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GUIDELINES FOR HANDLING AND PRESERVATION OF FRESH FISH FOR FURTHER PROCESSING IN VIETNAM

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ABSTRACT

Fish from catching has an important role in international fisheries as well as in developing countries like Vietnam. Therefore maintaining good quality in fish raw material is necessary. This project focuses on how to handle and preserve the fish especially during the process from catching the fish at sea to landing and transporting the fish to the processing plant. This project establishes guidelines for these activities. In addition some experiments were carried out to determine the insulation ability of different types of fish boxes used for storing fish and to validate the guidelines by evaluating the fish quality during ice storage in the worst and best scenario cases.

Based on data collected in Vietnam as well as fish preservation techniques in Iceland, problems in the handling and preservation process in Vietnam are pointed out and solutions presented. Choosing the appropriate fish containers like boxes or tubs is considered one significant factor contributing to fish freshness and quality. The Sæplast insulation plastic boxes or tubs are very suitable containers, which can possibly be used in the Vietnamese fisheries industry in the near future.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

1 INTRODUCTION

Vietnam has great potential for fish exploitation with a coastline over 3,260 km long. The inland water area is about 226,000 km^2 and the Exclusive Economic Zone (EEZ) is over 1 million km^2, three times the mainland area. Marine capture is divided into two fishing seasons with different characteristics, the south season (from March to September) and the north season (from October to February). There are more than 2000 fish species in Vietnamese marine waters, of which about 130 are of economic value. These are species like tuna, mackerel, swordfish, mahi mahi, scads, herring, sardine and demersal fish like sole, hair tail, pomfret, sea bream, grouper, sea perch and snapper. Coastal fisheries are characterised by high species diversity and small short-lived species. The resources have high potential for recovery and can sustain high levels of harvest. Besides marine fish, there are over 1600 species of crustaceans and about 2500 species of molluscs where squids and octopus are of significant economic value. There are some fresh water fishes with high economic value like catfish, snakehead, perch, tilapia and eel. There is also potential for fish aquaculture in Vietnam with its long coastline, many lagoons, straits and bays, estuaries, canals and thousands of small and big islands. In the inland area, many rivers, canals, irrigation and hydroelectric reservoirs have created a water surface area of about 1,700,000 ha. Fish production in Vietnam is developing quite fast (Figure 1) reaching 3.2 million tons in 2004. Out of this total, capture fisheries contributed 1.7 million tons, mainly from coastal fisheries (1.1 million tons). Although the contribution of capture fisheries is high in terms of volume the bulk of the catch is made up by low value fish, except for cephalopod and tuna. Fish aquaculture product yield was 1.5 million tons in 2004 with the main species being catfish (basa and tra fish) and black-tiger shrimp (Ministry of Fisheries 2005).

Today in Vietnam the consumption rate of fish for food is about 50% of the total protein food. The people prefer seafood products more and more. Fish consumption per person is still rather low at 8 kg/year. Therefore this amount needs to increase. Fish products are exported to many countries in the world, in which the main markets are the EU, USA and Japan. The total exporting value has been increasing for many years. The total fish products export value in 2004 was USD 2.35 billion for products mainly from finfish, shrimp and squid. The increase of capture fisheries is declining as stocks are becoming fully exploited. Therefore, maintaining the quality of fish raw material is more and more important. If the fish quality can be maintained the value from each trip for catching at sea can continue to increase. The fish product volume for export and domestic consumption can increase if the raw material used for processing is of higher quality (Ministry of Fisheries 2005).

Quality of fish raw material plays an important role for the quality of the end-product. Once the fish raw material freshness and nutrition value is lost, it can not be recovered in the processing stages. Products that are processed from low quality raw material are not always a safety risk, but the quality (nutrition value) and shelf life is significantly decreased.

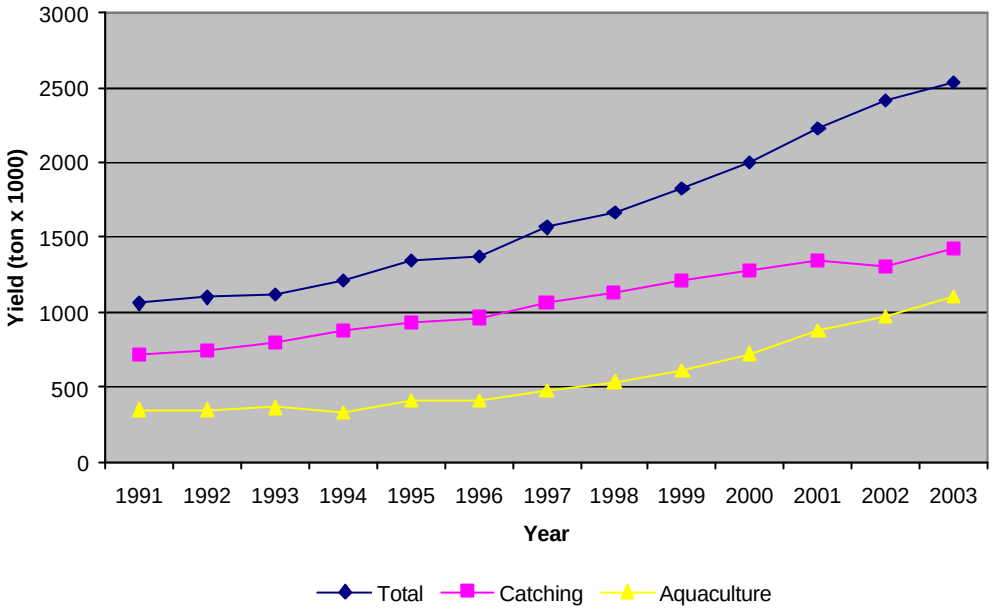

Figure 1: Fish production in Vietnam (FAO 2003).

In Vietnam, maintaining fish raw material quality is still a challenge. The time from catching to reception at a processing plant can be prolonged while the temperature of the raw material usually is not low enough to prevent spoilage. Some exporters have experienced product rejection due to quality problems e.g. microbiological criteria or extraneous matter. So the Vietnamese fishing sector is facing at least two serious problems, one is stagnation in catching and the second is deterioration in raw material quality. In light of the decrease in catch rates, quality improvements become especially important. There are three main reasons for quality deterioration and spoilage: autolysis, bacterial activity and rancidity (Huss 1995) and in some cases physical damages (mechanical stress, direct sunlight etc) can lower the quality considerably. The quality deterioration can start right away during fishing and it continues all the way to the final user.

The main objective of this project is to introduce new and validated guidelines for handling and preservation of fish in Vietnam in order to improve the quality of the raw material. Some studies on quality changes in fish under best and worst case scenarios were carried out to validate these guidelines. Some tests for the suitability of different plastic boxes or tubs for storing fish, were also carried out to examine these best and worst case scenarios in terms of retaining quality.

2 LITERATURE REVIEW

2.1 Reasons for spoilage of fish

Maintaining good quality of fish raw material for processing is very important. Therefore, the reasons for quality deterioration leading to spoilage need to be determined carefully. Just after death, fish can be soft for a few hours but then it becomes stiff. This phenomenon is called "rigor mortis". The fish stays in the "rigor mortis" condition for a while, but then its flesh muscles become relaxed again. At that time 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 and are therefore called sensory changes. One of the differences between fish appearance before and after rigor mortis is that the fish muscle is more elastic before rigor mortis. 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.. Generally, it is preferred to extend the time before and during rigor mortis. There are some reasons for deterioration of quality and spoilage; they are autolysis, bacteria spoilage, rancidity and mechanical damage (Huss 1994). Lowering the temperature by icing not only slows down the rigor mortis process, but also reduces the spoilage rate. Therefore maintaining low temperature during the handling and preservation process is very important.

2.1.1 Autolysis

The autolysis process relates to enzyme activities in fish (autolysis means self-digestion). Commonly the spoilage due to autolysis occurs first and is followed by spoilage due to bacteria and rancidity but sometimes they overlap (Gram and Huss 1996). Unlike most fish, autolysis occurs very quickly in some shellfish like lobster and shrimp (Hobbs 1982). When the fish dies adenosine-triphosphate (ATP), which is the energy-rich organic compound in its muscle, will mostly be synthesised from glycogen, but also from creatine-phosphate (for finfish) and from arginine-phosphate (for cephalopods) under anaerobic conditions. The glycolysis (glycogen reduction process) still occurs continuously to create the end product of lactic acid. Because the end product of this process is lactic acid, the pH of the muscle will decrease. The ATP concentration gradually decreases and when it goes below 1 µmol/g in the muscle tissue the enzyme ATP-ase is activated. This leads to the stiffing of the muscle which will be constant (rigor mortis). The ATP is gradually degraded during time to some degraded products e.g. adenosine diphosphat, adenosine monophosphat, inosin monophosphat, inosin and hypoxanthin. Hypoxanthin is considered to cause the off-flavour in spoiled fish. When the fish raw material is handled carelessly cells may be broken, which leads to the release of autolytic enzymes and this leads to the production of some spoilage substances. These substances create a very good environment for micro-organisms. Cathepsin, chymotrypsin, trypsin, cacboxypeptidase, calpain, collagenase and TMAO- demethylase are all autolytic enzymes. Therefore, in order to maintain fish quality, enzyme activities should be prevented. Using low temperature is the most frequently used measure to limit enzyme activities (Huss 1994).

2.1.2 Bacteria

Bacteria are capable of causing spoilage because of two important characteristics. First they are psychotropic and thus multiply at refrigeration temperatures. Secondly they attach various substances in the fish tissue to produce compounds associated with off-flavours and off-odours. When the fish is alive the bacteria are found on the gill and skin and in the intestines but can not attack the fish muscle. But when the fish dies the bacteria can penetrate into the flesh muscle of the fish. When fish is preserved by icing the rate of bacterial penetration into the flesh muscle is much slower. Fish spoilage occurs when the enzyme of bacteria diffuses into the flesh muscle and the nutrition substances from the flesh muscle diffuse to the outside. Spoilage will happen more rapidly for fish species with a thin skin layer. The number of bacteria in fish caught in temperate waters can develop even when in ice but the bacteria caught in tropical water grow slowly for one or two weeks in icing preservation (Gram and Huss 1996).

There are many bacteria species present in spoiling fish but there are only certain types that are considered to cause spoilage. The bacteria use their enzyme to change fish odour and flavour to sour, gassy, fruity and finally ammonia and faecal odour appear. Bacteria can still develop during icing as indicated by Hobbs (1982) (Table 1).

Table 1: The relative change in abundance of different groups of bacteria in cod stored in ice (Hobbs 1982).

Bacteria	0 day (%)	5 days (%)	10 days (%)	15 days (%)
Pseudomonas	14	17	50	82
Achromobacter	33	49	38	14
Flavobacterium	4	0	0	2
Coryneform	41	33	12	2
Micrococcus	8	1	0	0
Total	100%	100%	100%	100%

Not all the growing bacteria are involved in the spoilage process. There are just a few bacteria species that become predominant and are mainly responsible for spoilage. For example in gutted cod, chilled by ice the specific spoilage organism (SSO) is *Shewanella putrifaciens* and in packaged cod fillet it is *Photobacterium phosphoreum* (Connell 1995). If the fish is preserved by icing or in lack of air the amount of *Pseudomonas* and *Shewanella putrifaciens* bacteria is not very high but *Photobacterium phosphoreum* bacteria becomes quite high. After a certain time in ice in aerobic conditions the *Pseudomonas* and *Shewanella putrifaciens* bacteria will become the predominant bacteria. In general in low temperature (0-5°C), *Shewanella putrefaciens*, *Photobacterium phosphoreum, Aeromonas* spp., and *Pseudomonas* spp. cause spoilage but in higher temperature (15-30°C) other species like *Vibrionaceae, Enterobacteriaceae* and the positive Gram bacteria cause spoilage (Gram and Huss 1996). The bacteria produce a high amount of volatile compounds. These are trimethylamine, volatile sulfur compounds, aldehydes, ketones, esters, hypoxanthine as well as other low molecular weight compounds. The bacteria *S. putrefaciens* and some *Vibrionaceae* produce H_2S but *Pseudomonas* and *Photobacterium*

phosphoreum do not produce significant amounts of H_2S. The volatile sulphur-compounds have a very bad odour so even minimal quantities are considered to affect quality. The low temperature is very important in preservation of raw material. Especially in the range of 0-25°C the temperature strongly affects the bacteria activity (Figure 2). At 0°C the bacteria grow very slowly. The typical spoilage bacteria like *Shewanella putrefaciens* develop 10 times less in comparison with growing at the optimal temperature. Raising the keeping temperature thus increases the spoilage rate rapidly. Therefore it is important to decrease the temperature to 0°C as soon as possible after catching. For fish in the tropical water area where the ambient temperature is around 25 – 30°C the rate of spoilage can be 25 times higher than when kept at 0°C (Huss 1994).

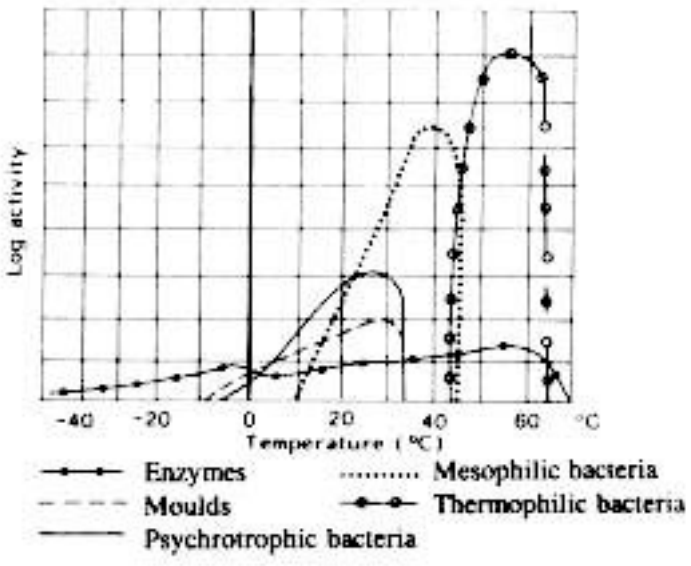

Figure 2: Change in micro-organism and enzyme growth by temperature (Huss 1994).

2.1.3 Rancidity

Fat oxidation usually occurs after autolysis and bacterial spoilage. The lipid concentration in fish can contribute to the spoilage process in fish. The fats in fish are mainly unsaturated fatty acids that are easily oxidised by oxygen from the atmosphere. High temperature or exposure to light can increase the oxidation rate. For fatty fish preserved in ice, spoilage due to rancidity is mainly caused by oxidation. This produces a bad and unpleasant odour as well as a rancid taste (Hobbs 1982). Fat fish species like herring, mackerel, and salmon are mostly affected by rancidity. The lean fish fat content is about 0.1-0.9% and the fat fish fat content is higher than 0.9% (Love 1982)

2.1.4 Mechanical damage

If the fish is broken by harsh handling, it will be subject to mechanical or physical damage and become bruised and defected in outside appearance. But it is more

important that some small cells will break leaving the enzymes free to react with other substances. Mechanical damage gives good conditions for some enzymatic activities. Fish kept in thick layers in a box with ice can cause high pressure between the ice and fish causing cells to break. All careless handling of fish raw material can result in bruised fish. This also opens channels for the micro-organisms to enter the fish flesh and enables quicker spoilage of the fish (Huss 1995). In general, in order to maintain the fish raw material quality after catching, some measures for handling and preservation are needed to prevent all the quality change processes mentioned above.

2.2 Fish raw material handling and preservation

Immediately after catching the fish start to spoil in one way on the other. However the rate of spoilage is different depending on ambient conditions, fishing technology, fishing equipment, species of fish, catching season and handling and preservation activities (Hobbs 1982). Using low temperature with ice is a popular method for fresh fish preservation. The chilling temperature of nearly 0°C can maintain freshness quality for a long time. When the temperature decreases the bacterial growth is slower, the reaction rate of enzymes is also decreased and the rigor mortis time can be extended. If the shelf life of some fish products stored at 0°C is known, the shelf life at different temperatures can be calculated by a certain formula e.g. if the fish can maintain quality for six days at 0°C the shelf life at 5°C will be 2.7 days or if another fish can maintain quality for 10 days at 0°C the shelf life at 15°C will be only 1.6 day (Table 2).

Table 2: Shelf life of cod stored at 0°C and predicted shelf life at 5, 10 and 15°C (adapted from Huss 1994).

Shelf life at 0°C of stored temperature (days)	Shelf life at different temperature (days)		
	5°C	10°C	15°C
6	2,7	1,5	1
10	4,4	2,5	1,6
14	6,2	3,5	2,2
18	8	4,5	2,9

Fish chilling should be carried out quickly and the fish raw material should not be exposed to sunshine or wind. Sunshine and wind can speed up not only autolytic and bacterial spoilage but also the oxidation process especially in fatty fish species. Fish handling and preservation can be carried out on board of the fishing vessel or on land. The first pre-processing stages for whole fish include some stages e.g. bleeding, gutting, icing and freezing. Some fish species can be bled and gutted on board, but this work can take much time and some fish species are only primarily washed and put into boxes or tubs with ice and stored in the hold of the vessel (Kelman 1992)

There are a lot of enzymes in the fish intestine which can be activated strongly when the fish dies. Fish intestines contain many enzymes catalysing autolysis and spoilage in fish. Fish intestines also contain many undesirable micro-organisms which can contaminate the fish flesh. Removing intestine can eliminate these undesirable enzymes and micro-organisms. Thus it is preferred to bleed and gut the fish, before

chilling and storing. However, after gutting the inside of the belly area is exposed to air, which can lead to oxidation and discolouration of the fish. Therefore, some fat fish species are not always gutted before chilling especially the small sized fish, as gutting them takes too long time. For the lean fish species, gutting is usually carried out because this can retain the quality for extended time periods. The chilled sea water (CSW) that includes ice and seawater can chill the fish raw material very fast. However if the fish is kept in water for a long time some colour pigments from the skin as well as some soluble and nutrition substances can be released and loaded into the environment. Using CSW can also create sensory changes in the fish e.g. higher salt content after chilling and storing. The chilled water (CW) is also often used for chilling fish and this does not affect to the fish salt degree (Huss 1994).

Different types of ice can be used for chilling fish like liquid ice, flake-ice, tube ice, and block ice (Table 3). Block ice should be grinded before use. Liquid ice has the highest cooling rate, the second is flake ice but grinded block ice is the slowest. Liquid ice has uniform particle size and large surface squares which means better heat transfer. Following Huss (1994) the crushed block ice and the tube ice is suitable for the chilled sea water (CSW) system. The rate of chilling is important. For some big fish species (e.g. ocean tuna) chilling is carried out by gutting and putting ice into the belly of fish to increase the chilling rate.

Table 3: Physical characteristics of ice utilised in chilling fish (Huss 1994).

Types	Approximate Dimensions	Specific volume (m^3/t)	Specific weight (t/m^3)
Flake	10/20 - 2/3 mm	2.2 -2.3	0.45-0.43
Plate	30/50 - 8/15 mm	1.7 - 1.8	0.59-0.55
Tube	50(D)- 10/12 mm	1.6 - 2.0	0.62-0.5
Block	Variable	1.08	0.92
Crushed block	Variable	1.4 - 1.5	0.71 -0.66

A common way to chill the fish is to arrange it with ice in a fish box. There are some specific requirements for these boxes. Research from a long time ago shows that the fish box should be made by material that is easy to clean; therefore wooden boxes should not be used as they are porous and with a rough surface (Jørgensen 1965). Some experiments show that the plastic container is better than aluminium, wood or wicker basket containers to maintain low temperature and retain the fish quality stored in these containers (Vyncke 1965). In general fishermen like using larger containers or tubs because there are fewer units to handle, saving time for unloading but the disadvantage in terms of fish quality is the high pressure on the fish in the bottom of the tub. However the box size must also depend on whether they are transported by hand or machine force. Tubs with iced fish should have good drainage to discharge water from the melting ice (Valdimarsson 1992). For a box or a tub containing fish the thermal insulation is essential to minimise ice consumption and to keep inside temperature more independent of outside temperature. A prototype from the 1970s was lined with glass fibre on a frame of iron. Prototypes lined with aluminium plates were also made. Both prototypes became rather clumsy and there were heat leakages because of metal contact from the inner to the outer lining. Polyurethane can be injected in between a double wall for insulation and to give

increased strength, especially if the polyurethane can be made to adhere to the linings (Røyrvik 1982). Some requirements for fish boxes are recognised. For example, the box should have good insulating effects, but the difference between polystyrene boxes, fibre board boxes and wooden boxes is not well distinguished (Wignall 1982). However, one of the main requirements for a fish box, tub or container is how to maintain fish freshness quality and extend the shelf life of fish. Shelf life of fish relates to handling and preservation methods and some other factors, even to the fishing season (Kolakowska 1992). In general the fish raw material is stored in the fish container with ice until reception at the processing factory. To evaluate the raw material quality like freshness or shelf life, various methods are used. They are outlined in the following section.

2.3 Analysis methods for quality evaluation

The methods of assessing freshness can be divided to two groups: sensory methods and non-sensory methods, where non-sensory methods include microbiological, chemical and physical analysis. Sensory assessment is a direct measure but the non-sensory methods are indirect measurements. They should be used in combination (Howgate 1982). The disadvantage of the sensory method is that it is subjective depending on the person who evaluates and people (panellists) have to be trained for fish sensory evaluation. The non-sensory methods are biological, chemical, physical. Their disadvantage is complexity because they require laboratory equipment (Jonsdottir 1992)

2.3.1 Sensory method

Sensory evaluation is a systematic assessment of the odour, flavour, appearance and texture of food. The Quality Index Method (QIM) is a seafood freshness quality control system that was developed by European fisheries research institutes. It is considered to be a rapid and reliable method for assessing freshness. (Martinsdottir *et al.* 2001) QIM is based on the significant sensory parameters for raw fish when using many parameters and a score system from 0 to 3 defect points (see QIM form in Appendix 1, Table 7). QIM is a practical rating system where the defect points are recorded. The sum of scores for all the characteristics is the overall sensory score. QIM gives scores of zero for very fresh fish, while increasingly larger totals result as the fish deteriorates. The description of evaluation of each parameter is written in a guideline. When the score is 18 or more the fish is considered spoilage.

2.3.2 Microbiological methods

There are a lot of microbiological methods to determine fish bacteria e.g. plate count, direct microscopic count, ATP measuring, but the plate count is a traditional and common method with some different media like plate count agar or iron agar. Some spoilage bacteria can produce H_2S (e.g. *Shewanella putrefaciens*) and reduce TMAO. The iron agar medium can be used in order to isolate spoilage bacteria that produce H_2S and form black colonies on the agar media. Black and white colonies are observed and counted respectively. The black ones are referred to as spoilage bacteria, while the totals (black + white) are referred to as the total count. The pour plate

method is often used with plate count agar, which is a common method to determine the total content of bacteria in seafood. The iron agar method can sometimes detect higher bacteria amounts than plate count agar (Gram 1992).

2.3.3 Chemical methods

Chemical methods to measure freshness quality have been considered to be objective methods and therefore superior (less variable) to methods involving sensory evaluation. During post mortem storage microbiological spoilage causes the formation of volatile bases, which can be determined to measure indirectly the freshness quality of such seafood. There are a few substances that are usually determined to evaluate fish raw material freshness, e.g. total volatile basic nitrogen (TVB-N), trimethylamine (TMA), ammonia, biogenic amines, ethanol and indol. The TVB-N remains constant for the first days of storage or increases slowly but it rises fast later in the spoilage process. Therefore TVB-N is a very good indicator of spoilage in fish (Oehlenschlager 1992). For some types of ground fish species like Atlantic cod (*Gadus morhua*), European hake (*Merluccius merluccius*), and haddock (*Meranogrammus aeglefinus*), the TVB-N determination is not as good to detect the early stages of deterioration in freshness quality like the TMA measurement, but it can be used for measuring later stages of deterioration (Botta 1995).

3 DESCRIPTION OF THE PRESENT SITUATION AND PROCEDURES IN VIETNAMESE AND ICELANDIC FISHERIES

Handling of raw material can roughly be divided into two categories: the artisanal type and the industrial type. In general, in the modern industry system the activities are automated, using little human force. But in Vietnam, the handling is mainly artisanal and uses mainly human force. The gutting stage is not carried out. After landing, the fish raw material still has to go through many stages before entering the receiving area of the factory. This takes a long time and the temperature can easily fluctuate during the process. In order to keep the ice melting rate slow, using insulation boxes for storing and transportation of fish with ice is important especially in tropical areas. In Vietnam very little attention has been paid to the effect storage boxes that influence the quality of raw material. In Vietnam there are some experiences in proper handling and processing from catching to factory reception. This chapter provides some data on temperature and time during the flow chain in processing fish raw material as well as information related the handling process. The knowledge which has been gained in Iceland for the last six months is also applied to point out the main problems in handling and preservation as well as the way to improve the present situation in Vietnam. It is of prime importance to analyse the operating procedures along the whole chain from catch to the consumer and to suggest changes in order to improve the quality of Vietnamese fish.

3.1 Present situation of fish handling in Vietnam

Today in Vietnam, the exploitation fisheries situation is confusing. The off-shore vessels are not working effectively and fish catch is not high. The catching process at

sea usually takes a long time, so if the fish is not handled properly, it loses a lot of value, and the economic gain for the fishermen can be very low. At the fishing port or fishing market, the middlemen[1] sometimes press the price to the fishermen down. After buying raw material from the fishermen, the middlemen will sell it to trading establishments[2], the more trading time the more quality decrease. The factory could still have to buy this raw material for a high price, especially at times of low supply. A solution to this may be the formation of an auction market, where the fishermen could sell at a price according to their fish value and quality. In order to retain high value, the fishermen and all the other people involved have to know about fish raw material quality and how to maintain the freshness as high as possible.

In practice the fish can go through a lot of middle stages before entering the processing factory. This increases the holding time which results in quality loss. If other conditions are also undesirable such as temperature, hygiene or methods of handling and transportation the fish quality may decrease much more. The temperature and time parameters are very different in the flow chain depending on the type of catching vessel. There are three main types of such vessels in Vietnam: big, medium and small size vessels. The big vessels usually operate on fishing trips at sea lasting up to 10 days. The medium vessels stay at sea for about three to seven days and the small vessels' fishing trips last for less than three days. For all of three types of vessels there are four ways to transfer the raw material to the factory (Figure 3). The minimum way (way 1) from catching to processing plant takes 4 hours ($\tau 6$) (Table 4) i.e. when the fishermen sell their fish directly to the factory. For the big vessels the time can be as long as 270 hours ($\tau 1 + \tau 2 + \tau 3$) in the worst case, i.e. a lot of middle stages before the fish arrives at the factory. The time may be shortened by transferring the catch at sea to other vessels coming back earlier.

Table 4: Time and temperature parameters in each stage from catching to the fish processing plant (Tam *et al.* 2004).

Time (τ) and Temperature (t)*	Type of catching vessel		
	Small size	Medium size	Big size
$\tau 1$	4- 10 hours	36-168 hours	168-240 hours
t1	15 -30 ^{0} C	0-15^0 C	5-15^0 C
$\tau 2$	1-3 hours	3-24 hours	3-24 hours
t2	15 -30 ^{0} C	15 -30 ^{0} C	15 -30 ^{0} C
$\tau 3$	1-2 hours	1-6 hours	1-6 hours
t3	0-6^0 C	0-6^0 C	0-6^0 C
$\tau 4$	1-3 hours	3-24 hours	3-24 hours
t4	10-25 ^{0} C	10-25^0 C	10-25^0 C
$\tau 5$	4- 10 hours	36-144 hours	72-240 hours
t5	0-6^0 C	0-6^0 C	0-6^0 C
$\tau 6$	4- 10 hours	36-144 hours	72-240 hours
t6	15 -30 ^{0} C	0-15^0 C	5-15^0 C

* See Fig. 3

[1] Middlemen are the persons who lend the fishermen money and cover their product
[2] The trading establishments buy fish from the middlemen and supply to the factory

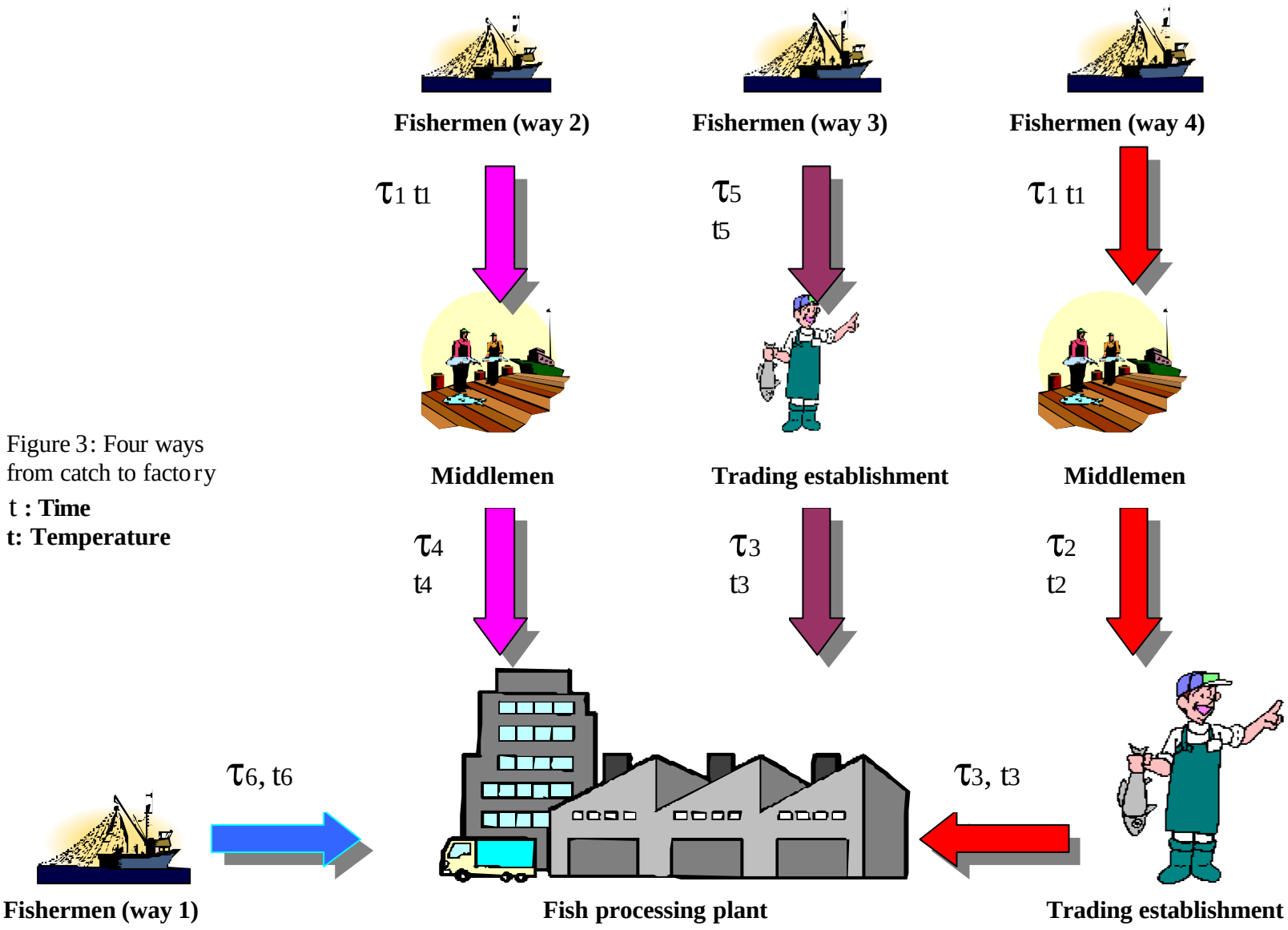

Figure 3: Four ways from catch to factory

t : Time
t: Temperature

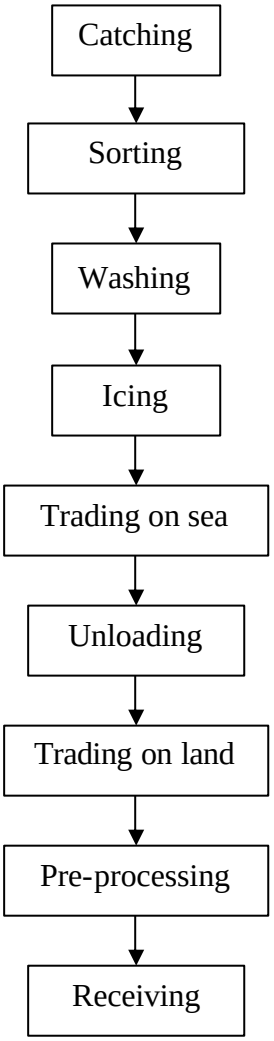

Figure 4: Flow chart for fish in Vietnam.

Each handling step affects the quality of fish (Figure 4, Table 5). Already at the catching stage the fish can lose quality by the fishing method. Bottom trawling for a long time can damage and scratch the fish and it can be shocked and stressed for too long before dying. The ambient temperature in the summer can be 30 – 35°C. In addition, handling and chilling by ice is carried out slowly and late, leading to temperature rise. Unloading is carried out only by human labour, so it is time consuming. Ice for chilling the raw material is usually not sufficient, which results in a temperature higher than 4°C. Some middlemen or trading establishments carry out re-handling and pre-processing raw material, where the raw material is made better in appearance in spite of poor quality. The containers used for storage and transportation are not always suitable as they are not specialised fish containers. These containers have poor insulation and the fisherman has to use more ice for the preservation of fish. This causes uncertainty in the shelf life and the products quality.

Table 5: The identified risk as low, medium and high in each handling stage of raw material in the flow chain.

Problems in each stage	Autolysis	Bacteria	Oxidation	Mechanical damage condition
1. Catching method	Medium	Low	Low	High
2. Sorting	High	Medium	Medium	High
3. Primary washing	High	Low	Medium	High
4. Icing and putting into the box	Medium	High	Low	Medium
5. Trading and transporting at sea	Medium	High	Medium	Medium
6. Unloading	Medium	High	Medium	High
7. Trading and transporting on land	Medium	Medium	Medium	High
8. Re-icing and pre-processing	Medium	Low	Low	Low
9. Receiving at factory	Low	Low	Medium	High

3.1.1 Catching method

The main problems are autolysis and mechanical damage. The fish breaks, is bruised or has scratched skin and the fish is stressed. Some other problems related to physical conditions are significant like extraneous matter, foreign bodies or hookers. There are a lot of different fishing methods like trawling, purse seine, gillnetting, lift net and long line. These methods can influence the gravity of fish. If fishermen carry out bottom trawling for a long time at certain gravity the fish can press each other causing breaks in the flesh, bruises and scratches on the skin. In addition when the fish is broken the outside organism can have easier access to the fish flesh. The catching process commonly takes about 7-8 hours or more, so the fish is seriously stressed before death. At that time the glycosis phenomenon happens and lactate acid is produced rapidly. This causes the rigor mortis process to be shortened leading to quality defects. The raw material temperature is similar with seawater temperature (20-25°C). This is a good temperature for the development of bacteria.

3.1.2 Sorting

The main problems are autolysis, bacteria growth, oxidation, and mechanical damage. The fish is crushed, fish temperature is high. Other hazards are smaller, like extraneous matter (physical) or chemical like lubricant contamination, but this does not often happen. Most of the vessels in Vietnam are wooden and not very big, which makes it difficult to use modern automatic systems like conveyer belts or a crane on board. On board, the fish is spilled out to the board of the vessel and later piled up to

a bulk or mass for a period of time. Although the fishermen try to sort the fish quickly, the time for sorting usually takes 2-3 hours. Therefore the raw material mass temperature stays relatively high or even increases, giving good conditions for growth of spoilage bacteria and autolysis. Unsuitable sorting tools are often used by the fisherman like iron rakes, which can cause mechanical damage of the fish. When the fish handling causes physical damage (mechanical stress), cells can be ruptured and this enables the autolytic enzymes to react with substrates and produce some spoilage substances. When the fish is stored in bulk, the temperature increases. This facilitates enzymatic protein reactions which produce substances like low molecular weight peptides and free amino-acids. These substances create a good environment for growth of micro-organisms. In addition, the sorting process is carried out in the open air in sunshine and wind. Those conditions favour the oxidation process as well as autolytic and bacterial spoilage. The fish temperature is too high especially bulk-stored in the summer and exposed to the wind and sunshine (30-35°C).

3.1.3 Primary washing

The problem here is mechanical damage due to strong flushing, autolysis and oxidation. The fish is put into a plastic basket and then washed by spraying strongly with water. The fishermen move the fish by throwing the basket with fish inside, risking bruises or breakage, similar to the sorting stage. The temperature of the washing is high as this is normally the vessel engine cooling water. This favours rapid autolytic and bacterial spoilage rates. The fish temperature is still 30-35°C and the time can be long (1-2h) if the catch volume is big.

3.1.4 Icing in boxes

Mechanical damage, autolysis and bacteria are the main quality risks at this stage. Boxes are often unclean, the fish is put into the box in a wrong way, icing is delayed, and polyethylene (PE) bags full of fish are piled up. Fish can be crushed by the ice or by stuffing so this affects the edibility and filleting yield seriously and stimulates autolysis. In the case of the fish plastic box, the ice is sufficient in the beginning but soon starts to melt especially at the sides of the box. Parts of the fish are then exposed to air, resulting in a temperature increase and drier fish. Ice and fish can become one integrated block which is easily subject to chemical damage in the transport process. Sometimes the fish is not chilled by ice immediately, so temperature is high for a prolonged time period and even increases, especially when the fish is piled up to bulk. Some boxes are used with a lid made of corrosive material which contaminates the fish. In all these cases above, the fish container is important. The container may be a PE bag, plastic box, tin box, styropore (foam) box or a bamboo basket. Commonly the fish box is made of plastic without insulation or made of tin with styropore insulation foam. The dimensions of the plastic box are 510x130x350 mm and can hold about 15-20 kg of fish, while the tin and styropore box is usually bigger. The low value fish caught is normally kept in a plastic tank. As most of the box types are not well insulated, the raw material can not be maintained at a low temperature. This leads to rapid growth of bacteria and bad quality. The fish boxes in Vietnam are usually made of non-sustainable material, providing poor physical protection for the fish during transport. The boxes are not specially designed for easy cleaning and the

raw material may be contaminated (Tam *et al.* 2004). However the temperature of fish can be maintained in around 0°C but the time sometimes is too long (10 days).

3.1.5 Trading and transporting at sea

The problem here is mechanical damage, autolysis and bacteria due to delayed icing. The catching trip is usually long especially for the big vessels where the time can be one month or more. Therefore the fish is transferred to another vessel that is going to land. In this case, care is not always taken to provide enough ice on the fish. The transfer is usually carried out carelessly leading to mechanical damage. The temperature in this stage is 0 - 10°C and the time is around one day.

3.1.6 Unloading

The problem here is also mechanical damage, autolysis, oxidation and bacteria due to delayed icing. The unloading process usually takes a long time as this is carried out by artisanal labour. The ice melts very fast especially in the summer and there is no supplementary ice. The temperature at this stage is 0 - 10°C and the time is around 2 – 3 hours.

3.1.7 Trading and transporting on land

The problem here is mechanical damage, autolysis, oxidation and bacteria due to delayed icing and unsuitable transportation facilities. The fish box is still made of material that is very difficult to clean e.g. bamboo baskets. Crushing of the fish by ice or by the other fish usually happens in the weighing and transportation process. The raw material is sometimes exposed to the air, wind and sunshine with high temperature. The temperature in this stage is 5 - 15°C and the time is around 1 – 2 hours.

3.1.8 Re-icing and pre-processing in the trading establishment or middlemen

The problem here is autolysis and oxidation physical damage. The transport and handling is carried out too carelessly. In the trading establishment the fish can be pre-processed e.g. headed, gutted, scaled and, washed. Sometimes it is soaked in water or brine with added oxidants or hydrogen peroxide (H_2O_2) for bleaching. This makes the fish look fresh but in reality its quality has seriously decreased. The fillet pieces can become discoloured to yellow after freezing. The hygienic conditions in the trading establishment handling area are sometimes not acceptable which can lead to bacterial contamination. The temperature at this stage is 0 - 6°C and the time is around 12 – 24 hours.

3.1.9 Reception at the factory

The problem here is mechanical damage due to careless practices. At the reception area transportation is carried out carelessly. In some factories the fish raw material is soaked again in water with salt and antioxidants or big block of ice are used, that can crush the fish. The temperature at this stage is 0 - 4°C and the time is around 1 – 2 hours.

3.2 Present situation of fish handling in Iceland

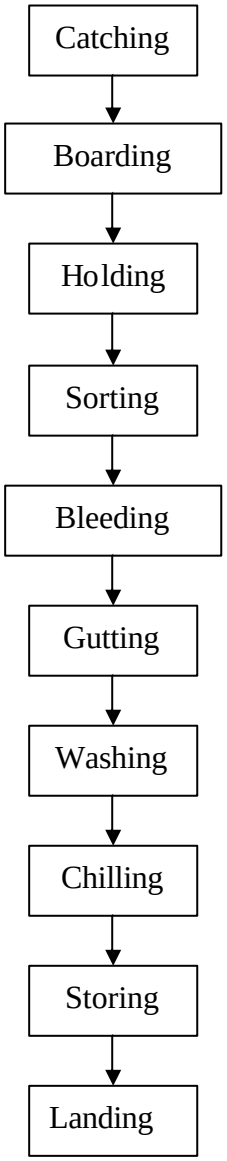

Figure 5 presents a flow chart from a typical Icelandic vessel with some main stages of advanced fish handling. The human labour force is minimal and the whole process is carried out with the helping of machines. This procedure is applied on an Icelandic vessel (trawler) that captures about 80 tons of fish per fishing trip. The crew on the vessel is only 16 people, of which the workers are 10 persons. Standard operation procedures for fish raw material are applied for maintaining fish quality as long as possible. The handling on board is not only focused on preventing fish spoilage but also pays attention to the hazards related to food safety. Depending on certain conditions (catching area, distance from catching area to land, species of fish, the size of the vessel, time of the catching trip, demand from market) some stages or their order in Figure 5 can be changed a little bit. Similarly some activities can be carried out by human labour or machine, depending on conditions.

Figure 5: Flow chart for handling of fish and processing in a typical trawler in Iceland catching mainly cod and haddock.

3.2.1 Catching method

In Iceland trawling is the main fishing gear (Figure 6) but the time of trawling is not very long or around 3-4 hours. If the haul size is big the fish can be pressed, so there might be some risk of mechanical damage. In addition, the time of the autolysis process is also reduced. The fish caught by bottom long-line and purse seine gear can give better quality. In general the time keeping fish in the net should not be very long. Shorter time reduces the amount of shocked, stressed or dead fish in the net.

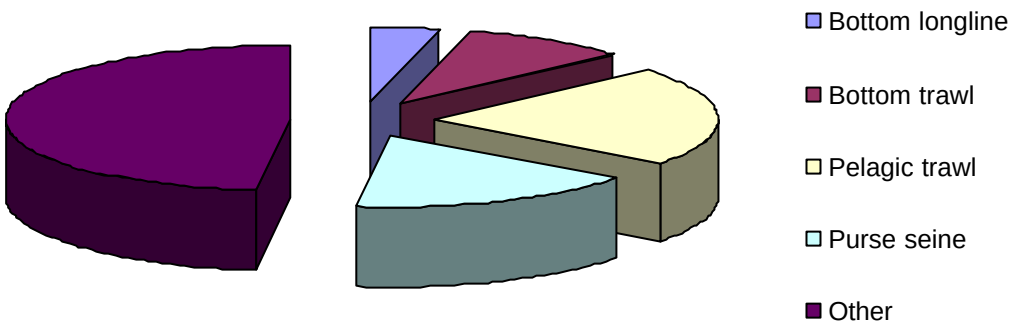

Figure 6: Catch of trawler by fishing gear in Iceland 2005 (Statistic Iceland 2005).

3.2.2 Boarding

Tackles are used for transferring the catch from the gear to vessel or hauling in the trawl. Then the net bottom is opened and the fish can fall down into a steel container below.

3.2.3 Holding

This stage is carried out especially when the volume of fish caught is quite big. The fish is put into a tank with chilled seawater (CSW) that includes ice and seawater slurry or refrigerated seawater (RSW). This stage is very important because it lowers the fish temperature rapidly and limits the activities of enzymes and bacteria. Depending on conditions, the sorting stage can be carried out after or right before this stage. The fish can be boarded by the tackle or using the pump to transfer the fish into the tank with CSW or RSW. This work is carried out by machine in order to save time and worker labour. The suitable tools can be used for transferring fish into the tank with care to avoid mechanical damage. In Iceland this work is carried out very fast. The fish is poured from the net into the big hold and then the fish is transferred by conveyer to the sorting stage.

3.2.4 Sorting

The fish is sorted quickly by hand parallel to the processing stage (see sub- section 3.2.5). This work is carried out below the deck with a conveyor belt. The by-catch is sold in an auction market on shore.

3.2.5 Bleeding and gutting

In order to the make the fish fillet maintain a good appearance, the fish has to be bled. Blood stains are regarded as defects, as the fillet should be white. Gutting removes the fish intestines limiting access of most spoilage bacteria. However, for small fish like pelagic species, the bleeding and gutting stage is not carried out. But for some lean fish gutting always is carried out. Then the CSW system is used to decrease fish temperature quickly.

3.2.6 Washing

After bleeding and gutting the fish is transferred to the washing stage. This stage cleans blood and viscera residues. The washing stage is carried out in a tub with ice and seawater. It is carried out quickly in order to avoid losing the nutrition substances.

3.2.7 Chilling

The fish is cooled down by liquid ice, therefore the fish temperature decreases very fast. This stage is short, about 30 minutes. The liquid ice has a lot of advantages to flake ice including high regular size, larger surface square and the ability to fill the entire tub/box and cover the fish. The disadvantage of liquid ice affects the taste of the fish a little bit because it is made by sea water it can make the fish salted. The liquid ice is also relatively expensive.

3.2.8 Storing

The fish is iced and arranged in layers in insulated tubs with tube ice for storing. The tubs are stacked in the hold and are easy to lift by crane when landing. A label is attached to each tub for traceability at further stages of the process.

3.2.9 Landing to plant or auction market

The raw material is unloaded from the boat by a crane. Transport and weighing is carried out quickly and carefully. In case of landing to land (e.g. Brim vessel) the raw material can be processed immediately. In other cases the fish will be transferred to the auction market. In Iceland there are several auction markets e.g. "Fiskmarkadur Islands". Most of the catch traded there are from fishing trips lasting one day, so the fish is quite fresh. The catch is sold on a daily basis before landing. At reception the fish is inspected for sufficient ice and arrangement in the tubs. The fish temperature is also measured and recorded. Then the fish is size graded and weighed, put in the tubs with fresh ice and dispatched in the evening of catch date. An internet auction where buyers can log in and participate from anywhere in the world has been established.

4 MATERIALS AND METHODS

Two experiments were carried out including a) determination of the ice melting rate and b) quality comparison between fish stored in an insulated plastic box and in an open box without insulation. The open box without insulation was chosen to imitate boxes commonly used in Vietnam. The first experiment was carried out to determine the effectiveness of different types of fish boxes in terms of keeping the contents cold. Based on the first experiment, the best and worst fish boxes were found. The second experiment was a comparison of fish quality in these best and worst scenarios.

4.1 Experiment for determining the insulation ability

Five types of containers were chosen for the experiment: two types of Sæplast tub 70 l with lid and without lid and two types of Sæplast cooler 65 l with seal and without seal; and the plastic box regarded similar to a typical fish box in Vietnam (VN box) (Figure 7). The Sæplast container commonly has some advantageous properties like durable, good insulation layer with the closed lid, comfortable bottom hole for water drainage, and easy to clean.

(a) (b) (c)

Figure 7: The types of box/tub used for experiment (a) Sæplast tub 70 l, (b) Sæplast cooler 65 l, (c) Vietnamese-like box (VN box).

Determination of ice melting was carried out by measuring the amount of ice after each period (time recorded). At first all the tubs/boxes were weighed. The grinded tube ice was filled into all the containers and each one weighed again. At each measuring point, melted ice was removed by opening the bottom hole and then the box and tubs weighed. Results were recorded and applied according to the formula:

$$Mi = Mio - K.\ t \quad \text{(Huss 1994)}$$

In which:
Mi: kg of ice left in the tub
Mio: kg of ice at the beginning
t: time (hours)
K: melting rate (kg/hour)

The time (t) and Mi, Mio is found by weighing and recording the time. Therefore the amount of ice melting every hour can be calculated.

4.2 Experiment for the quality change in fish

4.2.1 Fish preparation

Small size cod was used for the experiment. It was caught by the Brim trawler Hardbakur on 3 January and sampled on 5 January. The fish was gutted and stored in ice onboard until sampled at the Brim raw material storage. The ice type was tube ice supplied by Brim. Sæplast 70 l tub and the VN box were chosen for storing fish in the experiment because these boxes or tubs resulted in the highest and lowest ice melting rates based on the previous experiment. The fish and tube ice were put in layers into the box and tub as follows: VN box 1 layer of fish, 2 layers of ice, Sæplast tub 2 layers of fish, 3 layers of ice (each layer was around 10 cm); a lid was secured on the Sæplast tub. These two fish boxes and tubs were transferred from the Brim Company to Akureyri University and placed in the wet laboratory room of the University MRI. Room temperature was chosen for storing the boxes to create conditions more similar to Vietnam.

4.2.2 Measurements

Two fishes from each container were taken each time of sampling until all the ice had melted. Three methods were chosen for evaluation: microbiological, chemical and sensory. Each method is described in details in Appendix 1. The experiment schedule is shown in Table 6. All the analysis methods (sensory, microbiology and chemical) are following the IFL procedures described in Appendix 1.

Table 6: Sampling schedule for evaluation.

	VN box	Sæplast box
Day 0		**X**
Day 1	**X**	
Day 2	**X**	
Day 3	**X**	
Day 4		**X**
Day 5		
Day 6		
Day 7		**X**
Day 8		
Day 9		
Day 10		**X**
Day 11		
Day 12		
Day 13		**X**

The sensory evaluation followed the QIM method (form in Appendix 1, Table A) (Martinsdottir *et al.* 2001). The panel included three people. There are five main criteria for evaluation: appearance, eyes, gills, bloods and fillets. Every main criterion

includes some more detailed criteria. The score for each criterion is from 0 to 3. Lower scores signify higher quality and the total score can show the general fish quality.

The micro-biological evaluation is based on the pours plate method (Appendix 1). Samples from individual fish were bacteriologically assessed for aerobic plate count using plate count agar and iron agar. This medium was used in order to isolate spoilage bacteria that produce H_2S. Then the petri dishes were put into the incubator for incubation at 22°C for IA media and 30°C for PCA media for three days.

The chemical evaluation was a determination of TVB-N following the Kjeldahl distillation method (Appendix 1).

5 RESULTS AND DISCUSSION

The results include experiment results for ice melting and experiment results for a quality comparison between fish stored in the Sæplast insulation plastic box and the Vietnamese-like box. The first experiment was carried out to determine the effectiveness of different types of fish boxes. Based on the first experiment the best and worst fish box according to ice preservation was found. The second experiment focuses on comparing fish quality in the best one (Sæplast box) and the worst one (Vietnamese-like box) in order to make validations of the guidelines for handling and preservation of fish raw material in terms of keeping a low temperature.

5.1 Ice melting experiment

The change of ice left in the box after certain amounts of time is shown in Figure 8.

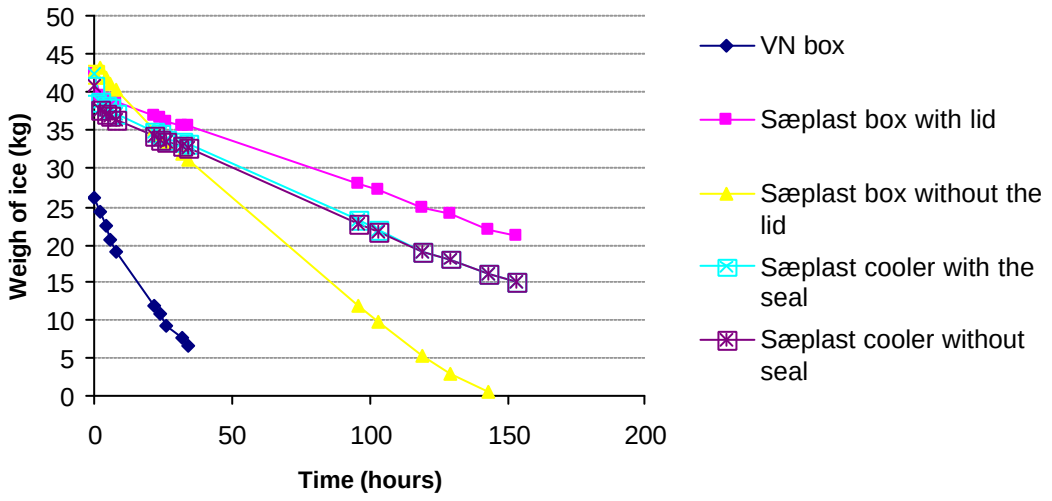

Figure 8: Weight of ice per container by time for the five different containers.

The ice in the Vietnamese-like box melted (Figure 8) fastest in comparison with the other fish boxes. Figure 8 shows that the graph can be estimated by a linear model and the amount of ice melted per hour is around 0.5 kg because the equation for the line is y = -0.5482x + 26.435 (Figure 14 – Appendix 5). Therefore we can calculate that after 48 hours (two days) the ice will be fully melted based on this formula. Weight measurements are most fluctuant (R2 value is lowest) in the VN box.

In the Sæplast insulated plastic box with lid, the amount of ice melted each hour is around 0.12 kg according to the linear equation y = -0.1222x + 51.338 (Figure 15 – Appendix 5). This is the lowest rate in comparison with the others i.e. this type of box has the highest insulation ability. Therefore from the formula we can calculate that after 428 hours (approximately 18 days) the ice will be fully melted. For the Sæplast insulation plastic box without the lid the amount of ice melted per hour is around 0.31 kg and the equation is y = -0.3069x + 51.152 (Figure 16 – Appendix 5) i.e. after 167 hours (approximately seven days) all the ice will be melted. For the Sæplast plastic cooler without the lid the amount of ice melted per hour is around 0.16 kg and the equation is y = -0.1594x + 49.713 (Figure 17 – Appendix 5) i.e. after 312 hours (approximate 13 days) all the ice will be melted and for the Sæplast plastic cooler without the seal on the lid the amount of ice melted each hour is around 0.15 kg (Figure 18– Appendix 5).

In both coolers the rate of melting is most stable in comparison with the others. The reason may be that the hole for water drainage is smallest so the influence from ambient temperature is minimised. In general the Vietnamese-like box is the worst type and the Sæplast box with the lid is the best one (Figure 9). Therefore these two were chosen for the second experiment.

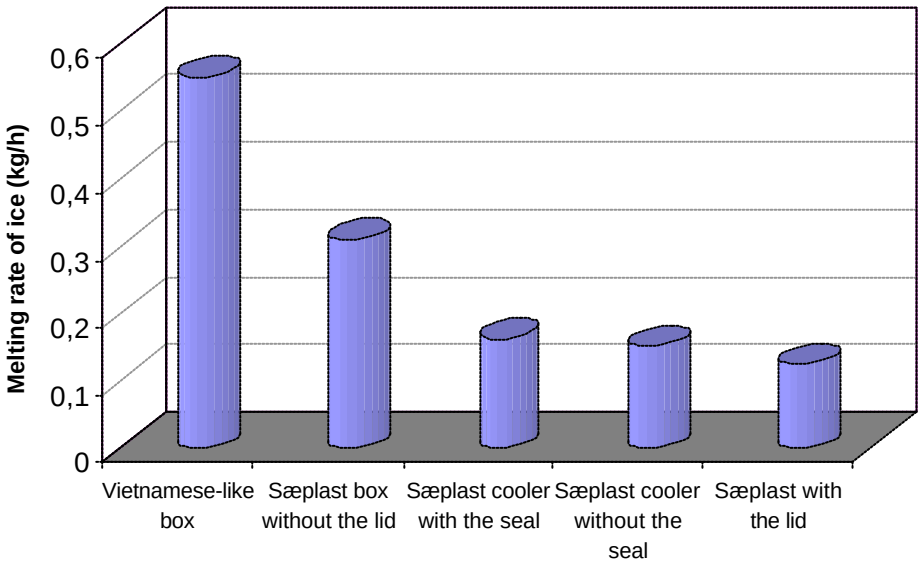

Figure 9: Melting rate of ice in box used in the experiment.

5.2 Difference of fish quality in the worst and best scenario case

5.2.1 Sensory quality

The sensory experiment shows that fish stored in ice in the VN box has started to spoil nearly after three days of preservation (Figure 10). On the third day the average QIM method score is around 16 and all the ice was melted. Therefore the experiment had not been carried out. Following Martinsdottir (2001) the fish is not considered fit for human consumption when the score is higher than 18. The line can be presumed to have extended to the unacceptable limit after 4.5 days.

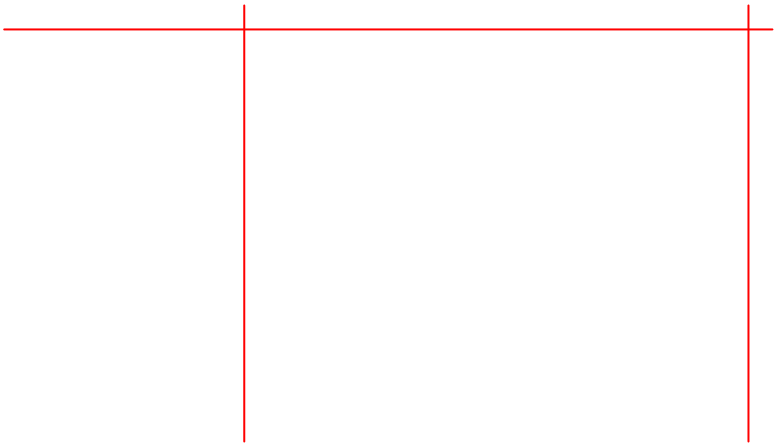

Figure 10: Sensory score (QIM) of fish stored in a VN box and Sæplast box.

Regarding the fish stored in the Sæplast insulated tub the quality of fish is maintained for longer periods of time. After 13 days of preservation the fish was unacceptable according to the QIM criteria and all the ice was melted. According to Huss (1995) the shelf life can be predicted at 15 days. This means that the results corresponded with Huss's evaluation because at that time the total days preservation by ice was 15 days (including two days on the boat and 13 days of experiment).

It is worth noticing that the fish quality score at the beginning is about 1 on the QIM scale, i.e. it is very high quality. The fish is caught just two days before and kept at very good conditions. Therefore the Sæplast insulation box can store fish better than the VN box as predicted.

5.2.2 Chemical analysis

Figure 11 shows the change in TVB-N during storage in ice.

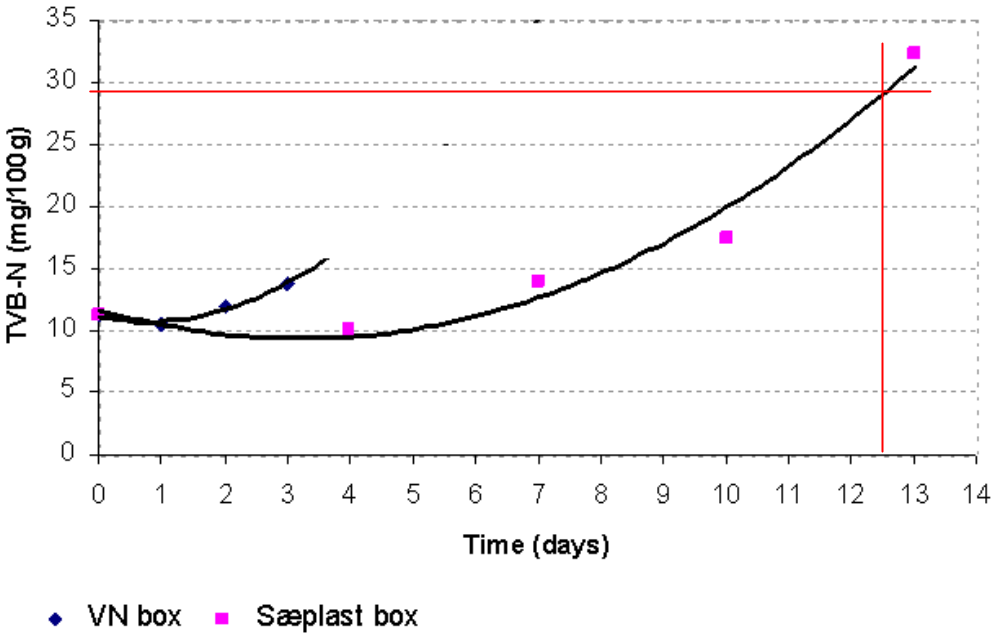

Figure 11: TVB-N contents of fish in ice stored in VN and Sæplast containers.

The increase is very slow for the first days. This corresponds with the conclusion of Oehlenschlager (1992) TVB-N remains constant for the first days of storage or increases slowly but rises fast later in the spoilage process. The reason may be explained that the main process leading to spoilage is autolysis (this is corresponding with the results of microbiology). The fish TVB-N value at the beginning is even higher than after one day, but the deviation between the two individual fish samples was also high. So this can be considered as normal deviation in between individuals. The experiment with the VN box should have been carried out for a longer period for clearer results. But at that time all the ice had melted.

After three days ice storage the value of TVB-N is still very low in the Sæplast tub, so the fish quality is maintained longer like predicted. The TVB-N value does not increase much until around 10 days of storage, but then it develops quite fast to around 33 mg TVB-N/100 g fish flesh on day 14. The limit for TVB-N given by Huss (1994) is 30 mg/100 g fish flesh and according to that the fish has reached the unacceptable limit. The trend of these curves shows that it is highly likely that the VN curve will rise sooner than the Sæplast curve.

5.2.3 Microbial analysis

Figure 12 shows the change in total bacterial count during storage.

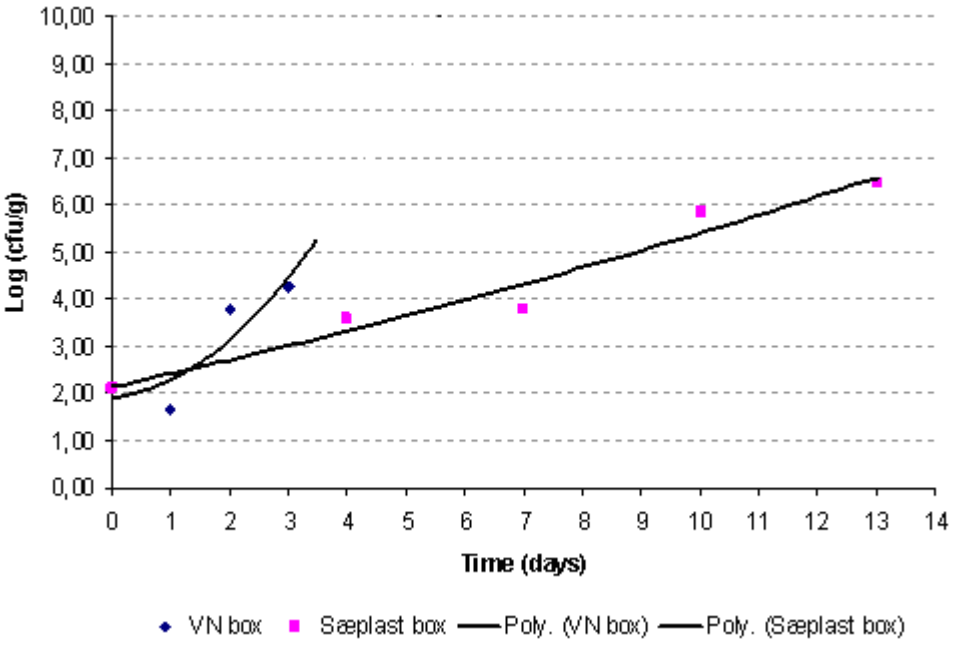

Figure 12: Total bacteria counts (PCA, 30°C) in the VN box and the Sæplast box.

The amount of cfu (colony forming units) increased very fast on the second day of storage (Figure 12) for the fish stored in the Vietnamese-like box. The quality of the fish sample at the beginning is even higher than the fish on the first day (with PCA media) and days 1 and 2 (with IA media). This can be explained because the fish is taken randomly so there are some small differences in quality between individuals.

The amount of colonies incubated in PCA media is less than in IA media. This is reasonable because the IA media is richer in nutritional substances than PCA media. In addition the temperature incubation for IA media is 22°C, which is lower than for PCA media (30°C) so conditions are better for psychrophilic spoilage bacteria growing (Gram 1992) (Figure 12 and 13).

Regarding the significant spoilage organisms (SSOs) with black colour on IA media at day 13 the total count is about 10^5 - 10^6 cfu/g (Figure 14).

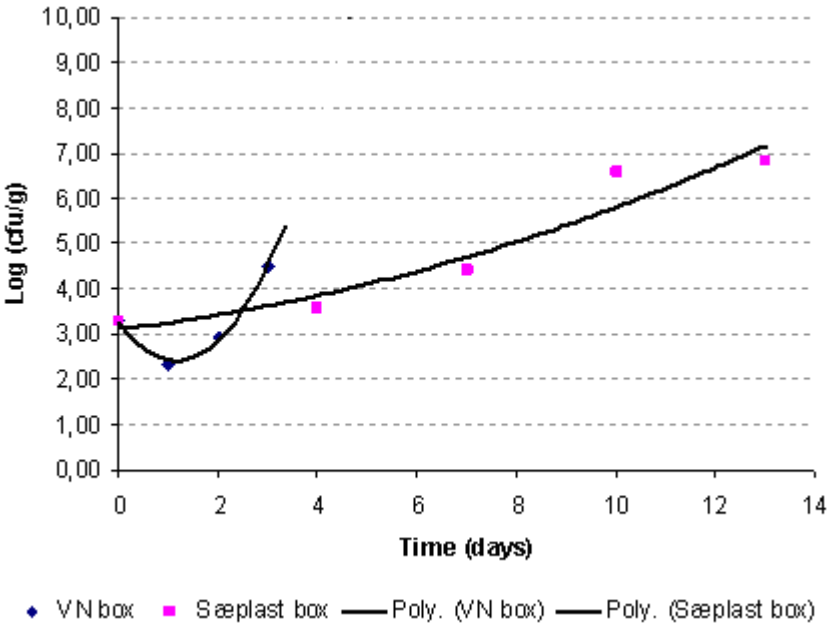

Figure 13: Total bacteria counts (IA, 22°C) in the VN box and the Sæplast box.

In case of the fish stored for a long time like storing in the Sæplast tub the bacteria can grow up to around $10^6 – 10^7$ cfu/g. The experiment with the VN box should have been extended for a few days until the bacteria growth became higher than the limit. Following Huss (1994) the fish can be considered unacceptable when the total bacterial count is 10^7 cfu/g.

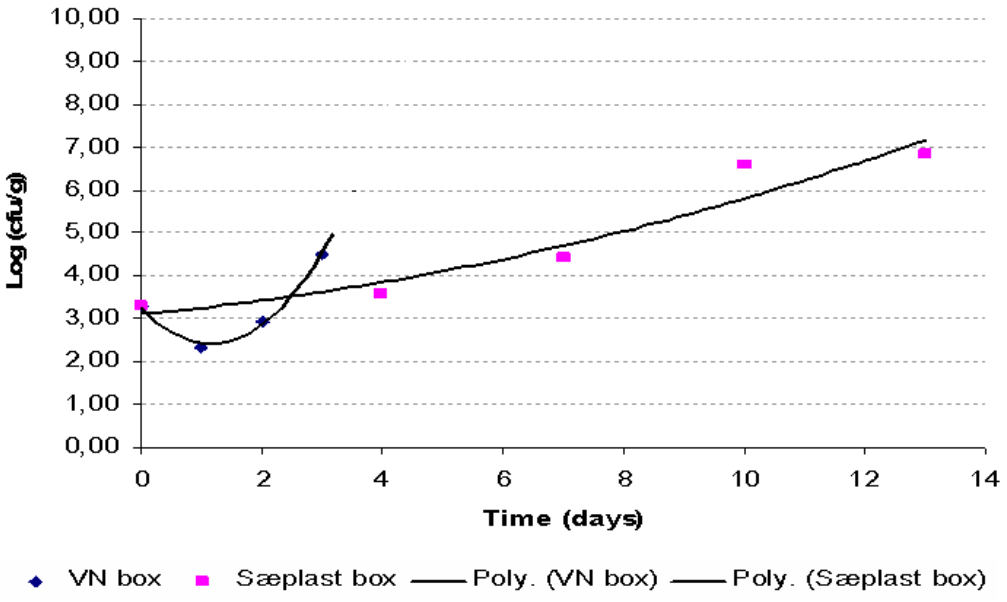

Figure 14: Black colony counts (IA, 22°C) in the VN box and the Sæplast box.

Thus for all three evaluation methods the results show that if the fish is stored with ice in an insulated container like the Sæplast tub with lid, it can for hold up to 13 days. It is obvious that the fish quality decreases slowly in the Sæplast tub but rapidly in the VN box preservation.

6 RECOMMENDATIONS FOR IMPROVEMENTS IN VIETNAMESE FISHERIES

In Vietnam, some fishermen and people involved in the handling of raw material do not have sufficient knowledge of the importance of raw material quality. Avoiding spoilage is one part of food safety assurance. Therefore the fish handling activities must have the objective of maintaining maximum quality. It is important to keep a continuous work flow and avoid any delay of icing i.e. the temperature and time is always controlled. The understanding of quality importance should be strengthened with the people involved in fresh fish handling. They need to be trained and educated on good handling practice and preservation. Below are some guidelines on the handling and storing of fish raw material to preserve quality.

Catching the fish can be affected by fishing gear e.g. bottom trawling carried out for a long time can result in damaged or stressed fish. The fishermen should reduce the trawling time. A bad odour can appear in fish caught in some specific sea areas. Avoid catching in these areas. These off-odours can be originated from the food supply of the fish or some specific bacteria in the fish or from sea pollution e.g. oil released from oil transportation vessels. In Vietnam today the tackle is only used to draw the catch on board. This process should be performed carefully to avoid physical damage to raw material.

Chilling on board often starts late. This leads to temperature increase in the raw material and exposure to sunlight. If the fish is poured out on to the board floor it can be affected both by high temperature and physical damage. The vessel floor should not contact the raw material directly. A layer of clean plastic or rubber can be used for covering it. This can avoid heat transfer from the boat floor and contamination from the wooden floor.

Sorting should be carried out quickly. Avoid direct sunlight and wind because it increases the fish temperature very fast. In addition the fish can dry on the surface due to the wind, leading to loss of value. Use specialised and clean plastic tools for sorting. Avoid use of hard corrosive tools like iron hooks to sort the fish. This can damage the fish. Keep all equipment clean. Consider the use of transport facilities (conveyers) for saving time at this stage.

If the fish is not gutted at this time, the temperature should be lowered as soon as possible. Chilled sea water (CSW) is very suitable for this purpose. It is always available with ice and can lower fish temperature very fast due to large surface heat transfer. The fish should preferably be put into insulated plastic tanks or tubs e.g. the Sæplast tub type with the slurry made by ice and sea water. Flake-ice or grinded

block ice with sea water should be used. Big block ice should not be used, as the blocks' sharp edges can damage the fish physically. It can also press the fish too much and decrease the temperature slowly. The NaCl and some other dissolved mineral substances in the sea water contribute to lower the temperature further. This activity should not go on for extended time, because the fish can lose nutritional value.

For some fish species quality and shelf life can be reduced much more if they are not gutted and the viscera removed. E.g. gutting should be carried out for lean fish and big fish like mackerel and tuna. However for the small size fish species, gutting will take a lot of time, leading to delayed icing. Washing should be carried out quickly with low water temperature. The CSW system can be used for washing, which will lower the fish temperature as well. Avoid flushing water strongly.

There are two methods of icing raw material: direct contact icing, which is often applied to fish and shrimp and non-contact icing which is often applied to squid, cuttlefish and octopus. The raw material and the ice are arranged in the fish box layer by layer. Each layer of fish should not be too thick. The fish layer in the tubs should not be too thick, as it will slow down the chilling. One ice layer can be put in the bottom and one on the top and one or two layers of fish in between. The icing is an important stage and care should be taken to bring enough ice for the whole fishing trip and make sure the icing in each container is sufficient because it is difficult to add more ice during the trip. If ice is added, it is only on the top of the box. The heat transfer can be uneven if the ice melts too much on the sides or bottom of the box, the fish temperature can go up although plenty of ice is still on the top. Liquid ice can be an option to consider for Vietnam in the future

The fish should be stored in insulated plastic boxes or tubs. This can help make the ice last longer and save the place for storing fish. Handle carefully while putting the fish into the box. Polyethylene bags should not be used to store the fish unorganised in the chilling hold. Systems for conveying fish are desirable to avoid physical damage to the delicate fish tissue. Avoid using lids made of corrosive material; it can contaminate the raw material. The box or tub should be designed for easy stacking or organising without pressure to the fish to save storing space. For traceability in further stages of the process it is necessary to label each tub or box.

In the chilling hold plastic trays or small boxes can be used to replace the PE bag. Systems for conveying or craning fish are optimal to unload the fish from the vessels to avoid damage and save time. Unloading time will shorten if it is performed by crane. It is very convenient and should be applied in Vietnam in the future.

The premises of middlemen or trading establishments must assure hygienic conditions. In the future, auction markets could be an option to cut some middle stages.

At the factory reception, systems for conveying or trolleying fish are preferable to avoid physical damage to the fish tissue.

7 CONCLUSIONS

There are a lot of stages in the fish raw material flow chain in Vietnam. In each stage some problems have been identified. There are some defectives that usually occur in the process from catching on board through a lot of middle stages. At the end in the factory the raw material quality has decreased. Iceland is a developed country in fisheries. Based on experiences from Iceland and the present situation in Vietnam guidelines have been written in order to contribute to the improved fresh fish handling situation in Vietnam and through that the quality of raw material can be also improved.

In general the main problems in Vietnamese handling of fish are poor chilling; and thus a lot of fish are already unacceptable at landing. All the activities should be carried out more quickly but carefully and tenderly to avoid crushing the fish; keep the fish always in low temperature conditions (around 0°C) and in hygienic conditions.

It is should be seriously considered to use insulated plastic boxes or tubs. Insulated tubs are better than open thin boxes for keeping fish; prolonged shelf life is very valuable. The boxes/tubs like the Sæplast insulated plastic tubs can be applied in Vietnam. All the results and the analysis above (see Section 5) show a big difference between the Vietnamese-like box and the Sæplast insulation tub. Therefore the worst and best scenario for storing fish here is storing fish in Vietnamese-like box and Sæplast tub with the lid. The experiments for validation of the guidelines in term of keeping low temperature show that as predicated fish can be stored in the Sæplast tub for extended time (up to 13 days). The fish stored in the Vietnamese-like box, on the other hand, lost quality rapidly.

It is very suitable in practice for storing the fish to chill it in ice. Ice chilling of raw material has to be improved throughout the process chain. For the catching vessel the time for the catching trip should be shortened. It should not take a lot of time (10 days or more). In Iceland the trip only takes 5-6 day at sea.

Training employees is an underestimated problem and should be prioritised. The captain should be trained to realise assurance of fish quality and he has responsibility to train again his workers. Avoid soaking raw material for personal profit because the quality will decrease in reality. This is economic fraud. It is necessary that the premises should be upgraded to ensure hygienic conditions.

Auction markets might be an option for better control of raw material flow. In the near future the middle stages should be replaced by a fishing auction market. This can solve a lot of problems because at that time the fish quality is corresponded with its value and its price, i.e. the fishermen's profit is clear. This leads to higher quality and more profit. This is a motivation for the fishermen to realise how important keeping the fish quality is.

More automation is favourable for future improvements in Vietnamese fisheries. The machine (e.g. conveyer, crane) is considered to gradually replace human labour or

artisanal activities. For the middle stages (middlemen, trading establishment) the activities to handle the fish should be mechanised (e.g. using a trolley) for saving time and human labour. In the future the design of equipment on the vessel should be changed to replace the wooden or corrosive surface contacting the fish by the stainless steal material to keep it hygienic and avoid contamination.

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APPENDIX 1: ANALYSIS METHOD (SENSORY, MICROBIOLOGY AND CHEMICAL)

1. Sensory method

1.1 Material

- Samples: whole fish and fish fillets
- Nylon sheet: 1 unit
- Table for evaluation: 1 unit
- Sticky paper for coding: 1 unit
- Evaluation form: 3 forms

1.2 Method

2 fishes were taken from the box and put into the PE bag and the sample coded. (Then the 2 samples are taken to the sensory room, put on the table and coded). Three people from the fellow group carried out the sensory evaluation on whole fish and on the fillet.

Appearance of skin, firmness of flesh, slime formation, colour and form of eyes and finally colour, smell and mucus formation of gills was evaluated according to the Quality Index Method (QIM).

Table Ia: Sensory evaluation form

Quality parameter		Description	Points	Score of each code					
Appearance	**Skin**	Bright, iridescent pigmentation	0						
		Rather dull, becoming discoloured	1						
		Dull	2						
	Stiffness	In rigor	0						
		Firm, elastic	1						
		Soft	2						
		Very soft	3						
Eyes	**Cornea**	Clear	0						
		Opalescent	1						
		Milky	2						
	Form	Convex	0						
		Flat, slightly, sunken	1						
		Sunken, concave	2						
	Colour of pupil	Black	0						
		Opaque	1						
		Grey	2						
Gills	**Colour**	Bright	0						
		Less colour, becoming discoloured	1						
		Discoloued, brown spots	2						
		Brown, discoloured	3						
	Smell	Fresh, seaweedy, metallic	0						
		Neutral, grassy, musty	1						
		Yeast, bread, beer, sour milk	2						
		Acetic acid, sulphuric, very sour	3						
	Muscus	Clear	0						
		Milky	1						
		Milky, dark, opaque	2						
Blood	**Colour**	Red	0						
		Dark red	1						
		Brown	2						
Fillets	**Colour**	Translucent, bluish	0						
		Waxy, milky	1						
		Opaque, yellow, brown spots	2						
Quality Index (0-23)			**Sum**						

2 Microbiological method

2.1 Material

- Iron Agar: 1000ml
- Petri dishes: 40 units
- Glass tube: 10 units
- Pipette: 10 units
- Blender: 1 unit
- Homogenizer (stomacher): 1 unit
- Analysis scale: 1 unit
- Alcohol 70%: 1000ml

2.2 Method:

The fish is aseptically filleted and minced to 100g. (25g were kept for microbiological analysis and the remains put into the refrigerator for chemical evaluation) A 1/10 dilution is made by mincing and mixing 25g of mince with 225g of dilution buffer and homogenizing in the stomacher for 60 seconds to make 1/10 dilution. 2 petri plates are used for each dilution, 2x1ml are then inoculated into the first pair of plates (1/10) and 2x0,1ml on the next pair (1/100), and similar with (1/1000) and (1/10000). Melted 45°C Iron agar is poured on the plates, stirred and left to cool. Agar plates are incubated at 22°C for 48 hours and the number of colony forming units (c.f.u.) is counted. Black and white colonies were observed and counted respectively. The counts were calculated against the dilution factors that contains at least 25 up to 250 colonies (IFL method)

3 Chemical method

3.1 Material

TVB-N method by steam distillation:

- Aqueous tricloacetic acid 7,5%: preparing 1000ml (each time using: 200ml)
- NaOH 10% preparing 100ml (each time using: 10ml)
- Acid boric 4% preparing 100ml (each time using: 10ml)
- Methyl red preparing 10ml (each time using: 0,04ml)
- Sulfuric acid 0,025N preparing 100ml
- Water distilled 1 unit
- Analysis scale 1 unit
- Waring blender: 1 unit
- Distillator: 1 Kjeldahl-type 1 unit
- Burette 0,05ml graduated
- Flask 250 ml.
- Beaker 250 ml
- Pipette 10 ml.
- Glass cup 100ml

- Filter paper

3.2 Method:

Spoilage chemical indicators are evaluated by measuring total volatile basic nitrogen (TVB-N) levels in the minced fish species. The determination of TVB-N by steam distillation is used. In this method:

- 200 ml of 7,5% aqueous trichloroacetic acid solution is added to 100 grams of fish muscle and homogenized in the blender.
- The mixture is filtered by filter paper.
- Steam distillation is carried out using a Kjeldahl-type distillator.
- 25ml of the filtrate is put into the distillation flask and 6 ml of 10% NaOH is added to it.
- An beaker containing 10 ml of 4% aqueous boric acid solution and 0.04 ml of methyl red and bromocresol green indicator are used for titration of ammonia and placed at the end of the condenser.
- Distillation is done until the final volume of 50 ml was obtained in the beaker (40 ml of distillate). This process take about 10 minutes
- The boric acid solution turned green when alkalinized by the distilled TVB-N. This solution is titrated using a 0,05 ml graduated burette containing 0.025N H_2SO_4 and complete neutralization is obtained when the colour turned pink on the addition of a further drop of H_2SO_4 (IFL method).

Calculation: (mgN/100g):

$$\frac{14\text{mg/mol}*a*b*300}{25}$$

a: ml of sulphuric acid
b: normality of sulphuric acid

APPENDIX 2 - SENSORY TESTING RESULTS

Table IIa: The QIM method score for Vietnamese-like box

VN box		Panellists			Average	Standard deviation
		Quang	Ofred	Yen		
Day 0	Fish 1	2	0	1	1	1
5/1/2006	Fish 2	0	2	1,5	1,17	1,04
Day 1	Fish 1A	2	8	2	4	3,46
6/1/2006	Fish 1B	3	2	1,5	2,17	0,76
Day 2	Fish 2A	11	11	7	9,67	2,31
7/1/2006	Fish 2B	8	14	8		
					10,00	3,46
Day 3	Fish 3A	19	21	11	17,00	5,29
8/1/2006	Fish 3B	18	15	10	14,33	4,04

Table IIb: The average value of QIM method score for Vietnamese-like box

VN box		Score average	General average
Day 0	Fish 1	1	1,09
5/1/2006	Fish 2	1,17	
Day 1	Fish 1A	4	3,09
6/1/2006	Fish 1B	2,17	
Day 2	Fish 2A	9,67	9,84
7/1/2006	Fish 2B	10,00	
Day 3	Fish 3A	17,00	15,67
8/1/2006	Fish 3B	14,33	

Table IIc: The QIM method score for Sæplast box

VN box		Panellists			Average	Standard deviation
		Quang	Ofred	Yen		
Day 0	Fish 1	2	0	1	1	1
5/1/2006	Fish 2	0	2	1,5	1,17	1,04
Day 4	Fish 4A	8	7	5,5	6,83	1,26
9/1/2006	Fish 4B	4	8	4,5	5,50	2,18
Day 7	Fish 7A	14	17	18	16,33	2,08
12/1/2006	Fish 7B	9	12	11	10,67	1,53
Day 10	Fish 10A	9	16	14,5	13,17	3,69
15/1/2006	Fish 10B	11	20	18	16,33	4,73
Day 13	Fish 13A	20	23	19,5	20,83	1,89
18/1/2006	Fish 13B	22	21	20,5	21,17	0,76

Table IId: The average value of QIM method score for Sæplast box

VN box		Average	General average
Day 0	Fish 1	1	1,09
5/1/2006	Fish 2	1,17	
Day 4	Fish 4A	6,83	6,17
9/1/2006	Fish 4B	5,50	
Day 7	Fish 7A	16,33	13,5
12/1/2006	Fish 7B	10,67	
Day 10	Fish 10A	13,17	14,75
15/1/2006	Fish 10B	16,33	
Day 13	Fish 13A	20,83	21
18/1/2006	Fish 13B	21,17	

APPENDIX 3 - CHEMICAL TESTING RESULTS

Table IIIa: The TVB-N value for Vietnamese-like box

VN box		H_2SO_4 (ml) First time	H_2SO_4 (ml) Second time	H_2SO_4 (ml) Average	TVB (mg/100g)
Day 0	Fish 1	2,7	2,3	2,5	10,5
5/1/2006	Fish 2	2,9	2,8	2,85	11,97
Day 1	Fish 1A	2,4	2,5	2,45	10,29
6/1/2006	Fish 1B	2,5	2,6	2,55	10,71
Day 2	Fish 2A	2,6	2,8	2,7	11,34
7/1/2006	Fish 2B	3,1	2,9	3	12,6
Day 3	Fish 3A	3,2	3,2	3,2	13,44
8/1/2006	Fish 3B	3,3	3,4	3,35	14,07

Table IIIb: The Average of TVB-N value for Vietnamese-like box

VN box		TVB (mg/100g)	Average	STDEV
Day 0	Fish 1	10,5	11,24	1,04
5/1/2006	Fish 2	11,97		
Day 1	Fish 1A	10,29	10,5	0,27
6/1/2006	Fish 1B	10,71		
Day 2	Fish 2A	11,34	11,97	0,89
7/1/2006	Fish 2B	12,6		
Day 3	Fish 3A	13,44	13,76	0,45
8/1/2006	Fish 3B	14,07		

Table IIIc: The TVB-N value for Sæplast box

VN box		H_2SO_4 (ml) First time	H_2SO_4 (ml) Second time	H_2SO_4 (ml) Average	TVB (mg/100g)
Day 0	Fish 1	2,7	2,3	2,5	10,5
5/1/2006	Fish 2	2,9	2,8	2,85	11,97
Day 4	Fish 4A	2,6	2,4	2,5	10,5
9/1/2006	Fish 4B	2,3	2,4	2,35	9,87
Day 7	Fish 7A	3,5	4	3,75	15,75
12/1/2006	Fish 7B	3	2,8	2,9	12,18
Day 10	Fish 10A	3,5	4	3,75	15,75
15/1/2006	Fish 10B	4,4	4,6	4,5	18,9
Day 13	Fish 13A	8,2	8,5	8,35	35,07
18/1/2006	Fish 13B	7	7,1	7,05	29,61

Table IIId: The Average of TVB-N value for Sæplast box

VN box		TVB (mg/100g)	Average	STDEV
Day 0	Fish 1	10,5	11,24	1,04
5/1/2006	Fish 2	11,97		
Day 4	Fish 4A	10,5	10,19	0,45
9/1/2006	Fish 4B	9,87		
Day 7	Fish 7A	15,75	13,97	2,52
12/1/2006	Fish 7B	12,18		
Day 10	Fish 10A	15,75	17,35	2,23
15/1/2006	Fish 10B	18,9		
Day 13	Fish 13A	35,07	32,34	3,86
18/1/2006	Fish 13B	29,61		

APPENDIX 4 - MICROBIOLOGY TESTING RESULTS

Table IVa: Cfu count after 48 hours incubation - VN box and Sæplast tub day 0

Day 0 (48h)

5.1.2006

VN box			D1/10	D1/100	D1/1000	D1/10000
	Fish 1	Petri 1	3	0	0	0
PCA		Petri 2	8	0	0	0
30°C	Fish 2	Petri 1	11	0	0	0
		Petri 2	13	0	0	0
	Fish 1	Petri 1	>250	19	0	0
IA (White)		Petri 2	>250	14	1	0
22°C	Fish 2	Petri 1	52	2	1	0
		Petri 2	40	2	0	0
	Fish 1	Petri 1	0	0	0	0
IA (Black)		Petri 2	0	0	0	0
22°C	Fish 2	Petri 1	0	0	0	0
		Petri 2	0	0	0	0

Table IVb: Cfu count after 72 hours incubation - VN box and Sæplast tub day 0

Day 0 (72h)

5.1.2006

VN box			D1/10	D1/100	D1/1000	D1/1000 0
	Fish 1	Petri 1	7	0	0	0
PCA		Petri 2	16	0	0	0
30°C	Fish 2	Petri 1	11	0	0	0
		Petri 2	19	0	0	0
	Fish 1	Petri 1	> 250	30	0	0
IA (White)		Petri 2	> 250	29	1	0
22°C	Fish 2	Petri 1	73	7	1	0
		Petri 2	68	3	0	0
	Fish 1	Petri 1	0	0	0	0
IA (Black)		Petri 2	0	0	0	0
22°C	Fish 2	Petri 1	0	0	0	0
		Petri 2	0	0	0	0

Table IVc: Cfu count after 48 hours incubation - VN box day 1

Day 1 (48h)

6.1.2006

VN box			D1/10	D1/100	D1/1000	D1/10000
	Fish 1A	Petri 1	4	0	0	0
PCA		Petri 2	3	0	0	0
30°C	Fish 1B	Petri 1	0	0	0	0
		Petri 2	0	0	0	0
	Fish 1A	Petri 1	19	0	0	0
IA (White)		Petri 2	22	0	0	0
22°C	Fish 1B	Petri 1	7	0	0	0
		Petri 2	6	0	0	0
	Fish 1A	Petri 1	0	0	0	0
IA (Black)		Petri 2	0	0	0	0
22°C	Fish 1B	Petri 1	0	0	0	0
		Petri 2	0	0	0	0

Table IVd: Cfu count after 72 hours incubation - VN box day 1

Day 1 (72h)

6.1.2006

VN box			D1/10	D1/100	D1/1000	D1/10000
	Fish 1A	Petri 1	10	0	0	0
PCA		Petri 2	5	0	0	0
30°C	Fish 1B	Petri 1	1	0	0	0
		Petri 2	2	0	0	0
	Fish 1A	Petri 1	30	1	0	0
IA (White)		Petri 2	42	2	0	0
22°C	Fish 1B	Petri 1	10	0	0	0
		Petri 2	7	0	0	0
	Fish 1A	Petri 1	0	0	0	0
IA (Black)		Petri 2	0	0	0	0
22°C	Fish 1B	Petri 1	0	0	0	0
		Petri 2	0	0	0	0

Table IVe: Cfu count after 48 hours incubation - VN box day 2

Day 2 (48h)

7.1.2006

VN box			D1/10	D1/100	D1/1000	D1/10000
	Fish 2A	Petri 1	8	0	0	0
PCA		Petri 2	8	0	0	0
30°C	Fish 2B	Petri 1	484	5	13	1
		Petri 2	> 250	169	24	9
	Fish 2A	Petri 1	40	5	1	0
IA (White)		Petri 2	60	0	0	0
22°C	Fish 2B	Petri 1	127	7	0	0
		Petri 2	129	9	0	0
	Fish 2A	Petri 1	0	0	0	0
IA (Black)		Petri 2	5	1	0	0
22°C	Fish 2B	Petri 1	0	0	0	0
		Petri 2	2	0	0	0

Table IVf: Cfu count after 72 hours incubation - VN box day 2

Day 2 (72h)

7.1.2006

VN box			D1/10	D1/100	D1/1000	D1/10000
	Fish 2A	Petri 1	8	0	0	0
PCA		Petri 2	8	0	0	0
30°C	Fish 2B	Petri 1	> 250	5	13	1
		Petri 2	> 250	169	24	9
	Fish 2A	Petri 1	40	5	1	0
IA (White)		Petri 2	60	0	0	0
22°C	Fish 2B	Petri 1	127	7	0	0
		Petri 2	129	9	0	0
	Fish 2A	Petri 1	0	0	0	0
IA (Black)		Petri 2	5	1	0	0
22°C	Fish 2B	Petri 1	0	0	0	0
		Petri 2	2	0	0	0

Table IVg: Cfu count after 48 hours incubation - VN box day 3

Day 3 (48h)

8.1.2006

VN box			D1/10	D1/100	D1/1000	D1/10000
	Fish 3A	Petri 1	> 250	125	16	1
PCA		Petri 2	> 250	112	8	0
30°C	Fish 3B	Petri 1	> 250	118	12	1
		Petri 2	> 250	102	8	0
	Fish 3A	Petri 1	> 250	> 250	25	4
IA (White)		Petri 2	> 250	> 250	44	4
22°C	Fish 3B	Petri 1	> 250	> 250	16	1
		Petri 2	> 250	40	35	2
	Fish 3A	Petri 1	> 250	> 250	27	3
IA (Black)		Petri 2	> 250	> 250	32	3
22°C	Fish 3B	Petri 1	> 250	38	1	2
		Petri 2	> 250	36	2	0

Table IVh: Cfu count after 72 hours incubation - VN box day 3

Day 3 (72h)

8.1.2006

VN box			D1/10	D1/100	D1/1000	D1/10000
	Fish 3A	Petri 1	> 250	246	25	1
PCA		Petri 2	> 250	142	15	1
30°C	Fish 3B	Petri 1	> 250	137	9	1
		Petri 2	> 250	159	13	1
	Fish 3A	Petri 1	> 250	> 250	49	7
IA (White)		Petri 2	> 250	> 250	37	4
22°C	Fish 3B	Petri 1	> 250	> 250	32	6
		Petri 2	> 250	46	19	4
	Fish 3A	Petri 1	> 250	> 250	31	3
IA (Black)		Petri 2	> 250	> 250	36	3
22°C	Fish 3B	Petri 1	> 250	840	1	2
		Petri 2	> 250	36	3	0

Table IVi: Cfu count after 48 hours incubation - Sæplast tub day 4

Day 4 (48h)

9.1.2006

Sæplast box			D1/10	D1/100	D1/1000	D1/10000
	Fish 4A	Petri 1	17	0	0	0
PCA		Petri 2	25	0	0	0
30°C	Fish 4B	Petri 1	17	0	0	0
		Petri 2	19	0	0	0
	Fish 4A	Petri 1	> 250	28	0	0
IA (White)		Petri 2	> 250	28	0	0
22°C	Fish 4B	Petri 1	> 250	22	1	0
		Petri 2	> 250	22	0	0
	Fish 3A	Petri 1	0	0	0	0
IA (Black)		Petri 2	0	0	0	0
22°C	Fish 3B	Petri 1	3	0	0	0
		Petri 2	0	0	0	0

Table IVj: Cfu count after 72 hours incubation - Sæplast tub day 4

Day 4 (72h)

9.1.2006

Sæplast box			D1/10	D1/100	D1/1000	D1/10000
	Fish 4A	Petri 1	30	1	0	0
PCA		Petri 2	39	3	0	0
30°C	Fish 4B	Petri 1	36	1	0	0
		Petri 2	46	4	0	0
	Fish 4A	Petri 1	> 250	40	2	0
IA (White)		Petri 2	> 250	38	5	0
22°C	Fish 4B	Petri 1	> 250	31	2	0
		Petri 2	> 250	32	0	0
	Fish 3A	Petri 1	3	0	0	0
IA (Black)		Petri 2	6	0	0	0
22°C	Fish 3B	Petri 1	6	0	0	0
		Petri 2	2	1	0	0

Table IVk: Cfu count after 48 hours incubation - Sæplast tub day 7

Day 7 (48h)

12.1.2006

Sæplast box			D1/10	D1/100	D1/1000	D1/10000
	Fish 7A	Petri 1	> 250	36	1	0
PCA		Petri 2	> 250	45	0	0
30°C	Fish 7B	Petri 1	> 250	56	1	0
		Petri 2	> 250	43	0	0
	Fish 7A	Petri 1	> 250	243	21	0
IA (White)		Petri 2	> 250	231	32	2
22°C	Fish 7B	Petri 1	> 250	123	5	0
		Petri 2	> 250	156	9	0
	Fish 7A	Petri 1	54	1	0	0
IA (Black)		Petri 2	63	1	0	0
22°C	Fish 7B	Petri 1	15	0	0	0
		Petri 2	19	0	0	0

Table IVl: Cfu count after 72 hours incubation - Sæplast tub day 7

Day 7 (72h)

12.1.2006

Sæplast box			D1/10	D1/100	D1/1000	D1/10000
	Fish 7A	Petri 1	> 250	48	2	0
PCA		Petri 2	> 250	53	2	0
30°C	Fish 7B	Petri 1	> 250	67	4	1
		Petri 2	> 250	64	4	0
	Fish 7A	Petri 1	> 250	> 250	27	2
IA (White)		Petri 2	> 250	> 250	38	4
22°C	Fish 7B	Petri 1	> 250	178	12	1
		Petri 2	> 250	162	13	0
	Fish 7A	Petri 1	64	4	0	0
IA (Black)		Petri 2	71	3	0	0
22°C	Fish 7B	Petri 1	27	1	0	0
		Petri 2	29	1	0	0

Table IVm: Cfu count after 48 hours incubation - Sæplast tub day 10

Day 10 (48h)
15.1.2006

Sæplast box			D1/10	D1/100	D1/1000	D1/10000
	Fish 10A	Petri 1	> 250	> 250	> 250	20
PCA		Petri 2	> 250	> 250	> 250	19
30°C	Fish 10B	Petri 1	> 250	> 250	> 250	98
		Petri 2	> 250	> 250	> 250	75
	Fish 10A	Petri 1	> 250	> 250	> 250	> 250
IA (White)		Petri 2	> 250	> 250	> 250	32
22°C	Fish 10B	Petri 1	> 250	> 250	> 250	> 250
		Petri 2	> 250	> 250	> 250	> 250
	Fish 10A	Petri 1	> 250	5	3	0
IA (Black)		Petri 2	> 250	15	1	0
22°C	Fish 10B	Petri 1	> 250	> 250	7	0
		Petri 2	> 250	> 250	15	1

Table IVn: Cfu count after 72 hours incubation - Sæplast tub day 10

Day 10 (72h)
15.1.2006

Sæplast box			D1/10	D1/100	D1/1000	D1/10000
	Fish 10A	Petri 1	> 250	> 250	664	23
PCA		Petri 2	> 250	> 250	728	22
30°C	Fish 10B	Petri 1	> 250	> 250	> 250	116
		Petri 2	> 250	> 250	> 250	82
	Fish 10A	Petri 1	> 250	> 250	> 250	624
IA (White)		Petri 2	> 250	> 250	> 250	572
22°C	Fish 10B	Petri 1	> 250	> 250	> 250	164
		Petri 2	> 250	> 250	> 250	153
	Fish 10A	Petri 1	> 250	5	3	0
IA (Black)		Petri 2	> 250	16	1	0
22°C	Fish 10B	Petri 1	> 250	> 250	8	0
		Petri 2	> 250	> 250	20	1

Table IVo: Cfu count after 48 hours incubation - Sæplast tub day 13

Day 13 (48h)
18.1.2006

Sæplast box			D1/10	D1/100	D1/1000	D1/10000
	Fish 13A	Petri 1	> 250	> 250	> 250	66
PCA		Petri 2	> 250	> 250	> 250	64
30°C	Fish 13B	Petri 1	> 250	> 250	> 250	> 250
		Petri 2	> 250	> 250	> 250	> 250
	Fish 13A	Petri 1	> 250	> 250	> 250	> 250
IA (White)		Petri 2	> 250	> 250	> 250	> 250
22°C	Fish 13B	Petri 1	> 250	> 250	> 250	125
		Petri 2	> 250	> 250	> 250	142
	Fish 13A	Petri 1	> 250	> 250	> 250	11
IA (Black)		Petri 2	> 250	> 250	> 250	10
22°C	Fish 13B	Petri 1	> 250	> 250	> 250	18
		Petri 2	> 250	> 250	> 250	15

Table IVp: Cfu count after 72 hours incubation - Sæplast tub day 13

Day 13 (72h)
18.1.2006

Sæplast box			D1/10	D1/100	D1/1000	D1/10000
	Fish 13A	Petri 1	> 250	> 250	> 250	82
PCA		Petri 2	> 250	> 250	> 250	76
30°C	Fish 13B	Petri 1	> 250	> 250	> 250	540
		Petri 2	> 250	> 250	> 250	432
	Fish 13A	Petri 1	> 250	> 250	> 250	956
IA (White)		Petri 2	> 250	> 250	> 250	39
22°C	Fish 13B	Petri 1	> 250	> 250	> 250	876
		Petri 2	> 250	> 250	> 250	744
	Fish 13A	Petri 1	> 250	> 250	> 250	14
IA (Black)		Petri 2	> 250	> 250	> 250	10
22°C	Fish 13B	Petri 1	> 250	> 250	> 250	22
		Petri 2	> 250	> 250	> 250	20

APPENDIX 5 - RESULTS OF ICE MELTING AMOUNT BY TIME FOR SOME TYPES OF BOX/TUB

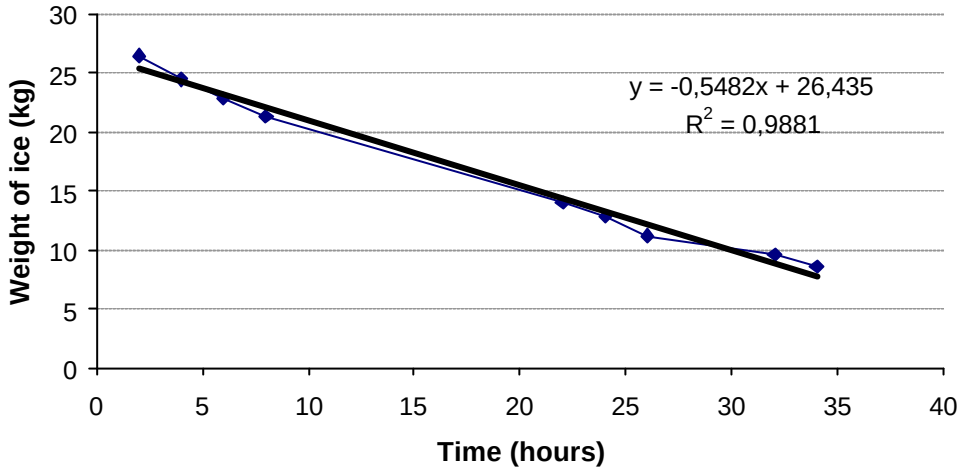

Figure Va: Weight of ice in container by time for Vietnamese-like box

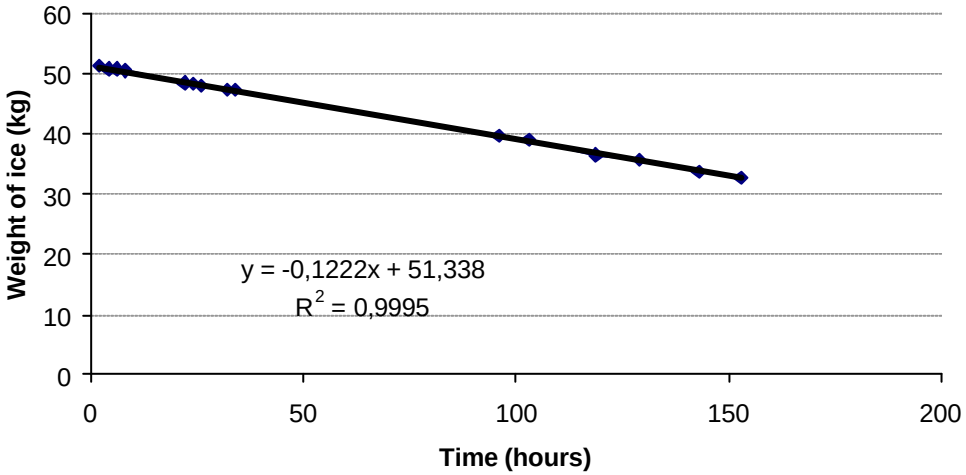

Figure Vb: Weight of ice in container by time for Sæplast box with the lid

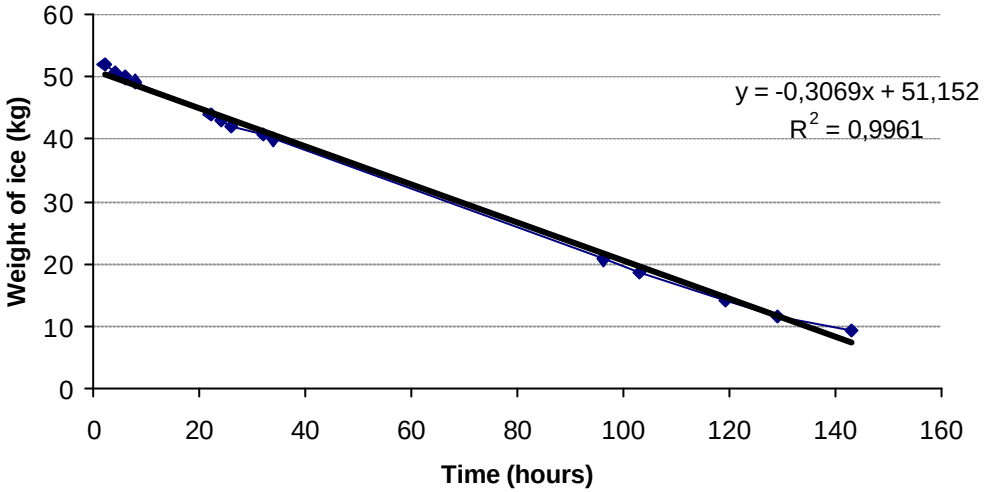

Figure Vc: Weight of ice in container by time for Sæplast box without the lid

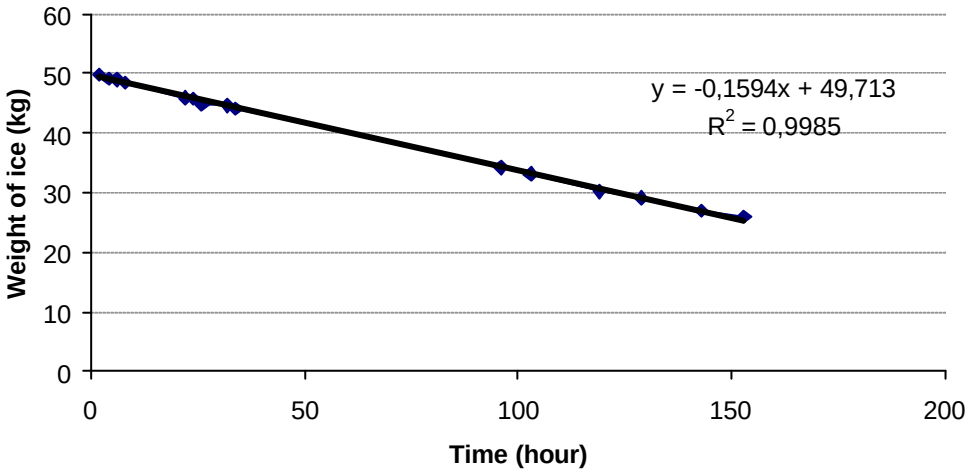

Figure Vd: Weight of ice in container by time for Sæplast cooler with the seal on the lid

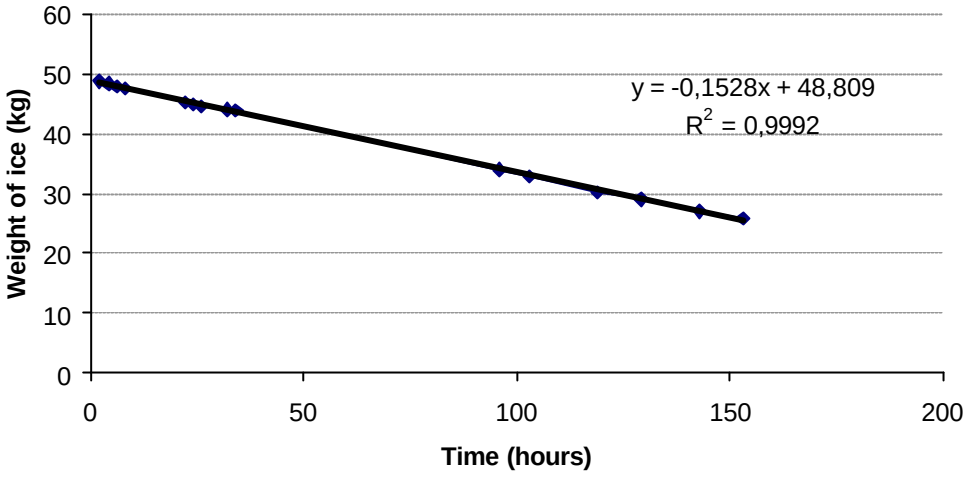

Figure Ve: Weight of ice in container by time for Sæplast cooler without the seal on the lid