ice to chill the fish. The industrial and semi-industrial vessels that stay out for long periods do take ice and some also have freezing compartments on-board to store the fish until landing. But one critical step is missing in the on-board handling, as the fish is rarely bled on-board the vessels.

This study therefore seeks to find out the importance of the bleeding, as well as the best and easiest procedure that Ghanaian fishermen can adapt in order to have top quality fish in terms of colour.

3 BLEEDING AND ON-BOARD HANDLING OF FISH

From the time a fish is caught until it is landed, it should ideally go through various steps in order to preserve the quality and also to prevent contamination that can lead to spoilage. Figure 4 shows typical on-board handling procedures for white fish on-board Icelandic vessels (pers. com). After the catch has been received, fishermen make a “deep cut” through vital arteries of the fish and then bleed it for 10-20 minutes, before gutting. But sometimes the “deep cut” and gutting is done simultaneously. The fish are then washed, iced and stored on-board before landing. Redfish, pelagic fish and many flatfishes are commonly not bled or gutted at sea.

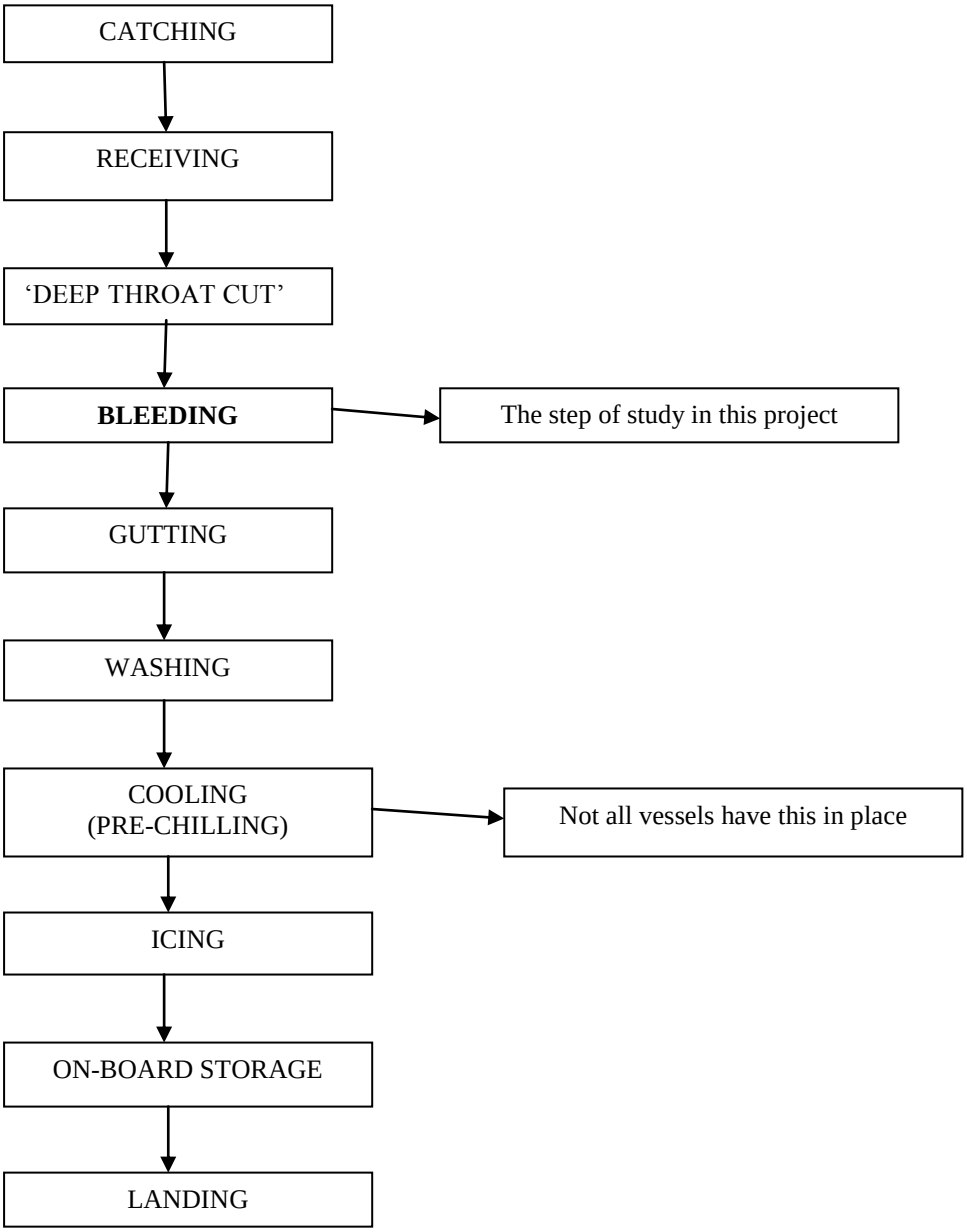

Figure 4: Flow chart of the handling of fish before it is landed.

When it comes to the appearance of the fish fillet a critical point in the process is the bleeding stage. Bleeding should be done as quickly possible after receiving, as the pumping action of the fish heart forces blood out of the fish through the cuts and prevents accumulation of blood in the muscle. This avoids discolouration of the flesh which may lead to undesirable changes in flavour and texture, as well as reducing shelf life.

3.1 Importance of bleeding

Many researchers have found that the bleeding of fish leads to improvement in the quality of flesh in terms of appearance, odour and shelf life. An example of this can be seen on the ocean tuna (Ben-Gigirey *et al*., 1998). Tretsven and Patten (1981) also arrived at this

conclusion after conducting experiments of bleeding using the rainbow trout (*Oncorhynchus mykiss*). Properly bleeding fish eliminates most of the haemoglobin from the tissue and delays rigor mortis (Sohn *et al.* 2007). Other advantages from the bleeding of fish include preventing the development of undesirable discolouration of the flesh and unpleasant flavour during ice storage. The process of bleeding has also been found to prevent microbial growth, lipid oxidation and the development of "fishy" odour in other species (Maqsood and Benjakul 2010, Richards and Hultin 2002, Olsen *et al*. 2008, Sohn *et al.*, 2005).

3.2 Methods of bleeding fish

Methods of bleeding vary from species to species. For example, species such as yellowfin and bigeye tuna have to be killed or stunned quickly and properly before bleeding is done. This is to avoid physical damage to the animal and also serves to retain the quality of the flesh. Killing or stunning is in the first place applied to induce a state of unconsciousness and insensibility of sufficient duration to ensure that the animal does not recover while bleeding to death. Also, stunning or killing produces sufficient immobility to facilitate the initiation of bleeding (Lambooij *et al*., 2006).

Bleeding can be done in at least three different ways; this usually depends on the individual's style, preference and relative comfort with the method of choice. These three most widely used cuts are (SNIC 2011):

1. The throat cut – cutting the blood vessel between the heart and the gill. Care must be taken not to sever the heart as this will lead to losing the pumping action the heart provides.
2. The gill cut – the cut is done by lifting the gill cover and then severing the gill arch or inserting the knife behind the gill through the gill membrane and then cutting up towards the spine severing the blood vessels at the top of the gills.
3. The pectoral cut – The cut is made from the base of the pectoral fin along the midline at both sides of the fish. Great care must be taken when using this method since the depth of the cut can affect the flesh of the fish severally reducing its value. This method is mainly used for tuna.

The throat cut is the most widely used method in the Icelandic fishing fleet, but on-board processes in bleeding and gutting can otherwise be divided in two main methods (pers. com). The first method includes the following steps (1) making a deep throat cut, (2) initiating the bleeding in seawater (sometimes mixed with ice) for a predetermined time followed by (3) making the belly cut for the purpose of gutting and finally (4) washing and icing. The second method includes just three steps as the deep throat cut and gutting are done simultaneously; this is followed by (2) bleeding and washing in seawater and (3) icing into tubs in the hold.

3.3 Consequences of improper bleeding

Not bleeding or inadequately bleeding the fish can have a big impact on the overall quality of the end product. It has for example effect on shelf life, taste and visual appearance as a result of the chemical reactions that takes place because of the residual blood in the tissues of the fish (Huss 1995).

Richards and Hultin (2002) reported that the blood residue catalyses lipid oxidation during storage of fatty fish. Another effect of non-bleeding as found by Ahimbisibwe *et al.* (2010) is that hypoxanthine, volatile basic nitrogen (VBN) and trimythylamine (TMA) were higher in the un-bled muscle tissues of amberjack (*Seriola dumerili*) and red sea bream (*Pagrus major*) after one week of storage.

The quality of the fish flesh depends more on the time that elapses between death and bleeding than the method of bleeding. The importance of bleeding fish immediately after catch cannot be stressed enough. This was for example made evident in an experiment conducted by Roth *et al.*, (2005) where overall quality of wild cod (*Gadus morhua*) was improved when it was bled before gutting.

3.3.1 Effects on colour

When white fish is bled and gutted at sea, washed and iced, the appearance of the flesh is white on landing because the fish would have had time to bleed properly. If however, they were filleted immediately after capture, then the flesh oozes with blood and it appears quite red; if fillets from such un-bled fish are frozen and thawed, they will be brown. When newly gutted whole fish is frozen, the blood similarly does not have time to drain completely resulting in a noticeably brown appearance of thawed fish fillets. When a fish is left un-gutted for some hours in the hold, it is impossible to effectively bleed the fish because of clotting in the blood vessels it will not run out after gutting. The flesh will have a brown appearance after freezing and thawing occurs; and in this instance the fish might be of inferior quality. It must be noted however that because residual blood in fish flesh can result in the darkening and browning of the flesh this does not necessarily mean that the fish fillet is of inferior eating quality (Huss 1995).

3.3.2 Effects on spoilage

When newly caught white fish is gutted and stored in ice it remains good to eat for several days. It will eventually acquire an undesirable taste and smell, and deteriorate to an advanced stage of spoilage if a red colour develops in the flesh along the backbone. This is a well-known feature of spoilage, and the fish is at this stage not really suitable for consumption. Spoilage begins as soon as the fish dies. If the fish has not been bleed properly the spoilage will happen faster, as blood is excellent nutrition for spoilage bacteria. Fish is a low acid food and is therefore very susceptible to the growth of food poisoning bacteria. Lowering the temperature with the use of ice or refrigeration also preserves the fish (Hultmann and Rustad 2004). For example, during storage, the microflora changes owing to different abilities of the microorganisms to tolerate the preservation conditions. Gram-negative, fermentative bacteria (such as *Vibrionaceae*) spoil unpreserved fish, whereas psychrotolerant Gram-negative bacteria (*Pseudomonas* spp. and *Shewanella* spp.) grow on chilled fish (Gram and Dalgaard, 2002).

3.3.3 Effects on bruising

Dark coloured patches in the fillet occur when 'whole' fish soon after capture are knocked against a hard surface. The result is bruising of the flesh which is evidenced by rupture of fine blood vessels in the flesh releasing blood into the muscle which does not drain away during gutting and icing. Effective bleeding reduces discoloration of the flesh, the start of spoilage and bruising (Slack-Smith 2001).

The flesh of some species of white fish is naturally darker than others; saithe in particular is noticeably darker than cod or haddock, and some farmed flatfish have slightly darker coloration of the flesh than their counterparts in the wild state. Comparing colour of flesh between species is therefore misleading.

3.3.4 Other issues that may affect colour and quality of seafood products

Flesh of various fish species differ in colour because of their natural characteristics, processing method and fat content. Many species of fish have a strip of dark brown muscle just under the skin and this colour remains unaffected by washing or draining in ice. The brown strip muscle contains more fat than the white muscle. The brown muscle can go rancid when fish is kept in cold storage and develop a characteristic flavour. If the flavour permeates the whole fish, it is strongest in the brown muscle. Variation in the amount of colour of brown muscle affects the appearance of the skinned flesh, and hence its value. The thickness of the muscle does not vary much from the head to the tail. Fish with very little brown strip like most flat fishes are also not fast swimmers and they tend to yield better white fillets. Other sluggish species like cod, haddock and whiting also have little brown muscle (Love 2001). But saithe, which is also a gadoid species, is known to be a fast swimmer and its fillets are therefore more brownish than those of cod and haddock (Smith and Hardy 2001). Fast swimmers who never stop swimming like mackerel and herring have very rich brown muscle. The processing method can also affect the colour tone of the products and the flesh of white fish can become dark as the result of the processing method employed. For example, heat processing, canning, or frozen storage under poor conditions can change the appearance of white fillets.

3.4 The Atlantic cod

Atlantic cod (Figure 5) is a demersal white fish that has traditionally been one of the most important commercial species in the North Atlantic Ocean. The cod fisheries are of a seasonal nature and today some cod stocks are in decline or depleted as a result of overfishing; while others are being harvested to their limits (Cook *et al.,* 1997; Hutchings 2000). Duun and Rustad (2007) claim in spite of its decline, the Atlantic cod has been the most important commercial species in the North Atlantic and is regarded as a promising species in cold water fish farming.

Cod are easily distinguished from most other marine fish by their three rounded dorsal fins and two anal fins that are mirror images of the second and third dorsal. They also have a prominent barbel ("whisker") on the chin (lower jaws). The primary habitat of the Atlantic cod is from near shore-areas to depths exceeding 400 m, but beyond this depth cod is rare. The cod found off shore tend to be larger than those observed inshore. Atlantic cod can live

up to the age of 20 years. Most cod enter the fisheries between the ages of 2-5 years. They can grow to lengths of 130 cm and can weigh up to 25-35 kg. The cod can be on average 26 cm by the end of their first year (O'Brien *et al.*, 1993). Fecundity is high and a large female may produce between 3 and 9 million eggs. Spawning occurs near the bottom during winter and early spring (Lough 2004).

Figure 5: The Atlantic cod (*Gadus morhua*)[1].

The colour of the cod varies, but usually includes many small spots and a pale lateral line. The colour can change; and is dependent on the bottom habitats of the fish. Cod do not swim in large schools but they do travel in small groups when searching for food.

4 MATERIALS AND METHODS

Atlantic cod was used to evaluate the effects of different bleeding methods on the colour of fish fillets in this project. The cod is a widely studied fish species with very white coloured muscle that should clearly show the effects of improper bleeding. The results are therefore likely to return good material that can be used when developing training and educational programmes for fishermen in Ghana. The experiment is divided into three parts where the samples used are fresh, frozen and salted fillets. Using these three processing methods gives an opportunity to study the effects of bleeding on different final products.

4.1 Sampling

The samples used for the experiment are grouped into two sets. For the first set, samples were caught by an Icelandic trawler (Stígandi VE) on the same day and were grouped into three (Table 1):

Table 1: Description of three treatment groups in the fresh fish experiment.

Group	Treatment
A	Fish bled and gutted simultaneously and then cleaned in two washing tubs for approximately 10 minutes before being stored in ice in the hold.
*B	Fish not bled at all. Only stored in ice in hold.
*C	Fish bled and then stored on ice in hold. Only deep throat cut without being allowed to bleed in bleeding tank was done.

**Groups B and C were gutted and cleaned the next day at Matis.*

[1] Figure sourced from http://www.hafro.is/undir.php?REFID=9&ID=39&REF=2&mID=54&mID=1

There were three cods in each group. The fish was caught on January 12th, 2011 landed on the next day and then groups B and C were gutted and cleaned. The samples were then stored in a cooler at 2-4°C for six days; filleted and evaluated on January 18th, 2011.

The second set of samples was collected by staff of Matis on-board an Icelandic longliner (Tjaldur SH) where the main catch is cod. The vessel is equipped with a bleeding tank in which the catch was bled in before being stored on ice. Samples were divided into seven groups. In each case the samples underwent different bleeding conditions. All the samples were stored on ice on-board the vessel before they were landed, inspected and processed.

The samples were bled by using 'deep throat cut'. Temperature, time and water flow in the bleeding tank and washing tank are shown in Table 2.

Table 2: Second set of samples – treatment of seven different groups.

Group	Bleeding Tank			Washing Tank		
	Temperature (^{o}C)	Time (Min)	Water flow (L/sec)	Temperature (^{o}C)	Time (Min)	Water flow (L/sec)
1	5.6	15	1.25	2.1	34	0.07
2	5.6	8	1.90	2.1	34	0.07
3	3.6	15	1.25	3.6	34	0.07
4	5.6	6	1.25	2.1	30	0.07
5	5.6	15	0.70	2.1	30	0.07
6	5.6	15	1.80	2.1	30	0.07
7	Samples did not go through the bleeding and washing tanks (bled outside, air bleeding) (control group)					

The conditions for bleeding are as follows:

1. The normal practice by the fishermen where they bleed the fish for about 15 minutes in seawater, then gut, wash and store on ice;
2. The fish were allowed to bleed in the tank for 8 minutes with rapid/increased water replacement;
3. There was an addition of slurry ice into the bleeding tank to decrease the temperature of the seawater in which the fish was bleeding in;
4. The time that the fish were allowed to bleed in the tank was reduced (6 min);
5. The volume of fresh seawater being pumped into the bleeding tank was decreased by almost 50%;
6. The volume of fresh seawater being pumped into the bleeding tank was increased by almost 50%;
7. The fish was bled without gutting (did not go into bleeding or washing tank; this is the normal method used by small day-boats in Iceland).

There were 30 cods in each group (25 that were salted and 5 that were frozen). Groups 1-3 were caught on November 15th, 2010 groups 4-6 were caught on November 16th, 2010 and group 7 were caught on November 17th, 2010. All the groups that were salted were filleted and brine salted on November 19th, 2010. Samples that were frozen were whole frozen on November 19th and then thawed up, filleted and inspected on December 21st, 2010.

4.2 Method of Analysis

The first set of samples grouped A, B, and C was treated fresh, filleted and visually inspected for blood spots colour (sensory evaluation and colour detection) in the muscle. Chemical compound (iron) in the fillet was also measured and photographs were taken of each fillet.

The second set of samples (groups 1-7) was examined through processing i.e. first after brine salting, then "half way" through the salting process (after twenty-five days in dry salt) and finally when the fillets were ready for packaging (90 days after brine salting).

Each group (1- 7) of samples for salting was passed through the following process:

1. Be-heading with a machine (Baader 434);
2. Filleting with a machine (Baader 184);
3. Salting –
   a. Injecting – each of the fillets was injected with a brine solution using an injecting machine;
   b. Brine salting – The injected fillets were immersed in a brine solution for two days;
   c. Dry salting – fillets were stacked with alternating layers of dry, coarse salt;
   d. Storage – the salted fillets were stored at temperature between 0-2^{o} C.
4. Grading of the salted fillets was done at the factory about half way through the processing (25 days) and again when the fillets were ready for packaging (90 days). The grading was done by an experienced appraiser in the processing company. Grading was based on the overall colour appearance of each group, ranging from B^{-} (dark) to A^{+} (light).

Frozen samples (at -25^{o}C for 30 days) from each of the categories of bleeding (1-7) was thawed, filleted and inspected for blood spots colour (sensory evaluation and colour detection) in the muscle to see if there was any change in appearance or colour. Chemical compound (iron) in the fillet was measured and photographs were taken of each fillet.

4.2.1 Colour Detection

The frozen and fresh fish samples were subjected to colour detection. The intensity of the flesh colour was measured with a Minolta CR-300 chromameter (Minolta camera Co., Ltd; Osaka, Japan) in Lab* system (CIE, 1976) with CIE Illuminant C. Measurement was done at the loin, middle and tail areas of each sample. In every group, three fillets were used and the mean calculated for that particular group. The instrument records the L* value, lightness on the scale of 0 to 100 from black to white (black 0, and light, 100); a* value, (+) red or (-) green b* value, (+) yellow or (-) blue. Whiteness was calculated using the equation L*-3b* (Shie and Park 1999). The a* values were not used in the final analysis of the results, as the main emphasis of the project was to measure whiteness. The L* and b* values are presented in Appendix 1.

4.2.2 Sensory analysis

A sensory panel, who are experienced and trained staff of Matis who perform sensory evaluation on regular basis, assessed the colour of the fresh and frozen thawed skin-on fillets using a ranking test where the groups where ranked from 1-7 according to overall colour

appearance (O'Mahony 1986). The sensory evaluation was based on overall colour in flesh, rather than individual blood stains or bruising. The form used by the evaluators can be seen in Appendix 2.

4.2.3 Chemical analysis (heme and non-heme iron content)

The frozen and fresh fish samples were measured for iron content. Blood is rich in iron and iron content in the flesh of fish is therefore an indicator of how much blood is present in the flesh. The iron measurements were done in two separate tests where iron in the haemoglobin (blood compound) was measured in one and iron not associated with haemoglobin in the other.

Heme iron content

The heme iron content was determined according to the method of Gomez-Basauri and Regenstein (1992) with a slight modification with the main reagents being 40 mM phosphate buffer (pH 6.8) (Disodium hydrogen phosphate).

A grounded sample (2 g) was weighed into 50 ml centrifuge tube and 20 ml of cold 40 mM phosphate buffer (pH 6.8) was added. The content was then homogenized at 13.500 rpm for 10 sec and centrifuge at 3000 g for 30 min at 4^{o}C. The supernatant was filtered using Whatman No. 1 filter paper and the filtrate read directly at 525 nm using a spectrophotometer (Shimadzu UV-1800).

Myoglobin content was calculated from the millimolar extinction coefficient 7.6 and a molecular weight of 16.110. Heme iron was calculated based on Myoglobin, which contains 0.35% iron. The heme iron content was expressed as mg/100 g sample.

Non-Heme iron content

The non heme iron content was determined according to the method describe by Schricker *et al.*,(1982) with the main reagents being 0.39% (w/v) sodium nitrate, 40% trichloroacetic acid solution, 6 M HCl solution and 2 ppm iron colour reagent. The colour reagent was prepared by mixing a 1:20:20 ratio (w/v/v) of Bathophenanthroline (0.162 g dissolved in 100 ml of double deionised water with 2 ml thioglycolic acid (96- 99%)), double deionised water and saturated sodium acetate solution.

A grounded sample (1 g) was weighed into 50 ml screw cap test tube and 50 µl of 0.39% (w/v) sodium nitrate added. A 4 ml freshly prepared mixture (ratio 1:1, v/v) of 40% trichloroacetic acid solution and 6 M HCl solution was added. The tubes were tightly capped and placed in an incubator shaker at 65^{o}C for 22 hours. After the 22 hours, the mixture was allowed to cool down to room temperature for 2 hours. The supernatant (400 µl) was mixed with 2 ml of the iron colour reagent (freshly prepared), vortex and stand for 10 min and the absorbance measured at 540 nm using a spectrophotometer (Shimadzu UV-1800).

The non-heme iron content was calculated from the iron standard curve. The iron standard solution, ranging from 0 to 2 ppm (400 µl) was mixed with 2 ml of the non-heme iron colour reagent. The concentration of non-heme was expressed as mg/100 mg sample.

4.3 Statistical analysis

Microsoft Excel 2010 was used to calculate the means and standard deviations for all multiple measurements and to generate graphs.

4.4 Photographic analysis

A digital camera (Canon EOS 10D with a 24-85 mm lens) was used to capture each group of samples to show the differences in the colour of the fillets.

5 RESULTS

The results from colour detection, sensory evaluation, chemical analysis, grading and pictorial evidence are presented in this chapter. Each processing method is discussed separately.

5.1 Fresh fish

The effects of three different bleeding methods on the colour of fresh fillets were examined and the results were measured using colour analysis, sensory evaluation and chemical analysis. Pictures were in addition taken of each fillet to provide pictorial evidence.

Colour analysis:

The whiteness of three fillets in each group was calculated by measuring lightness (ranging from black to white) and b* (ranging from yellow to blue). The results show that the colour of fillets in group A (Fish bled and gutted simultaneously and then cleaned in two washing tubs for approximately 10 minutes before being stored on ice in the hold) was significantly whiter than in the other two groups (Figure 6). The colour difference between fillets in group B (Fish not bled at all but only stored on ice in hold) and C (Fish bled and then stored on ice in hold after only deep throat cut without being allowed to bleed in bleeding tank was done) was not statistically significant, according to these measurements.

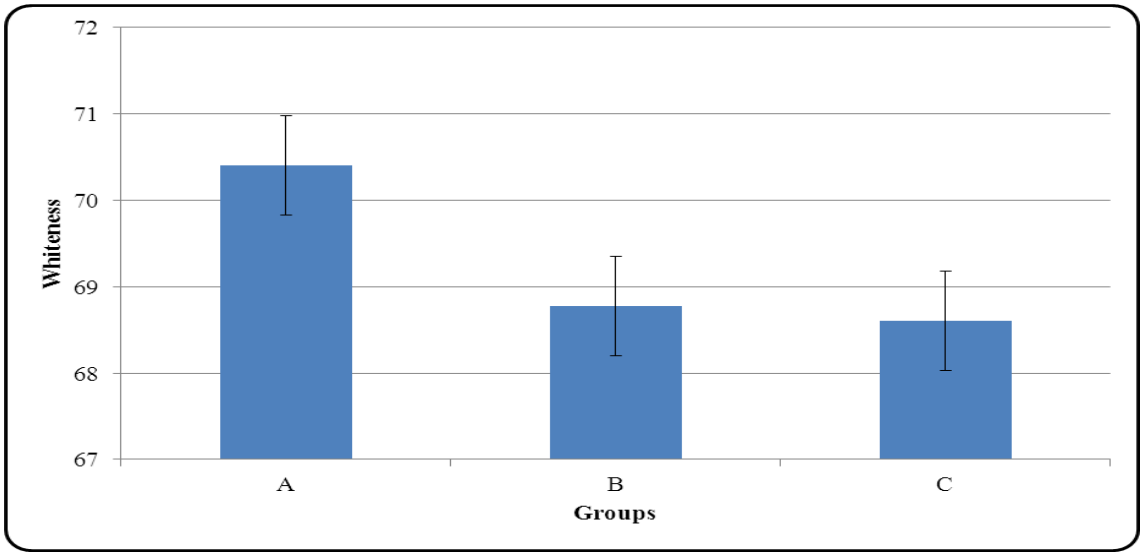

Figure 6 : The average whiteness levels of the three fresh fish groups calculated from the L* and b* values measured**.**

Sensory evaluation:

Sensory panel containing ten experienced panellists from Matís ranked the groups according to colour from lightest (1) to the darkest (3). All of the panellists agreed that group B was the darkest, and all except one agreed that fillets in group A were the whitest (Table 3). This means that the colour difference between the groups is statistically significant when applying 95% confidence level (O'Mahony 1986).

Table 3 : Evaluation of the sensory panel in the colour of fresh cod fillets.

Groups	Sum of Ranking	Remarks
A	11	Lighter
B	30	Darker
C	19	Intermediate

Iron content:

The non-heme iron in the samples is higher than the heme iron. Group A (bled, gutted and cleaned on-board) have the highest of the non-heme (1.209 ± 0.193 ppm) and the lowest of the heme iron (0.9 ± 0.094 ppm). Group B on the other hand have the highest (1.154 ± 0.128 ppm) of the heme iron and the lowest (1.095 ± 0.094 ppm) of the non-hem iron content (Figure 7).

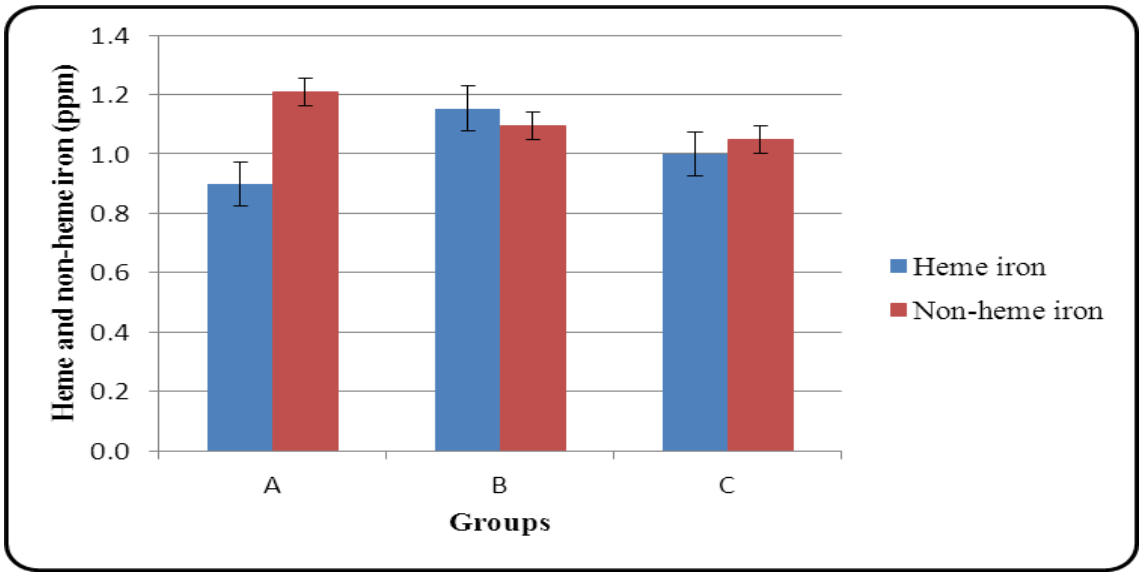

Figure 7: Mean of iron levels measured in the fresh fillets.

Pictorial evidence:

The difference in appearance between the three groups of fresh fillets can be clearly seen in Figure 8, where the colour of the fillets in group A is much whiter than the fillets in groups B and C.

Figure 8: Colour difference of the fillets from the fresh samples with group B showing the darkest[2].

Though the difference between group B and C is not as evident, the colour of the fillets in group B did appear to be somewhat darker than the fillets in group C.

5.2 Salted fish

The effects of seven different bleeding methods on the colour of salted cod fillets were examined using the expertise of experienced evaluators from the processing company to visually grade each group from A^+ to B^-. The grading was first done about half way through the salting process (25 days after filleting) and then again when the fillets were ready for packaging (90 days after filleting). The results from day 90 varied from the results on day 25, as some groups that appeared white half way through processing turned out to be spotted or dark when the final product was ready (Table 4).

[2]A - Fish bled and gutted simultaneously and then cleaned in two washing tubs for approximately 10 minutes before being stored on ice in the hold
B- Fish not bled at all. Only stored on ice in hold
C- Fish bled and then stored on ice in hold. Only deep throat cut without being allowed to bleed in bleeding tank was done

Table 4: Grading of the salted fillets at 25 and 90 days processing time.

Group[3]	Grade after 25 days	Remarks	Grade after 90 days	Remarks
1	A	Very bright colour, all fillets look good	B	Big variations within group, some fillets are white and some are dark
2	B^+	Rather dark in colour, but not too bad. Big variation between individual fillets	B^-	Dark overall colour, lots of dark bloodspots
3	B^+	Rather dark in colour, but not too bad. Big variation between individual fillets	A	Good white colour but some variability. Few fillets of poorer quality
4	A^+	Very good white colour. All fillets look really good	A^+	Good white colour with little variability
5	B	Dark colour, considerable variability	A	Good white colour but some variability. Few fillets of poorer quality
6	B	Much variability. Some fillets okay and others very dark	B^-	Dark overall colour, lots of dark bloodspots
7	B^-	Very dark. All fillets dark	B	Big variations within group, some fillets are white and some are dark

Group 4 is the only one that showed the same results after 25 and 90 days, but all the other groups either improved or got worse.

5.3 Frozen fish

The effects of seven different bleeding methods on the colour of fillets cut from frozen cod were examined and the results were measured using colour analysis, sensory evaluation and chemical analysis. Pictures were also taken of each fillet to provide pictorial evidence.

Colour analysis:
The whiteness of the fillets was calculated by using measurements of lightness (ranging from black to white) and b* (ranging from yellow to blue). The results show that fillets from group 1, 2, 5 and 7 where significantly (95% confidence limit) whiter that fillets from group 3, 4 and 6 (Figure 9). The samples from group 6, where volume of seawater in the bleeding tank was increased, returned the darkest fillets.

[3]1. The normal practice by the fishermen where they bleed the fish for about 15min in seawater, then gut, wash and store on ice;
2. The fish were allowed to bleed in the tank for 8 minutes with rapid/increased water replacement;
3. There was an addition of slurry ice into the bleeding tank to decrease the temperature of the seawater in which the fish was bleeding;
4. The time that the fish were allowed to bleed in the tank was reduced (6 min);
5. The volume of fresh seawater being pumped into the bleeding tank was decreased by almost 50%;
6. The volume of fresh seawater being pumped into the bleeding tank was increased by almost 50%;
7. The fish was bled without gutting (did not go into bleeding or washing tank, this is the normal method used by small day-boats in Iceland);

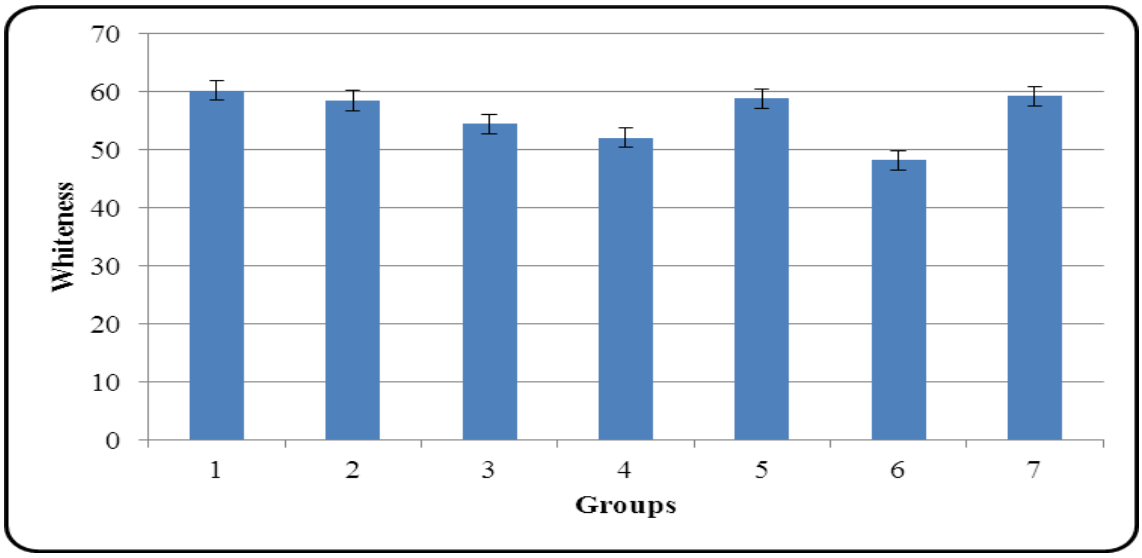

Figure 9: The average whiteness levels of the seven groups of fillets from frozen samples calculated from the L* and b* values measured.

Sensory evaluation:

Seven experienced sensory panellists at Matís ranked the groups from lightest (1) to darkest (7) which is based on the overall intensity of blood in the fillets. All of the panellists agreed that fillets from group 1 were lightest and that fillets from group 5, 6 and 7 were darkest (Table 5).

Table 5: Evaluation of the sensory panel in the colour of frozen cod fillets.

Bleeding Groups	Sum of Ranking	Remarks
1	7	Lightest
2	21	The difference between these groups and the rest is not statistically significant
3	29	
4	16	
5	36	Dark
6	44	Darkest
7	43	Darker

The results clearly show that fillets from group 1 (normal practice on-board Tjaldur SH, using seawater in bleeding tank for 15 min) returned the whitest fillets and that these results are statistically significant using a 95% confidence limit

Iron content:

The results from the iron measurement show that both heme iron and non-heme iron content ranged from 0.8 - 1.0 ppm (Figure 10). The heme iron levels are higher than the non-heme iron in all the groups except for group 3 (slurry ice added into the bleeding tank) and 5 (decreased volume of fresh seawater) where the non-heme iron is higher than the heme iron. The heme iron is 0.784±0.060 ppm while the non-heme iron is 1.027± 0.153 ppm in group 3.

In group 5, the heme iron is 0.835± 0.040 ppm while the non-heme iron is 0.967± 0.184 ppm. Both the heme and non-heme iron in group 4 (reduced time in bleeding tank with same level of water replacement) were low and of almost equal amount (Figure 10).

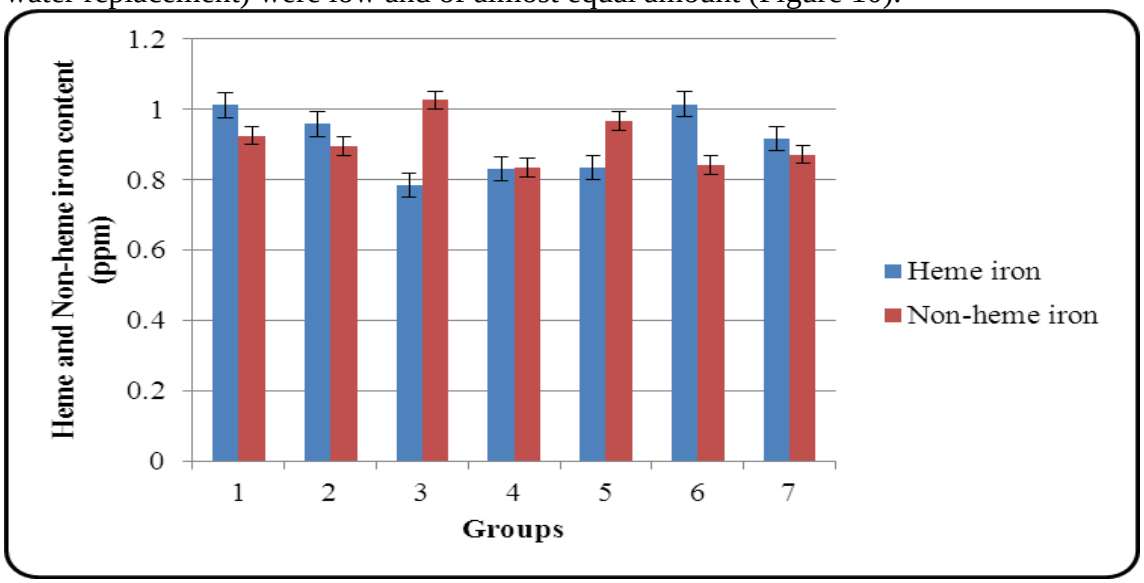

Figure 10: Mean iron levels measured in fillets from frozen samples.

Pictorial evidence:

The difference in appearance of fillets cut from whole frozen fish (stored at -25^{o}C for 30 days) shows that group 7 (bled without going into the bleeding tank) have more blood spots and looks much darker than the rest of the groups (Figure 11).

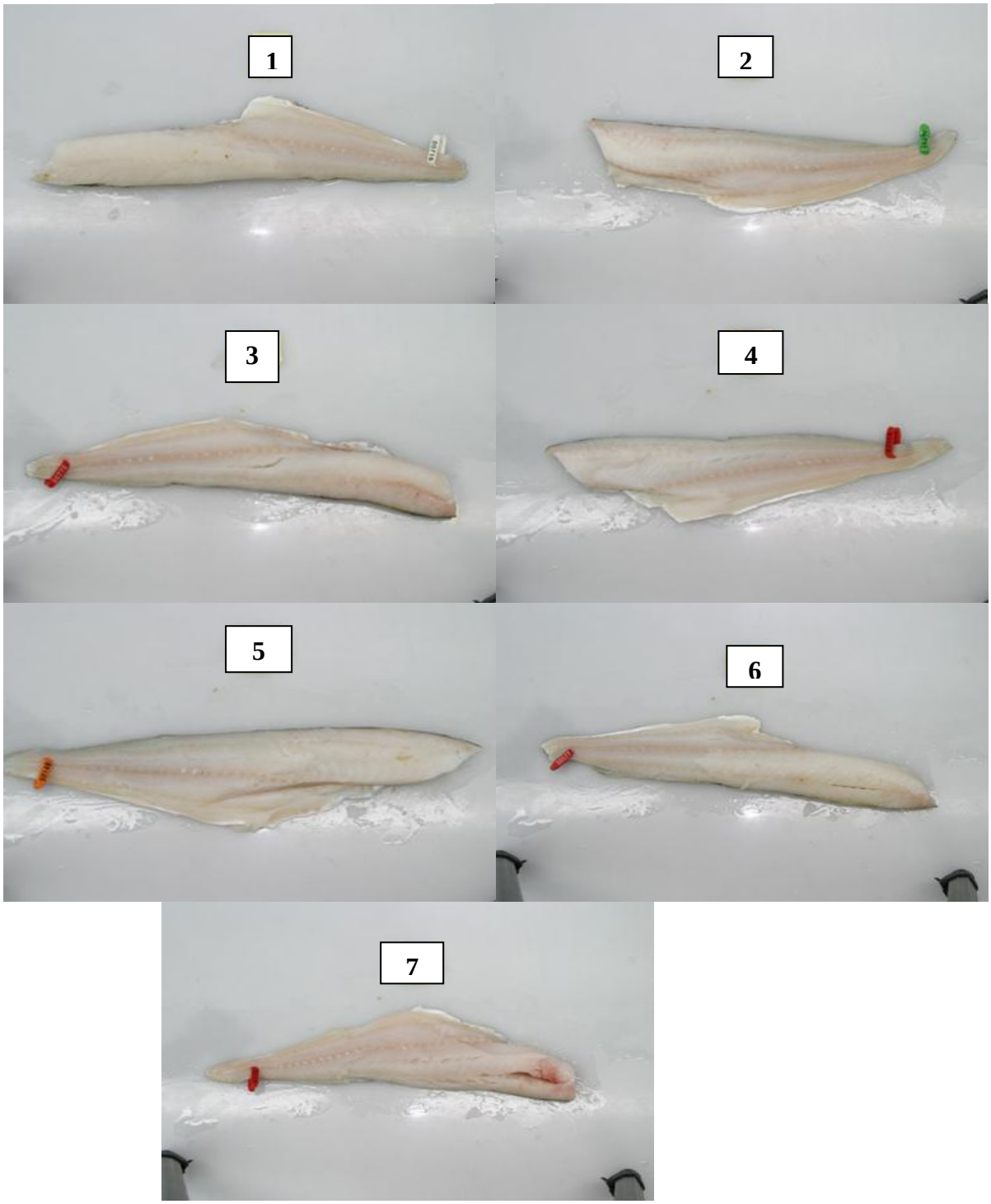

Figure 11: Colour difference of the fillets from the frozen seven group samples with group 7 showing the darkest.

Group 1 was lighter in colour and with fewer blood spots. There is not much variation in colour among the rest of the groups as they were neither dark nor light.

6 DISCUSSION

Fish being supplied to the processing sector in Ghana is generally landed un-bled. This affects and reduces the quality of fish i.e. colour tone of the flesh, odour, shelf life and lipid oxidation. Erikson *et al.,* (1999) reported that bleeding significantly improves the quality (appearance, odour and rancidity) of fish flesh. This suggests bleeding as an integral part of on-board handling of fish. The quantity of haemoglobin and myoglobin in fish muscle in addition to the state of heme iron dictate the colour of the fillet. Bleeding the catch immediately after capture effectively lowers the total heme and non-heme iron contents and gives the end product much better quality. Below is a discussion of the results of the project for each product and bleeding method tested.

6.1 Fresh Fish

The results from the analysis of the fresh fillets showed that group A (which was bled, cleaned and washed immediately after catch) was much whiter and contained less heme-iron content than group B (which was bled, gutted and washed the following day at the lab). These results confirmed that un-bled fish in whatever state (stress or un-stress) have higher heme-pigment content than the bled ones (Olsen *et al.,* 2008). One important contributing factor to the closeness of whiteness of group B and C is due to the washing process. Both samples were gutted and washed at the lab the day after catch even though group C was bled but not washed on board and stored. The extent of the washing after the bleeding may have affected the overall appearance of samples in group B and C. Proper washing after bleeding returns whiter fillets as this tends to remove more blood that contains the heme proteins (Botta *et al*. 1986, Chaijan *et al*., 2005). The results show that group C returns whiter fillets than group B.

This indicates that if fishermen in Ghana can change their procedures from their regular on-board handling practises (B) to bleeding the fish on-board their vessels (C) they could improve the quality of the catch they land. The method used for group C can easily be adapted for Ghanaian small vessels that do not have space for bleeding- and washing tanks. They simply need to do the ‘deep throat cut’ to bleed the fish and immediately put the fish on ice until they land and can wash the fish thoroughly.

6.2 Salted Fish

Salting was used as a processing method to evaluate the effect of different bleeding conditions on the final product. The appearance or colour of fish after salting is of great importance because the lighter the colour after salting the better the grade and price.

The main activity that takes place during the salting process is the decrease or extraction of water/fluid by transporting salt into the fish. Turan *et al*.,(2007) highlighted that diffusion, osmosis, and other series of chemical and biochemical processes take place during salting. A major quality problem in such ‘skin-on’ products is yellow/brown discoloration of the flesh surface, which can be contributed to insufficient bleeding.

The grading of the fillets returned confusing results as grades changed considerably from day 25 to day 90, where some improved while other got worse. The grade of the final product is however of most importance and there group 4 returned the best results and groups 2 and 6 returned the worst results. Group 4 was only allowed to bleed in the bleeding tank for six minutes with regular water replacement, compared to the normal 15 minutes. Group 2 was only allowed to bleed in the bleeding tank for eight minutes with increased water replacement and group six was allowed to bleed in bleeding tank for the normal 15 minutes with increased water replacement. This suggests that increasing the seawater throughput in the bleeding tank has an adverse effect, as it may increase pressure that prevents the blood draining out of the flesh. The reason why group 4 returns the whitest fillets is however very difficult to explain, as previous studies have showed that optimum bleeding time in running seawater is between 10-20 minutes. It is therefore likely that some other factors may have affected the experiment, such as the condition of the fish when it was bled i.e. the line may have been in the water for a whole day and some of the catch is therefore going to be extremely tired or even dead. A longline vessel was specifically chosen for this project to give homogenous examples, but handline or aquaculture would probably have been a better choice.

6.3 Frozen fish

According to the colour measurements groups 1, 2, 5 and 7 yielded the whitest fillets, and the sensory panellists all agreed that the fillets from group 1 were the whitest. Results from the iron measurements did not return very conclusive results as all values ranged from 0.8-1.0 ppm, which is a low value compared to results from other projects. It therefore looks like the normal practise used on-board Tjaldur SH is the best bleeding method i.e. bleeding with normal seawater for 15 minutes with medium replacement of seawater. Group 3, where the temperature in the bleeding tank was decreased, has a relatively low whiteness value, which suggests that decreasing the temperature in the bleeding tank can affect the rate of the blood flow out of the fish. The original idea was though to decrease the temperature down to 0-2°C, which would probably have returned more interesting results i.e. more extremes. Group 6, where there was increased volume of fresh seawater pumped into the bleeding tank, has the lowest whiteness value, a high amount of heme iron and very dark grade from the sensory panellists. This might be caused by an increased outside pressure from the water being injected into the bleeding tank.

The heme iron content in group 3, where the temperature in the bleeding tank was lowered, is significantly lower than for most of the other groups, but the non-heme iron is however the highest. This might be caused by a breakdown of the heme resulting in the release of the non-heme iron in the fillets. This same reason can be given to the high level of non-heme iron in group 5 where the samples were treated with decrease in the water flow in the bleeding tank. During freezing, heme and non-heme iron content changes and heme iron decreases while non-heme iron increases (Maqsood and Benjakul 2010, Turhan *et al.*, 2006). This may be due to the release of free irons from the heme. This was observed in samples in group 3 (Slurry ice added into the bleeding tank to lower the temperature) and 5 (decreased volume of fresh seawater being pumped into the bleeding tank).

In most of the groups the mean heme iron was lower than the non-heme iron during the storage. This may be due to the degradation of heme pigment and other iron-containing proteins that were possibly denatured during the storage and this affected the colour tone of

the fish fillets. Therefore bleeding of the cod under the various conditions in this study resulted in the removal of heme from the muscle to some degree.

Bleeding is an important process in handling of fish on-board vessels after catch, as it tends to affect the colour of the muscle negatively or positively depending on how the bleeding is done. The washing of the fish after bleeding is also an important factor in bleeding. The better the washing the more of the blood (which contain the haemoglobin and myoglobin that dictates the colour of the flesh) is removed and a better muscle colour is achieved.

Aside from the different conditions of bleeding treated in this study, other factors also play a role, for example, the season, time before bleeding, gear used, and quantity of haul at the time of catch, among others. The colour of cod is darker in summer than in winter due to more physical activity at summer times (Margeirsson *et al.*, 2007). Botta *et al.*, (1987) also reports that the fishing method is more significant than the season of capture of the Atlantic cod. Catching fish with hook and line the quality of the fish is preserved during icing since there is less stress and the fish is killed faster. Also the larger the haul (in the case of trawlers) the longer it takes for the fish to be bled and this result is retained blood in the muscle (Margeirsson *et al*., 2007).

7 CONCLUSIONS

The results from the project suggest that bleeding treatments have an effect on the colour tone of fresh, frozen and salted cod fillets. The myoglobin and haemoglobin (in the blood) contents in the muscle gives the colour tone of fish flesh. The results show that when the fish was bled (deep throat cut) just after catch, put in a bleeding tank for about 15 minutes and washed thoroughly before storage on ice, the fillets were white as compared to the other treatments procedures even though in this study the samples were not taken on the same day.

A better conclusion can be made when all the samples are considered under one particular processing method. From the study the different processing (fresh, frozen and salting) methods gave different colour tones of the, making comparison difficult. Bleeding is carried out to eliminate as much of the blood as possible from the tissues in order to reduce the discolouration development and muscle darkening of the flesh and the type of processing method have to be considered.

Vessels that do not have proper bleeding tanks on-board should try to install such devices if possible, as it will increase quality and value. This does not have to be very expensive equipment as simply using plastic tubs can give improved results. Many vessels (particularly in Ghana) are however too small to be fitted with bleeding- and washing tanks. Alternatively, they should bleed the catch immediately after capture with a deep throat cut and store it then on ice. The fish should then be cleaned and washed immediately after being landed. Fish should not be landed without having been bled out at sea, as it will affect the quality and colour of the fillets.

The importance of bleeding has been summarised in a leaflet intended for Ghanaian fishermen. This leaflet is a one pager that stresses the importance of bleeding and suggests how the practice of bleeding fish can be adapted in Ghana (Appendix 3).

8 RECOMMENDATIONS

The results from the project suggest that the sampling plan and the execution of the experiments could be improved. If the project is going to be repeated it is recommended that:

1. In this study, the bleeding treatments were done on different days and it did not help in coming out with concrete conclusions, hence the study should be repeated where all the samples would be collected on the same day.

2. Different gear for catching should be used to determine the impact of the gear type and the bleeding on the appearance of the fish colour tone.

3. Samples with various bleeding treatments should be studied under different freezing and storage temperatures when frozen and salted.

4. The following tests should be considered when this study is repeated: shelf life measurements (Torry, QIM and/or QDA), stress factor (lactic acid and pH), trimethylamine (TMA) and total volatile basic nitrogen (TVB-N).

5. It will also be interesting to see further the effects of cooling the fish during bleeding: for example at 0°C, 4°C, 8°C, 12°C and control group that uses same sea temperature at the time of experiment.

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APPENDIXES

Appendix 1: The L* and b* values of frozen and fresh fillets

FROZEN FILLETS			FRESH FILLETS		
Groups	**L***	**b***	**Groups**	**L***	**b***
1	59.98± 2.35	-0.08 ± 1.48	A	49.90 ± 3.38	-6.71 ± 0.45
2	57.63± 1.86	-0.28 ± 1.24	B	50.51 ± 2.25	-6.09 ± 0.51
3	57.46 ± 1.63	1.01 ± 1.09	C	49.32 ± 2.74	-6.43 ± 0.51
4	59.39 ± 3.28	2.42 ± 0.51			
5	57.57 ± 1.49	-0.43 ± 2.52			
6	57.74 ± 1.86	3.18 ± 1.43			
7	57.65 ± 3.62	-0.55 ± 1.60			

The equation for the calculations of the whiteness is L* -3b*

Appendix 2: Sensory evaluation of cod fillets.

Evaluating red/dark colour in flesh

Arrange the groups according to colour from one to nine. Each group consists of three fillets and the evaluation is supposed to reflect on the overall average colour of the group. Give each group a grade according to the scale below. Be sure to evaluate colour in flesh, rather than individual blood stains or bruising. Remember to sign your evaluation paper.

Order	**Number of group**
1	
2	
3	
4	
5	
6	
7	

Name: _____

UNITED NATIONS UNIVERSITY
Fisheries Training Programme

The importance of bleeding fish


Holding fish for bleeding


'Deep throat Cut'


Gutting


Gutted cod

Effects of not bleeding

Not bleeding or inadequately bleeding the fish can have big impact on the overall quality of the end product. It has for example effect on shelf life, taste and visual appearance.

Effects on colour

When white fish are gutted at sea, washed and iced, the appearance of the flesh is white on landing because they would have had time to be properly bled and blood residues will be reduced.

Effects on spoilage

If the fish has not been bled properly the spoilage will happen faster, as blood is an excellent nutrition for bacterial growth. Fish is a low acid food and is therefore very susceptible to the growth of food poisoning bacteria.

Effects of bruising

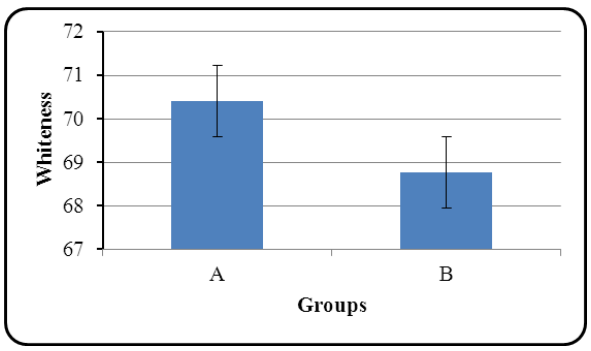
Dark coloured patches in the fillet occur when 'whole' fish, soon after capture is knocked against a hard surface. The result is bruising of the flesh which is evidenced by rupture of fine blood vessels in the flesh releasing blood which does not drain away during gutting and icing.

Methods adaptable in Ghana

Big vessels can build a bleeding and washing tank on the vessels to bleed and wash the fish. Alternatively it is possible to use conventional fish tubs as bleeding- and washing tanks, but this has to be done carefully so as not to overcrowd the fish in the tubs which will lead to improper bleeding.

Small vessels that do not have space to put bleeding- and washing tanks on-board can simply do the 'deep throat cut' to bleed the fish and immediately put the fish on ice until they land. Upon landing they can then wash the fish thoroughly and re-ice the catch.

Properly bled cod fillet (A) and poorly bled fillet (B)



There is statistically significant difference ($p<0.05$) in colour between fillets from properly bled fish (A) and poorly bled fish (B).

Ministry of Food and Agriculture
Fisheries Commission
Marine Fisheries Research Division

P.O. Box BT 62
Tema, Ghana
Phone: +233 244 745 197