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EFFECT OF DIFFERENT RATIOS OF ICE ON QUALITY AND SHELF LIFE OF COLD WATER SHRIMP (*Pandalus borealis)*

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ABSTRACT

This study was conducted to investigate the effect of different ratios of ice on quality changes and shelf life of cold water shrimp (*Pandalus borealis*). The samples were evaluated by sensory assessment, chemical analysis and bacteriological test. The samples of four groups without ice, ice and shrimp ratio 1:1, ice and shrimp ratio 1:3, and ice and shrimp ratio 1:5 were stored in a cooler at +0.5°C for seven days in styrofoam boxes. The temperature of the samples were recorded by temperature loggers. The evaluation of the samples were done on 0, 3, 5 and 7 days of storage. All the quality parameters TVC, TVB-N, QI score gradually increased with progress of storage days except pH. The pH value of all the sample groups slightly decreased on 7th storage day. The results of all the sample groups showed that there were no significance ($p<0.05$) difference between the quality parameters but they had a strong correlation between TVB-N, TVC, QI scores and H_2S producing bacteria with over storage days. All the experimental results suggested that the shelf life of the sample without ice was 0 day and the sample of ice and shrimp ratio 1:5 was 3 days. The shelf life of ice and shrimp ratio 1:1 and ice shrimp ratio 1:3 samples were 5 days. Finally the result revealed that the ice extends the shelf life of shrimp and had a direct effect on quality changes. The study concluded that the Ice and shrimp ratio of 1:1 and 1:3 were the best ratio for shrimp storage to maintain quality and extend of shelf life.

Keywords: shelf life, quality, shrimp (*Pandalus borealis*), chilled temperature, storage day.

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LIST OF ABBREVIATION

ADP	Adenosine Triphosphate
AMP	Adenosine Monophosphate
AOAC	Association of Official Agricultural Chemists
ATP	Adenosine Triphosphate
DMA	Dimethyl Amine
DoF	Department of Fisheries
FAO	Food and Agricultural Organization
GDP	Gross Domestic Product
IMP	Inosine Monophosphate
ISO	International Standard Organization
MoFA	Ministry of Fisheries and Agriculture
MRI	Marine Research Institute
PUFA	Poly Unsaturated fatty Acid
QIM	Quality Index Method
SPC	Standard Plate Count
TBV-N	Total Volatile Basic – Nitrogen
TMA	Trimethylamine
TMAO	Trimethyl Aminoxide
TVC	Total Viable Count

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1 INTRODUCTION

1.1 Background

Fisheries play an important role for supplying animal protein, creating employment opportunity and earning of foreign currency in Bangladesh. After independence in 1971, the aquaculture sector achieved remarkable progress. Total fish production of the country was 3.4 million tonnes in 2013 whereas in 1971 total fish production was 0.75 million tonnes. Fisheries sector contributes to 4.37% to the national GDP and 23.37% to the agricultural GDP. Fisheries sector performs highest GDP growth rate from the last 10 years in comparison to other agricultural sectors. Average GDP growth rate of this sector was 5.88% in 2013. The country's exports earnings from fisheries sectors was 535 million USD exporting more than 84 thousand tonnes fish and fishery products that was contributed about 2.01% of total foreign income in 2013. The fish and fishery products that were exported from the country for export earning, shrimp alone contributed 76% of total income in 2013. Per capita fish consumption is 19 kg per year and fisheries sectors provides 60% of the animal protein. More than 11% of the total population is directly or indirectly involved in this sector for their livelihoods (DoF 2014).

The fisheries resources of Bangladesh are highly diverse and is divided in to two sectors, capture fisheries and aquaculture. Capture fisheries is further divided in to inland capture fisheries and marine capture fisheries. The aquaculture is further divided into fresh water aquaculture and brackish water aquaculture. The brackish water is used for shrimp culture. Due to overfishing of post larva, absence of fisheries management and conservation measures, implementation of flood control and drainage projects, shrimp production in the open water, particularly in the rivers and estuaries, has declined significantly during the last three decades (DoF 2014).

Shrimp is an export oriented sector of Bangladesh. There are 2.75 million hectors area of fish farms in Bangladesh that are mostly located in south and south west coastal region of the country. According to DoF (2014), "shrimp production was 0.23 million tonnes from farm and 0.05 million tonnes were collected from sea by industrial and artisanal trawl. 95% of the culture shrimp that are produced from the shrimp farms are exported in the foreign countries". But Bangladesh has faced some problems on the issue of quality and food safety from the importers.

Most of the farmers never use ice for preserving their shrimp. Some big farmers preserve their shrimp by using little amount of ice for overnight. Most of them sell the shrimp to the near depot owners. Ice is not generally used at small depots. They collect the shrimp daily for all the day and sell it to the company or big depot owners at afternoon or evening. The shrimps that are sold in the auction market are- mainly collected by whole sellers or the company agents. They buy the shrimp from the market and preserve it on the concrete floor or in big bamboo basket by crushing block ice. The company agents take more time for transportation of shrimp to the company. The time mainly depends on distance and communication. During transportation 23% use traditional bamboo baskets, 2.5% use insulated Styrofoam boxes and rest of them use plastic baskets and drums (Alam 2010).

Ice is a major element of Bangladesh for preserving fish for a short time. Most of the ice factories are located beside the highway road or in the town, where fish markets are available. There is no ice factory in local area. About 88% of fisherman, 77% fish of farmer, 27% of retailer and 47% of fish vendors do not use ice to keep the shrimp (Alam 2010). Among the wholesalers or transporters, 12% use ice-fish ratio of 1:1 and 44% use a ratio of 1:2. Most of

wholesalers, retailers and vendors use ice-fish ratio of 1:3 to 1:5 that is very negligible. In case of depots holders 80% use a shrimp to ice ratio of 1:1 and 10% of the depot holders or transporters use a shrimp to ice ratio of 5:1. Most of the depot holders use bamboo baskets and plastic drums with crushing block ices (Alam 2010).

1.2 Rationale of the study

Cold water shrimp *(Pandalus borealis)* is a species of caridean shrimp found in cold parts of the Atlantic and Pacific Oceans at the temperature of 2-14°C. They live at the bottom of the sea at the depth of 50-700m usually at muddy bottom. They grow very slowly. The FAO (2012) refers to them as the northern prawn. Other common names of cold water shrimp are pink shrimp, deep water prawn, deep-sea prawn, great northern prawn and northern shrimp. They are mainly distributed at New England, Canada, Newfoundland, Labrador, Greenland, Iceland, Svalbard, Norway, and North Sea.

Shrimp is a highly perishable product and its shelf life is greatly influenced by enzymatic action and microbial changes. Due to small size, non-protein nitrogenous compound on shell and present of feed in gut, its post-mortem autolytic changes occur faster and spoiled the flesh rapidly (Shamshad *et al*. 1990). A lot of microbes are present on the external surface and in the gut of the shrimp. After death of the shrimp the micro-organisms and enzymes diffuse in to the flesh and react with the substance of the flesh (Lee and Um 1995). For extending the shelf life, the shrimp are stored in ice on board during fishing. Ice and temperature has a great effect on quality and shelf life of cold water shrimp. So a comprehensive study is needed to identify the shelf life and quality of shrimp at chilled temperature with different ratio of ice at different storage days.

1.3 Objectives of the study

The objectives of the study were to:

(1) Find out an efficient ratio of ice and shrimp for shrimp preservation.
(2) Find out the effect of chilling temperature on shelf life of shrimp.
(3) Determine the effect of ice on quality parameters and shelf life of shrimp.

2 LITERATURE REVIEW

2.1 Shrimp spoilage

Spoilage can be defined as an undesirable change in foods due to a reason of oxygen, moisture, light, microbial growth and temperature. Spoilage can be detected by smell, taste, touch or sight. Fish goes under spoilage due to combined effects of microbes, chemical and enzymatic action (Huis and Veld 1996) that is same in case of shrimp. According to Hayes (1985), “Spoilage can be defined as a change in fish or fish products that are unsuitable and unsafe for human consumption”.

Sea foods are highly perishable due to high moisture content, available of nutrients for growth of microorganisms and low tolerance of temperature. Spoilage can be easily identified by observing the change of physical characteristics. Changes in colour, odour, texture, colour of eyes, and colour of gills and softness of the muscle are some of the characteristics observed in

spoiled fish (Prabjiet *et al*. 1991). Colour, texture, odour and softness also indicate the quality of shrimp.

2.1.1 Microbial Spoilage

Bacteria are the main cause for the spoilage of sea foods (Shewan 1992). When shrimps die, they are primarily contaminated with a wide variety of microorganisms. These organisms grow rapidly in the food to a higher numbers. The spoilage of seafood has occurred because of these type of specific microbial community (Gram *et al*. 1996). Bacterial metabolism produces off-odours and off-flavours and these are common result of spoilage. There are few microbial flora participates in the spoilage (Castell and Anderson 1948). In an anaerobic condition, specific spoilage bacteria (*Shewunellu putrefuciens, Photobacterium phosphoreum, Vibrionaceae*) produce off-odours and off-flavours by using Trimethyl Amino Oxide (TMAO) due to formation of Trimethyl Amine (TMA) (Gram *et al*. 1987).

Pseudomonos sp are the main spoilage bacteria that are available on fish and shrimp and perform same activities as spoiler. According to Gram *et al*., (1990) "tropical fresh water fish that are preserved in ice, spoiled only by *pseudomonas sp"*. *Pseudomonos sp* and *P. putrefaciens* also are spoiler of marine tropical species stored in ice (Gram 1992). *S. putrefaciens* cannot play important role for the spoilage of ice fresh water species in tropical waters due to occurrence of very low numbers and the inability of the organism to compete with high numbers of antagonistic pseudomonads (Gram 1993).

2.1.2 Chemical spoilage

Generally, shrimps contain protein, fat, mineral, carbohydrate and high content of free amino acid. Swant (2012) reported that most of the marine fish species contain trimethylamine oxide (TMAO). Specific spoilage bacteria produce ammonia, biogenic amines, organic acids, and sulphur compounds from acids, hypoxanthine from ATP and acetate from lactate. Spoilage organisms produce off odour volatile base compounds from nitrogen compounds. Fat oxidation is a common chemical action in fatty species and contain a high level of polyunsaturated fatty acids (PUFA) and enhance oxidative changes (Swant 2012).

Chemical spoilage takes place in protein, carbohydrate and fat of the muscle but the most important chemical spoilage processes takes place in the lipid fraction by auto-oxidation process. The first step of the oxidation process leads to the production of hydro-peroxides, which are tasteless but can cause brown and yellow discolouration of the tissue. The degradation of hydro-peroxides gives rise to the formation of aldehydes and ketones. These compounds have a strong rancid flavour. Factors such as heat, light, and several organic and inorganic substances like copper or iron, can initiate and accelerate oxidation (Huss 1994).

2.1.3 Autolytic spoilage and fat oxidation

Enzymes are the main cause of autolytic spoilage. Higher enzymatic activities increase autolytic spoilage due to ionic strength. Icing and freezing reduce the autolytic and enzymatic activities. But freezing damages cell membrane because water freezes out of the mussel that improved access of enzymes to substrates. This increases drip, protein denaturation and oxidation and affect texture, juiciness, flavour and odour of the products (Huss 1995).

All aquatic species have a natural defence mechanism in the living condition, for this reason enzymes and bacteria never cause any deteriorative changes. When shrimp harvest then enzymes involve in autolytic changes. The bacteria that are available on skin and gill invade in the muscle and flesh loss its quality and freshness. According to Swant (2012) "the fish that have high food intake, contain a large amount of digestive enzyme in digestive tract, degrade quickly and spoil easily". In the autolytic process Adenosine triphosphate (ATP) degrades to adenosine diphosphate (ADP), adenosine monophosphate (AMP), inosine monophosphate (IMP), Inosine (Ino) and hypoxanthine (Hx), that are associated with bitter flavours . Hypoxanthine (Hx) has a characterized by undesirable, bitter flavour, that is an indication of spoilage product under chilled storage (Huss 1995).

The rapid post mortem change enhances lipid oxidation and muscle becomes very soft. The rancid flavour, off odours and discolouration grows up due to fat oxidation. Fat oxidation produces hydro-peroxide, which further degrades into aldehyde and ketones with a typical rancid flavour (Swant 2012).

2.2 The relationship between spoilage and temperature

The spoilage rate of shrimp mainly affected by temperature, autolytic reaction, bacterial activities and fat oxidation but temperature has great effect than all other spoilage factors. Higher temperature increases the higher rate of spoilage. Temperature helps to increase the bacterial activities and autolytic reaction that enhance the spoilage. Bacteria grow with certain range of temperature. For this reason psychotropic gram negative, rod-shaped bacteria such as *Pseudomonos, Moraxellu, Acinetobacter, Shewanellu, Fluvobucterium, Vibrionuceue* and *Aeroemonaduceue* grow dominantly of temperate water fish. The bacteria on tropical fish carries higher load of Gram-positive and enteric bacteria due to high temperature (Liston 1980). At chilling temperature *Shewanella putrefaciens, Photobacterium phosphoreum, Aeromonas spp.* and *Pseudomonas spp*. cause spoilage of seafood. However, at high storage temperatures (15º-30°C), different species of Vibrionaceae, Enterobacteriaceae and Gram-positive organisms are responsible for spoilage (Gram *et al*. 1987).

Different types of cooling agents and packaging methods like dry ice and ice packs are using to minimize effects of temperature fluctuations for transportation of sea foods by cargo (Terchunian *et al*., 1990). The marine temperate water seafood produces off-odours and flavours like rotten eggs whereas tropical and fresh water species produces fruity, sulfhydryl off-odours and flavours (Santos 1978).

2.3 Shelf life of shrimp

Shelf life for fish and fishery products is very important for processing and marketing of the products. Gutting, bleeding, transport, handling and storage condition affects the shelf life of fish but handling and storage conditions are the most important factors that affect the shelf life of fish and fishery products (Doyle 1995).

Ice is preferred cooling agent for preserving of fish on board. Rapid cooling and maintain of low temperature is essential for sea food to obtain desire shelf life. Temperature fluctuation has a considerable effect on quality and shelf life during processing, storage and transportation (Bao *et al*. 2007). If fish are left without ice for a few hours, their shelf life will decrease sharply compare to the fish that are preserve in ice (Jensen and Hansan 1973).

The shelf life of shrimp (*Peneous monodon*) which was collected from the farm and stored in ice had the acceptable condition up to seven days. The samples which were collected from the depots and stored in ice had the acceptable condition for four days (Haider *et al*. 2011). The estimated shelf life of deep water shrimp (*Pandalus borealis*) was 6 days (Martinsdottir *et al*. 2001). The shelf life of shrimp (*Peneous merguiensis)* which were harvested in Singapore and stored in ice was four days (Yamagata and Low 1995).

2.4 Methods of measuring spoilage

Different types of analyses such as sensory, chemical and bacteriological analysis have been developed to measure the loss of fish freshness and detection of spoilage. Measurement of these compounds provide an idea about the progress of deterioration (Connel 1975). The evaluation methods that are commonly used for assessment of spoilage can be classified into sensory methods, microbiological methods, biochemical methods and physical methods (Huss 1995).

2.4.1 Sensory evaluation

Sensory evaluation is an important and effective assessment method in sea food industry to assess freshness and quality deterioration. Sensory evaluation can be easily adopted at any place without any equipment in fish processing such as landing centre, fish market, fish plant, at the reception, or processing halls of the fish factories (Martinsdottir *et al*. 2001).Products price depend on its freshness and quality. The fish that are harvested in the sea are brought on shore at designated sites for sale and are graded on different price groups based on the freshness using sensory analysis (Chebet 2010). A well-equipped laboratory is needed for practicing the chemical and microbiological test for assessment of quality and freshness. According to Nielsen (2002) "Quality Index Methods (QIM) are easier to use compare to other some sensory methods like freshness scale and threshold methods". No equipment is needed in this method rather than human senses. On the other side limited training is needed to determine the accurate results because all the quality parameters are well defined. With the QIM, it is easily possible to give more detailed information of the sensory quality. The processors can use the QIMs to estimate the shelf-life and plan of the production more efficiently. QIM is easy to teach and easy to understand for the inexperienced people to evaluate the fish. Quality Index increased linearly with the storage time on the ice (Martinsdottir *et al*. 2001). QIMs have a good advantage over other sensory methods for purchasing fish from electric auction markets. The quality indexes can also be used in the traceability, because such data can be applied in the supply chain of the products to check the accuracy (Nielsen 2002).

2.4.2 Chemical evaluation

Chemical spoilage of can be measured by assessing trimethylamine (TMA), total volatile bases (TVB) and hypoxanthine contents of the flesh. TMA is produced during spoilage by bacterial breakdown of trimethylamine oxide (TMAO) that is naturally found in seafood (Pedraso and Regenstein 1990). According to Howgate (1982) "TMA level is all time lower at the early stage of spoilage. It is not consider for those species that are stored less than 6 days in the ice". Now a days TVB-N is only measured for detection of spoilage because TVB-N content is an alternative measuring of TMA including ammonia, dimethylamine (DMA) and TMA. Hypoxanthine can be formed by bacteria and autolytic decomposition of nucleotides but the bacterial formation is higher than autolytic action. Total volatile base nitrogen (TVB-N), biogenic amines, trimethylamine (TMA) and dimethylamine (DMA) are universally applicable (Gill 1990). Measurement of Total volatile basic amines (TVB) is one of the quickest and most

widely used tools in assessing the quality of seafood. TVB-N includes the measurement of trimethylamine (produced by spoilage bacteria), dimethylamine (produced by autolytic enzymes during frozen storage), ammonia (produced by the deamination of amino-acids and nucleotide catabolites) and other volatile basic nitrogenous compounds associated with seafood spoilage (Huss 1995).

2.4.3 Microbiological evaluation

Spoilage and pathogenic microorganisms are main problem for hygienic quality. Temperature and high nutrient content of fish assist them to grow on muscle. These microbes can easily detect and measure by using total viable count (TVC). TVC and counts of H_2S-producing bacteria can be used as one of the method of assessing fish freshness as well as to measure shelf life. According to Vyncke (1996) "the method is quick and should be used routinely". Total Viable Counts (TVC) or Standard Plate Count (SPC) methods are mainly measure colony forming unit (cfu/g) in a products. All the samples are placed in specific incubation condition for a definite temperature that are optimize temperature for culturing of the microorganisms for a definite time. The temperature has a great influence on the plate for colony developing in the sample. Generally, 25°C and 37°C are recommended for psychrophilic bacteria for 3-4 days incubation period (Huss 1995).

3 MATERIALS AND METHODS

3.1 Raw material and experimental design

3.1.1 Shrimp

Cold water whole shrimp was collected from Kampi, Isafjordur, Iceland on 23rd January 2015. The shrimp was received in Matis at 11:20 by a truck transport, two days after harvesting. During transportation the sample was preserved with flake ice. When the shrimp was received, the ice was melted and the temperature of the sample was 4°C. The temperature of the sample was recorded by an automatic thermometer type TFX410 (Ebro Electronic, Ingolstadt, Germany). The shrimp was stored in styrofoam boxes and the size of the boxes were 38×25×14 cm. The styrofoam boxes were collected from the company Promens Dalvik /Tempera, Iceland.

3.1.2 Temperature Measurements

The shrimp was immersed in flake ice and all the boxes were stored in a cooler in Matis laboratory with a chilling temperature of 0.5°C. Sixteen thermometer loggers were used for the temperature measurement in the boxes. Two thermometer loggers were used for each box for measuring the product temperature. The temperature reading was taken from the loggers at the end of the experiment. The temperature of the samples was recorded at 10 minutes interval.

3.1.3 Experimental groups and sampling

The shrimp was randomly divided into four groups in eight styrofoam boxes. The shrimp and the flake ice was measured by electric balance (Rp 00845, Kristinsson Hf, Langagerdi 7, Reykjavik) at the laboratory. Each box contained 2kg of shrimp. The shrimp was arranged in the Styrofoam box by adding one layer of ice and one layer of shrimp. A thin layer (1 cm.) of ice was kept in the bottom of every box. Then the Styrofoam box was filled up by using 2kg,

0.667 kg and 0.40 kg of ice. One group of sample was stored without ice. The flake ice was collected from the laboratory that was made from portable water by using an automatic machine (Scotsman, Kaeltaekni, Raudagerdi 25, 105, Reykjavik, Iceland). Temperature of the sample and cold chamber was monitored. The storage was done for 7 days. The duplicate samples were submitted for microbiological, chemical, and sensory analysis on 0, 3, 5, and 7 of storage days. All the samples were stored in the cooler at +5°C. The experimental groups and sampling plan are outlined in Table 1.

Table 1: Experimental groups and sampling plans.

Experimental groups	Ratio of Ice and shrimp	Sampling Days				Code
		0	**3**	**5**	**7**	
Shrimp without Ice (WI)	-	1	1	1	1	WI (Shrimp without Ice)
Ice: shrimp (SIE)	1:1		1	1	1	SIE (Ice and shrimp ratio Equal)
Ice: shrimp (SIM)	1:3		1	1	1	SIM (Ice and shrimp ratio Medium)
Ice: shrimp (SIL)	1:5		1	1	1	SIL (Ice and shrimp ratio Low)
Total		1	4	4	4	

3.2 Sensory evaluation

A Quality Index Method (QIM) (Table 2) was used to evaluate the quality of whole shrimp. One sample from each Styrofoam box was taken on days 0, 3, 5, and 7 days. The samples were kept in room temperature for 20 minutes before assessment. Three samples from each group were taken but the panellists had no information about the samples which were taken from same groups. The samples were placed in plastic plates then coded with random three digit numbers and randomly arranged on a table in sensory laboratory. The sample sizes were 10 shrimps per sample. Five panellists who had knowledge about the quality of shrimp and the characteristic of sensory attributes were selected for sensory evaluation.

Table 2: Quality Index method for deep water shrimp (Martinsdottir *et al*., 2001).

Quality parameter		Description	Score
Whole shrimp	Dark in the head	None	0
		Some (25%)	1
		Many (50-75%)	2
		All (75-100%)	3
	Colour	Pink/Red	0
		Pale pink	1
		Yellowish	2
		Yellow, green, grey wish, discoloration	3
	Odour	Fresh, Sea weedy	0
		Faint odour, reminds of tar	1
		Faint Amonia odour	2
		Obvious ammonia odour, sour, putrid	3
Roe	Roe colour	Copper green	0
		Discoloured faded	1
		Dark	2
Quality Index			0-11

3.3 Chemical evaluation

3.3.1 Protein measurement

Protein content of shrimp was determined by Kjeldhal method ISO 5983 (1997). Two grams of whole shrimp was digested in 17.5 ml. concentrated sulphuric acid solution. A copper catalyst (1000 kjeltabs Cu 3.5) was used to enhance the reaction. An excess sodium hydroxide was added to the cold digest to liberate ammonia. The solution was placed in steam distillation unit. Then the solution was titrated with hydrochloric acid solution to colour metric end point. The nitrogen content was calculated from the amount of ammonia produced. The crude protein content is obtained by multiplying the result by the conventional factor 6.25.

3.3.2 Ash measurement

Ash content was determined according to method described by AOAC (1990) Official method 942.0. Two grams of whole shrimp was taken in a crucible and it was burned in Muffle furnace at 550°C temperature for four hours. Water and other volatile materials were vaporized and organic substances were burned in the presence of oxygen in air. Then the sample was turned into white and free of carbon. The weight of the residue then was calculated.

3.3.3 Fat measurement

Fat content of shrimp sample was determined by the method of AOAC (1997) official Method Ba-3-98. The test portion of 5 g of sample was wrapped in a filter paper and 80 ml. of petroleum ether was added with the sample. The wrapped sample was placed in a Butt extraction tube. The sample was heated in an electric hot plate and the extraction was collected in a beaker. Then the petroleum ether from the extraction was evaporated on a steam bath. The extracted was weighted and the percentage of fat content was calculated.

3.3.4 Moisture measurement

Water content of the sample was determined according to the method of ISO 6496 (1990). The test portion of 5 g of sample was taken in a dish containing with dried silica. The sample was mixed properly with silica and it was heated in an oven at 103°±2°C for 4 hours to get a constant weight. Then the sample was placed in a desiccator to cool. After cooling the sample was weighed again and the moister content of the sample was calculated.

3.3.5 TVB-N measurement

Total Volatile Basic Nitrogen (TVB-N), was determined by using steam distillation Kjeldhal method that was described by Malle and Tao (1987). 50 g of whole shrimp sample was taken in a warring commercial blender (Rp 00612, Kristinsson hf, Langagerdi-7, Reykjavik) and then 100 ml. of 7.5% aqueous trichloroacetic acid solution was added. The mixture was homogenized in a laboratory homogenizer for one minute and then filtered through a Whatman no. 3 filter paper number. The filtrate of 25 ml. was transferred into a distillation flask followed by addition of 6 ml. 10% aqueous NaOH solution. Steam distillation was performed using a vertical distillation unit (Struer TVN Distillery). TVB-N was collected under a condenser in a beaker containing solution of 10 ml of 4% boric acid and 0.04 ml. of methyl red and bromocreol green indicators. Steam distillation took place for 4 minutes until final volume of 50 ml. was

obtained in the beaker. The alkalised solution was then titrated with aqueous 0.0324 sulphuric acid solution. The TVB-N content was calculated and expressed in mgN/100g.

3.3.6 pH measurement

The pH of the sample was determined by using a pH meter (Thermo Scientific, Orion star A111 Bench and star A121 portable pH meter, Germany). 100 g of the shrimp sample was minced in a laboratory blender and the glass electrode was inserted in the minced sample for about 1 minute. The pH meter was calibrated before measuring the pH of the sample by using the pH 4.0 and 7.0 buffer solution.

3.4 Bacteriological test

Total Viable Counts (TVC) and counts of H_2S-producing bacteria were performed on Iron Agar by the spread plate method modified from Gram *et al*. (1987). Twenty grams of shrimp sample was taken and minced in a blender (warring commercial laboratory blender, USA). The mixer was then diluted with 180 ml cooled maximum recovery diluent (mrd, oxoidi, UK) and homogenized in a stomacher bag for one minute. Serial 10-fold dilutions were performed for the 9 ml cooled MRD that was prepared before. After completing the 10-fold method, the solution of the iron agar plate was spread properly over the iron agar of the plate and then the plates were incubated at 17˚C for 5 days. The spoilage bacteria were formed black colonies on this medium. Total number of colonies were counted by colony counter (T-171, Labe line digital colony counter no. 1586,) and calculated the total viable bacteria and H_2S producing bacteria by cfu/g.

3.5 Data analysis

Microsoft excel programme was used for data analysis. The data of sensory score and other quality parameters were tested by using analysis of variance (ANOVA) to analyse if a difference existed within a group and among groups during the storage time. Linear equation and the correlation coefficients (R) of some indicators such as total volatile bases nitrogen (TVB-N), Total Viable Counts (TVC), and pH were calculated. P value <0.05 was considered as significant. The microbial results of the samples were converted to log value.

4 RESULTS

4.1 Temperature profile

The ambient temperature of eight Styrofoam boxes for four sample groups were measured by using sixteen temperature loggers. The average of each sample group for each box was calculated that are shown in Figure 1. When the samples were stored in the cooler, the temperature of WI (Without ice) sample was decreased very slowly but the temperature of other samples SIE (Ice and shrimp ratio 1:1), SIM (Ice and shrimp ratio 1:3) and SIL (Ice and shrimp ratio 1:5) were decreased quickly due to ice. The WI sample reached at 0°C after 16 hours whereas SIE, SIM and SIL sample reached at 0°C within 2 hours. The ambient temperature of WI sample was around 0°C, SIL sample was –0.5°C, SIE and SIM samples were -1°C. The maximum, average and minimum temperature of different sample groups are shown in the Table 3.

Table 3: Ambient temperature of different sample groups of Styrofoam boxes.

Sl.	*Sample groups*	*Max. Temperature (°C)*	*Average Temperature(°C.±SD)*	*Min. Temperature (°C)*
1	WI	2.33	0.05±0.51	-0.85
2	SIE	0.30	-0.70± 0.22	-1.01
3	SIM	0.53	-0.77±0.18	-1.0
4	SIL	1.93	-0.59±0.27	-.0.91

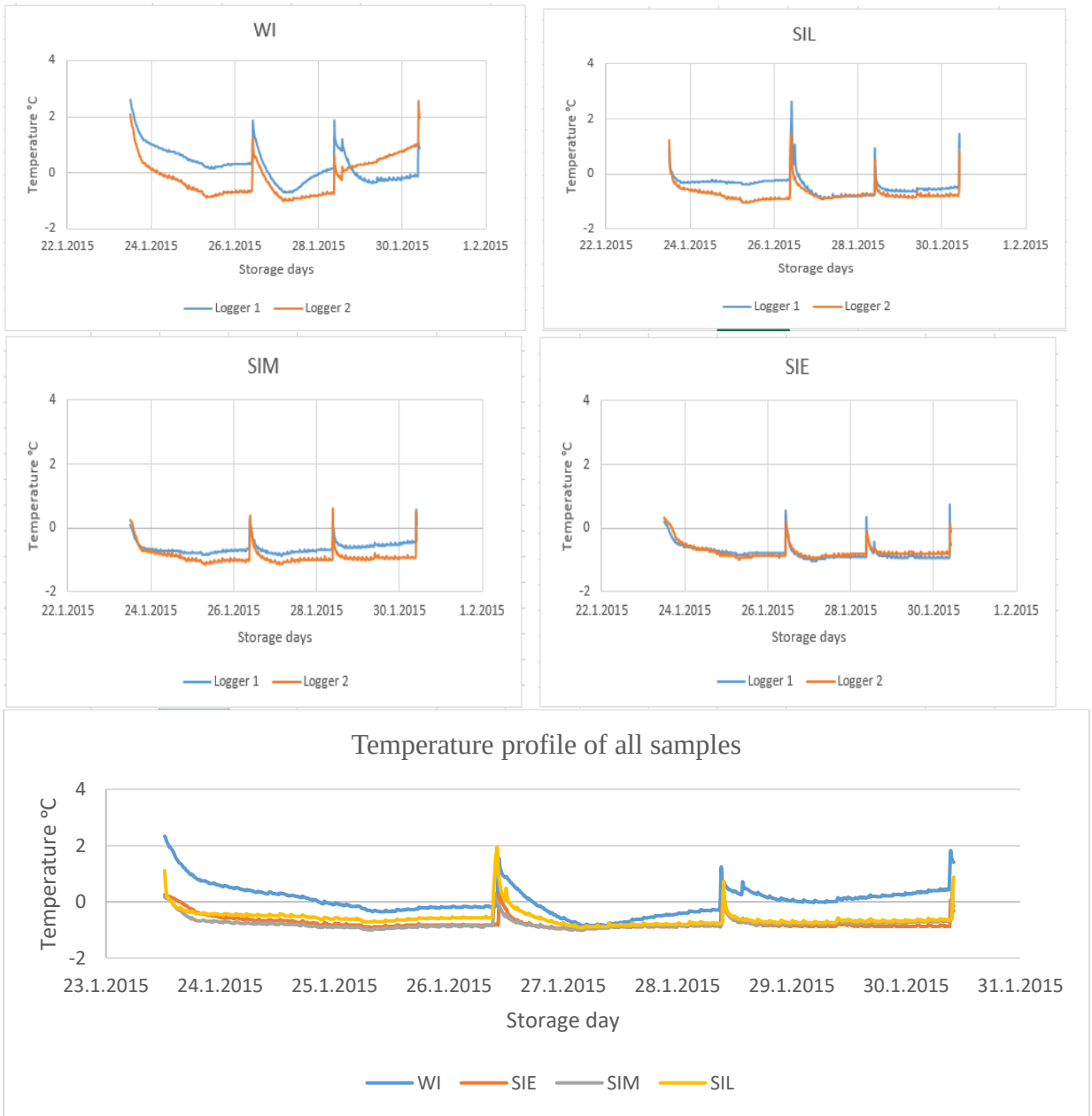

Figure 1: Temperature fluctuation of the sample groups (WI, SIE, SIM, and SIL) over storage days. Data shown is a mean value of two temperature data loggers positioned in two Styrofoam boxes (mean temperature fluctuation of four sample groups).

4.2 Basic Characteristics of the sample

After arrival of the shrimp in laboratory, the chemical composition, length and size of the shrimps were measured. In case of chemical composition, the protein content was 15%, fat content was 1.8%, ash content was 4.7% and moisture content was 78.4%. Additionally total volatile base nitrogen (TVB-N) and pH also determined and the results were 14.97±0.38 mgN/100 g and 7.5 respectively. The mean length of shrimp was 8.95±0.57 cm and mean weight of the shrimp was 4.3±0.91 g.

4.3 Sensory evaluation

After arrival of the sample, most of the panellists evaluated that 25% of the shrimps were dark in head, colour of the shrimps was pale pink, odour of the shrimps were faint odour and roe colour of the shrimps were discoloured faded. The average Quality Index from the assessment of the whole shrimps (Figure 2) indicated that the shrimp quality decreased with lapse of storage days. The QI score of the sample without ice was higher during the storage days compared to the samples with ice and shrimp ratio 1:1, ice and shrimp ratio 1:3 and ice and shrimp ratio 1:5. The QI scores for the sample groups of ice and shrimp ratio 1:3 and ice and shrimp ratio 1:5 were very close up to five storage days and the QI values were 6. After lapsed of five storage days, the QI score of all sample groups were increased rapidly. The sample group of without ice exceeded the rejection level of QI value 6 (approximately) before 3 days which were assumed that the shrimps were spoiled on the 3rd day. The sample group of ice and shrimp ratio 1:5 was reached at the rejection QI value after 3 days. The sample group of ice and shrimp ratio 1:3 exceed the acceptable QI value at 5th storage day whereas the sample group of ice and shrimp ratio 1:1 was very close to rejected QI value of 6. After 5 days of storage all the sample lost their acceptance and reached over the rejected QI value of 6.

The QI score of the whole shrimps were counted four attributes of appearance, colour, odour, dark in head and roe colour by five panellists on every sampling day. The quality and shelf life of the shrimps were assessed by using the mean QI scores. At the mean QI scores of 2 or above for each parameter of each sample (WI, SIE, SIM, SIL), most of the panellists evaluated the spoilage of shrimp ((Figure 3, Appendix 2). The appearance of shrimp at different storage days are shown in Appendix 6.

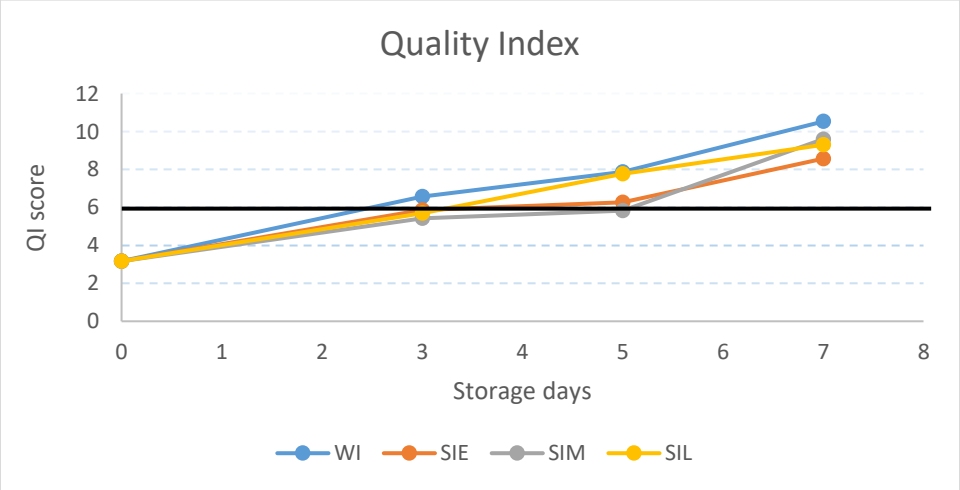

Figure 2: Quality Index (QI) of the shrimp groups at different storage days. Without Ice (WI), Ice and shrimp ratio = 1.1(SIE), Ice and shrimp ratio = 1:3 (SIM), and Ice and shrimp ratio = 1:5 (SIL).

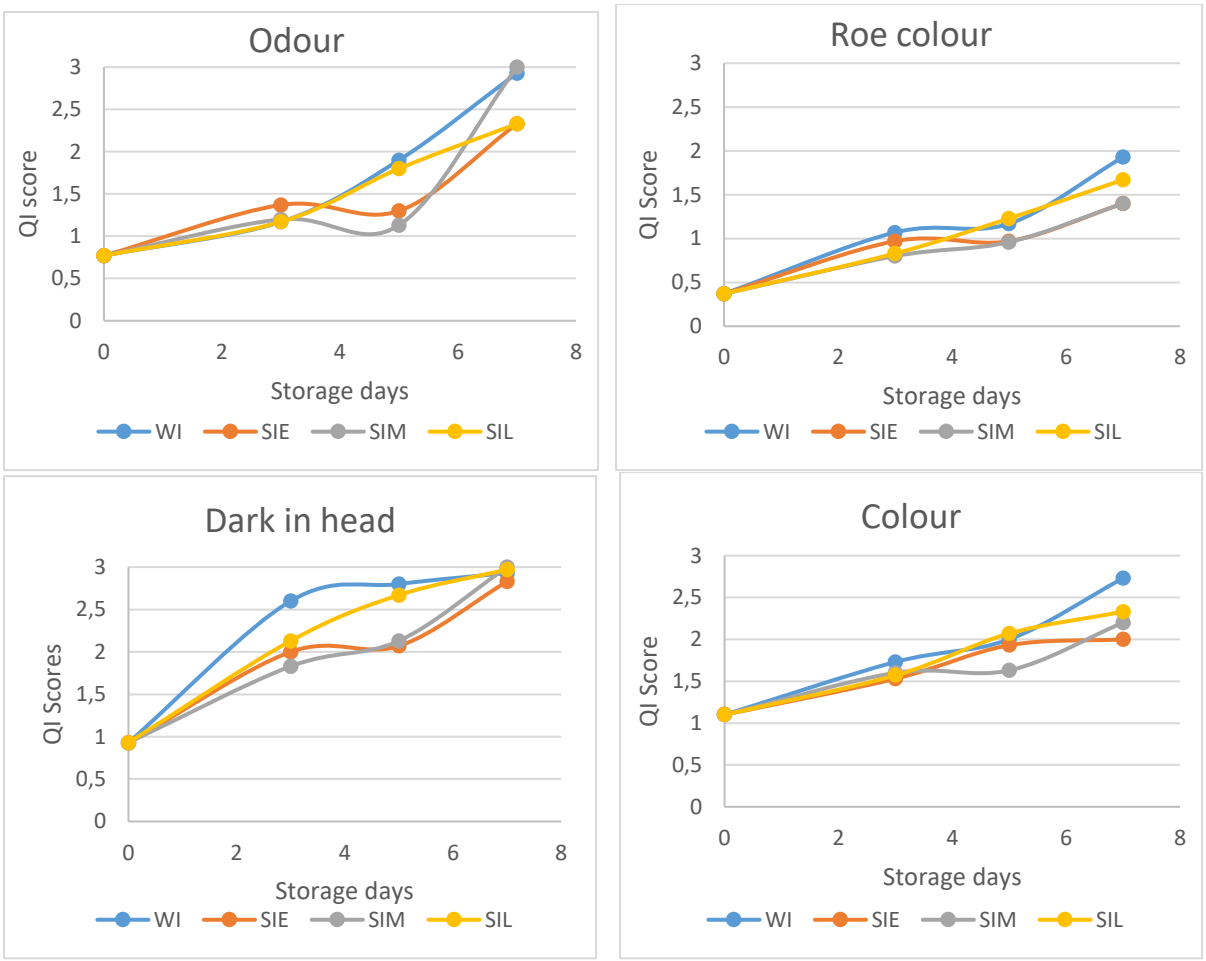

Figure 3: Mean QI scores of odour, roe colour, dark in head and colour of shrimp with different storage days (n = 5 panellist receiving duplicate sample).

4.4 Chemical evaluation

4.4.1 TVB-N

The initial TVB-N value of the sample was measured after the arrival of the sample and the TVB-N value was 15.24 mgN/100 g which was gradually increased with the progress of storage days (Figure 4). TVB-N value of the sample without ice was increased and reached at 31mgN/100 g at the 3rd storage day which was exceeded the upper limit of acceptable level 30 mgN/100 g. The TVB-N value of other sample groups of ice and shrimp ratio 1:1, ice and shrimp ratio 1:3 and ice and shrimp ratio 1:5 were beyond the rejection limit of 30 mgN/100g but TVB-N value of these samples were very close to the rejection limit at 3rd storage day. On sampling days 5, TVB-N value of the sample groups without ice and ice and shrimp ratio 1:5 were exceeded the acceptable limit and reached at 37 mg/100 g and 35 mg/100 g respectively. The sample groups of ice and shrimp ratio 1:1 and ice and shrimp ratio 1:3 were on the line of acceptable limit and the TVB-N value were 30 mgN/100 g and 29 mgN/100 g respectively. On 7th sampling day, the TVB-N value of the sample group without ice increased sharply and reached at 71 mgN/100 g but TVB-N value of other sample groups of ice and shrimp ratio 1:1, ice and shrimp ratio 1:3, and ice and shrimp ratio 1:5 increased slowly and the values were 37 mgN/100 g, 44 mgN/100 g and 47 mgN/100 g respectively. The TVB-N value of the sample ice and shrimp ratio 1:5, was lowest compared to other samples with lapse of storage days.

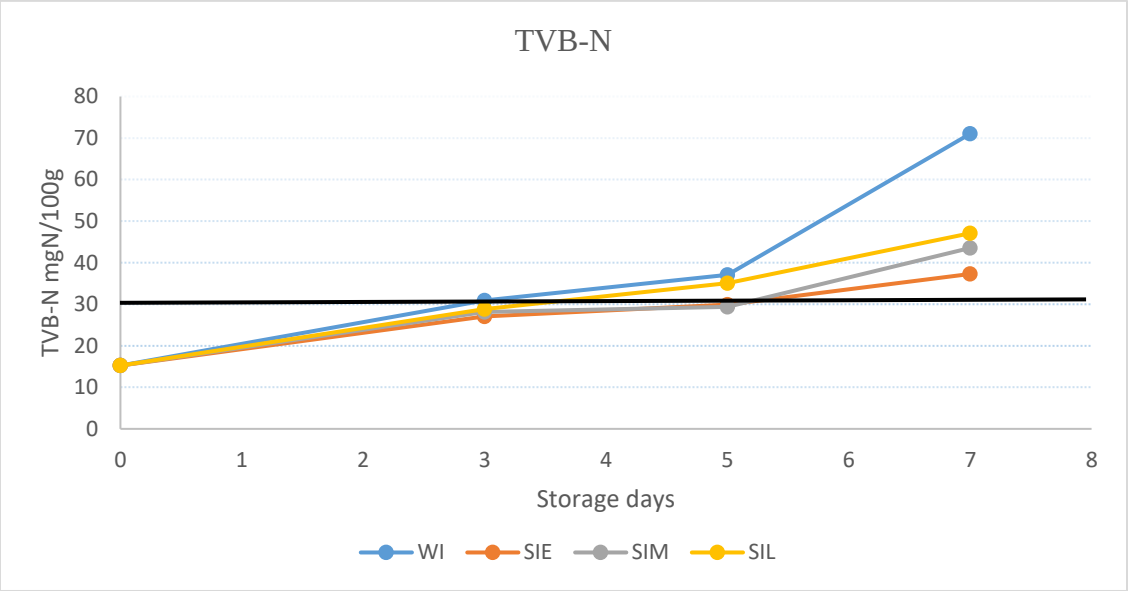

Figure 4: Total Volatile basic Nitrogen (TVB-N) mgN/100g of the shrimp over storage gays. Without Ice (WI), Ice and shrimp ratio = 1:1(SIE), Ice and shrimp ratio = 1:3 (SIM), and Ice and shrimp ratio = 1:5 (SIL).

4.4.2 pH

The pH value of the samples was conducted with the storage days that is shown in Figure 5. The initial pH value of the shrimp sample was 7.5 which was steadily increased with the lapse of up to 5 storage days but at 7th storage day the pH value of the samples were slightly dropped. The pH value of all the sample groups were close to 8.0 on 3rd sampling days and over 8.03 at 5th sampling day. On the 7th sampling day, the pH value of the sample groups without ice, ice and shrimp ratio 1:1, and ice and shrimp ratio 1:3 were around 7.90 whereas the pH value of the sample group ice and shrimp ratio 1:5 was 8.03.

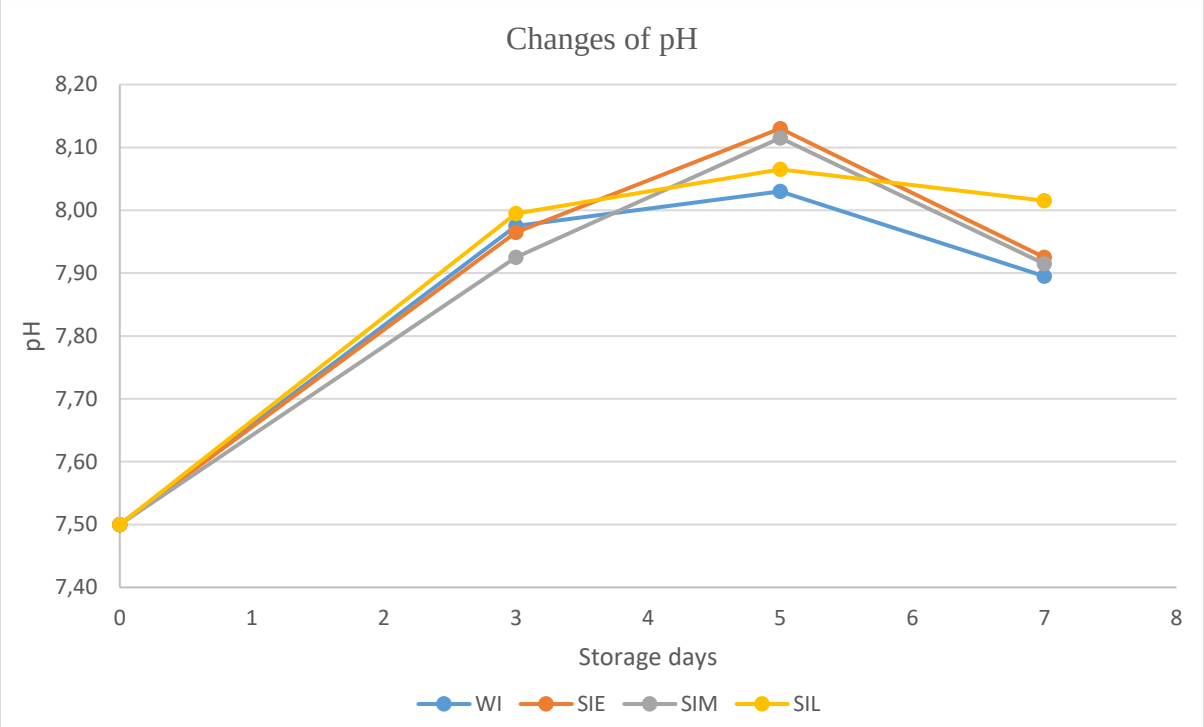

Figure 5: Changes of pH values of samples at different storage days. Without Ice (WI), Ice and shrimp ratio = 1:1 (SIE), Ice and shrimp ratio = 1:3 (SIM), Ice and shrimp ratio = 1:5 (SIL).

4.5 Microbial evaluation

4.5.1 TVC

The microbiological analysis that were conducted in the samples at different storage days are shown in Figure 6. Total volatile count (TVC) of all the samples were increased gradually. The microbiological growth rate of the sample without ice was faster than the other samples which were stored with ice. After arrival of the sample the TVC was measured and the value was $\log_{10}$ 5 cfu/g. The TVC of the sample without ice was increased to $\log_{10}$ 8 cfu/g at the end of 7th sampling day and the TVC of other samples ice and shrimp ratio 1:3 and ice and shrimp ratio 1:5 were $\log_{10}$7.6 cfu/g and $\log_{10}$7.7 cfu/g. The TVC of the sample ice and shrimp ratio 1:1 was the lowest at the end of the 7th sampling day and the value was $\log_{10}$7.4 cfu/g. TVC value of ice and shrimp ratio 1:1, ice and shrimp ratio 1:3 and ice and shrimp ratio 1:5 were below $\log_{10}$ 7 up to 5 storage days where as TVC value of WI sample was $\log_{10}$ 7.6.

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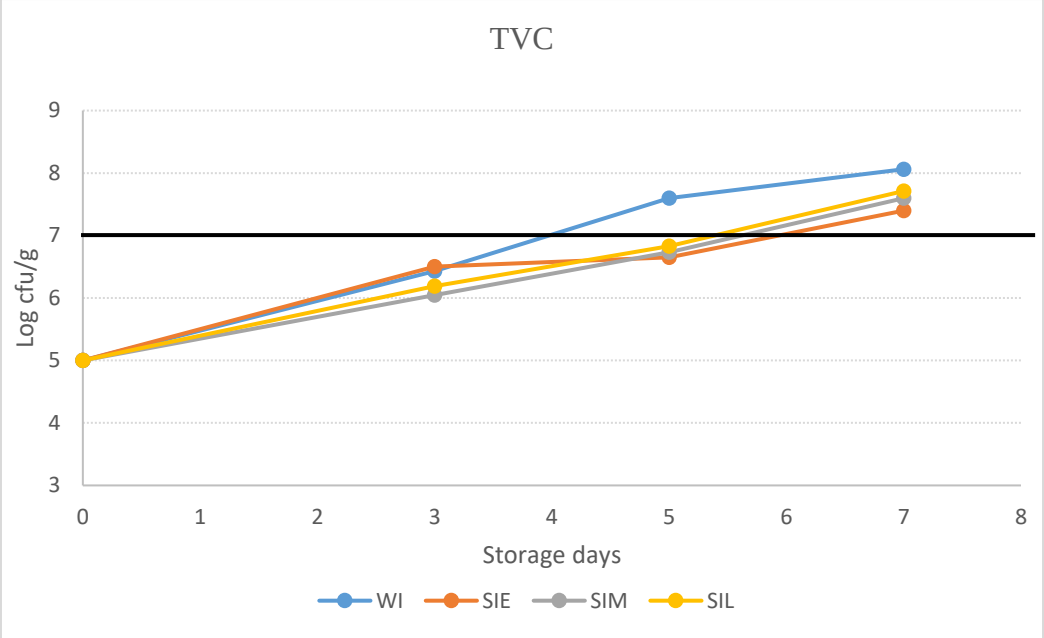

Figure 6: Total Viable Count cfg/g of shrimp at different storage days. Without Ice (WI), Ice and shrimp ratio = 1:1 (SIE), Ice and shrimp ratio = 1:3 (SIM) and Ice and shrimp ratio = 1:5 (SIL).

4.5.2 H_2S producing bacteria

The result of H_2S producing bacteria (Spoilage bacteria) of different samples are shown in Figure 7. Initially the spoilage bacterial count of the sample was $\log_{10}$ 4.15 cfu/g. The numbers of spoilage bacteria count of all samples steadily increased with lapse of storage days. After day 3 of storage the H_2S producing bacteria of all the samples were very close and the result of the samples without ice, ice and shrimp ratio 1:1, ice and shrimp ratio 1:5 and ice and shrimp ratio 1:5 were $\log_{10}$ 6.01cfu/g, $\log_{10}$ 6.20 cfu/g, $\log_{10}$ 5.39 cfu/g and $\log_{10}$ 5.51cfu/g respectively. The spoilage bacterial count of all the samples were gradually increased after 3 of storage days and the spoilage of the sample without ice was $\log_{10}$ 6 that was higher than other samples ice and shrimp ratio 1:1, ice and shrimp ratio 1:3, and ice and shrimp ratio 1:5. After 7 days of storage the H_2S producing bacteria counts of without ice sample was highest and the value was $\log_{10}$ 7.7 cfu/g whereas H_2S producing bacteria counts of ice and shrimp ratio 1:1 sample was lowest and the value was $\log_{10}$ 7.08 cfu/g. The H_2S producing bacteria count of the sample ice and shrimp ratio 1:3 and ice and shrimp ratio 1:5 were $\log_{10}$ 7.4 at the 7th storage day.

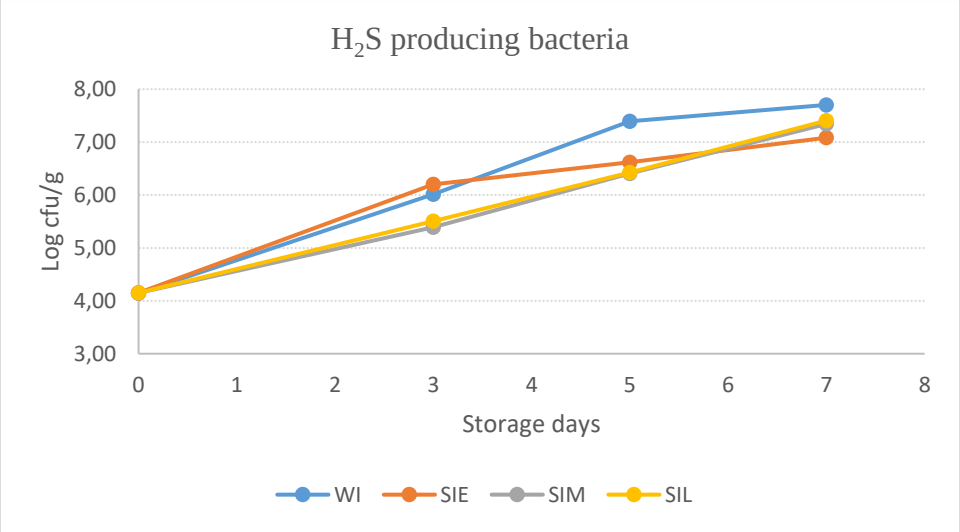

Figure 7: H_2S producing bacteria cfu/g formation of shrimp at different storage days. Without Ice (WI), Ice and shrimp ratio = 1:1 (SIE), Ice and shrimp ratio = 1:3 (SIM) and Ice and shrimp ratio = 1:5 (SIL).

4.6 Shelf life of shrimp

When the sample was received at 0 day in Matis it was two days old. The assessment of QI scores for sensory evaluation, TVB-N and pH value of chemical evaluation and TVC value of microbiological evaluation were given a clear result about the shelf life with compared to acceptable level. The shelf life of the sample groups are shown in the Table 4. The yellow colour indicated the acceptable level of quality parameters.

Table 4: The results of quality indicators and shelf life of different sample groups.

Sample groups	Quality parameters	Storage days				Acceptable limit	Shelf life		Comments on shelf life
		0	3	5	7	30 mg/100 g			**WI= 0 days,**
WI	TVB-N	15.24	30.88	37.08	71.03		WI	3	
SIE		15.24	27.07	29.93	37.28		SIE	5	**SIE= 5 days,**
SIM		15.24	28.15	29.39	43.10		SIM	5	
SIL		15.24	28.84	35.10	47.08		SIL	3	
WI	pH	7.5	7.98	8.03	7.90	-	WI	-	**SIM= 5 days,**
SIE		7.5	7.97	8.13	7..93		SIE	-	
SIM		7.5	7.93	8.12	7.92		SIM	-	**SIL= 3 days**
SIL		7.5	8.0	8.07	8.02		SIL	-	
WI	TVC	log 5	log 6.5	log 7.59	log7.80	Log_{10} 7	WI	3	
SIE		log5	log6.5	log 6.73	log7.39		SIE	5	
SIM		log5	log6.19	log6.82	log7.71		SIM	5	
SIL		log5	log6.19	log6.82	log7.71		SIL	5	
WI	QI	3.17	6.56	7.83	10.53	6	WI	0	
SIE		3.17	5.87	6.27	8.37		SIE	3	
SIM		3.17	5.43	5.83	9.60		SIM	5	
SIL		3.17	5.70	7.77	9.30		SIL	3	

(WI = Without ice, SIE = Ice and shrimp ratio 1:1, SIM = Ice and shrimp ratio 1:3, SIL = Ice and shrimp ratio 1:5).

4.7 Correlation between indicators

The correlation coefficient of the quality parameters TVB-N, pH, TVC, spoilage bacteria, sensory score were measured. The yellow colour highlighted the good correlation (Table 5).

Table 5: Correlation coefficient of quality indicators of different sample groups in different storage days.

Parameters	TVB-N	pH	TVC	Spoilage bacteria	Sensory Score
TVB-N	1				
pH	-0.55621	1			
TVC	0.943871	-0.58934	1		
Spoilage bacteria	0.762578	-0.56747	0.933337	1	
Sensory Score	0.728334	-0.87671	0.854715	0.892721253	1

5 DISCUSSION

5.1 Sensory evaluation

The panellists observed that the Quality Index (QI) for all parameters was increased with progress of storage days. Quality Index of the samples stored in ice increased linearly with the storage time (Martinsdottir *et al*. 2001). The QI score for dark in head was highest and QI score for roe colour was lowest with the lapse of storage days. So the quality of head lost faster. According to Rahman *et al*. (2001), "the head-on prawn lost its quality faster than headless prawn that are stored in ice". The QI scores for the sample without ice was faster than other sample group of ice and shrimp ratio 1:1, ice and shrimp ratio 1:3 and ice and shrimp ratio 1:5. The sample groups of ice and shrimp ratio 1:1, and ice and shrimp ratio 1:3 were lowest scores that means higher quality and lower spoilage than other groups of sample without ice and ice and shrimp ratio 1:5 throughout the 7 storage days (Figure 2). At the end of the 7^{th} day the QI scores were reached to the maximum value of Quality Index. This indicated that all the panellists evaluated all the sample groups were totally spoiled at 7^{th} day. The result from the QI scores (Figure 2) showed that the sample groups (QI score 6) of ice and shrimp ratio 1:1 and ice and shrimp ratio 1:3 had longer shelf life than the sample group without ice and ice and shrimp ratio 1:5. The shelf life of shrimp (*Pandalus borealis*) at sample groups of without ice, ice and shrimp ratio 1:1, ice and shrimp ratio 1:3, and ice and shrimp ratio 1:5 were varied considerably with storage days. The results of QI from the analysis of variance (ANOVA) of four attributes (Dark in head, odour, colour and roe colour) of the four sample groups without ice, ice and shrimp ratio 1:1, ice and shrimp ratio 1:3, and ice and shrimp ratio 1:5 showed that there were no significant different ($p<0.05$) among the attributes of other groups (Appendix 1). The result of Quality Index (QI) from sensory evaluation for each group of sample showed that the sensory scores of the sample increased linearly with the storage time. The same result was found by (Qingzhu 2003) where he found a good linear correlation among the groups of different cooling techniques. The linear equation and correlation coefficient of each sample groups are the mentioned in (Table 6). These equation indicated that the shrimp sample stored without ice had the highest spoilage rate (slope value is 1.01) and the shrimp sample stored with ice and shrimp ratio 1:1 had the slowest spoilage rate (slope value is 0.72). Qingzhu (2003) reported that the shrimp sample had lowest spoilage rate which was stored in liquid ice at the temperature of -1.5°C.

Table 6: Linear equation and correlation coefficient of different sample groups at different storage days.

Serial no.	Sample group	Linear equation	Correlation coefficient
1	Without ice	Y = 1.01x + 3.21	R^2=0.99
2	Ice and shrimp ratio 1:1	Y = 0.72x + 3.25	R^2 = 0.96
3	Ice and shrimp ratio 1:3	Y = 0.84x + 2.85	R^2 = 0.89
4	Ice and shrimp ratio 1:5	Y = 0.89x + 3.15	R^2 = 0.99

5.2 TVB-N

Total volatile basic nitrogen (TVB-N) was measured after arrival of the sample and the value was 15.24 mgN/100 g. TVB-N value was increased considerably with the storage days (Figure 4). According to Baldan (1961) "the combined effect of hydrolysis of protein, other nitrogenous compound, autolytic enzymes and bacterial activities lead to increase TVB-N values". Begum *et al*. (2011) reported that the initial TVB-N value of prawn was 10 mgN/100 g. Qingzhu (2003) found TVB-N value 33.5 mgN/100 g of whole northern shrimp at the beginning of the storage in ice. Initially the TVB-N value was high because the sample was two days old. The TVB-N value of all the sample jumped into doubled on 3rd storage day and then increased steadily. The TVB-N value of all samples were around 30 mgN/100 g. The TVB-N value on 5th sampling day was 30 mgN/100 g for the sample ice and shrimp ratio 1:1 and ice and shrimp ratio 1:3. The TVB-N value of ice and shrimp ratio 1:5 sample was 35 mgN/100g and without ice sample was 37 mgN/100 g. The increase of TVB-N in shrimp stored in ice was slower than the sample stored without ice due to restrained of the growth of spoilage bacteria (Qingzhu 2003). Ali *et al*. (2008) reported that the moderately acceptable limit of TVB-N for prawn was 33.50 ± 0.44 mgN/100 g and unacceptable limit of TVB-N for prawn was 39.55 ± 0.47 mg/100 g. The TVB-N values increased positively with the progress of storage days and after seven days the TVB-N values were determined within the limit of 30 mg/100 g in ice stored prawn (Rahman *et al*. 2001). At the end of 7th storage day the TVB-N value of the sample without ice was found the highest and the value was 71 mgN/100 g and the sample ice and shrimp ratio 1:1 was found lowest and the value was 37.28 mg/100 g. So all the samples were completely spoiled at the end of 7th storage day. Yiu-fai, (2000) reported that the average TVB-N value of shrimp (*Peneous monodon*) was 51.6 mgN/100 g at odour rejection level.

5.3 pH

The study was conducted on the cold water shrimp and initial pH of the sample was 7.5. There was a trend of increasing pH with storage days up to 5th day and the value was over 8.00 but on 7th storage day it was slightly dropped and the values were around 7.90 (Figure 5). pH value of 7th day declined due to hydrolysis of glycogen to lactic acid because some of the ice was melted in the Styrofoam boxes although the lactic acid was not measured in this study. This is supported by Haider *et al*. (2011) where they found that the pH of the shrimp muscle dropped due to hydrolysis of glycogen to lactic acid. Huss (1995) reported that breakdown of residual glycogen via glycolysis to pyruvic acid and then lactic acid production dropped of pH value during storage days. Qingzhu (2003) determined that the initial pH of cold water shrimp was 7.41 and it was steadily increase with storage days and after 6th storage day the pH was 8.26.

5.4 Microbiological evaluation

The growth of H_2S producing bacteria and TVC gradually increased with progress of storage days which enhance the spoilage rate. The initial measurement of the TVC and H_2S producing bacteria were low and the value were $\log_{10}$ 5 cfu/g and $\log_{10}$ 4 cfu/g respectively. According to Sveinsdottir *et al*. (2002) "at the initial stage, the total bacterial count is always low due to immune system and prevents of bacterial growth in the flesh. During post rigor mortise, the immune system collapsed and the bacteria that are present on the surface invade in the muscle". Begum *et al*. (2011) reported that the initial TVC of prawn sample was 3.02×10^6 cfu/g ($\log_{10}$ 6.3) and at unacceptable the TVC value was 2.33×10^8 cfu/g ($\log_{10}$ 8.2). The TVC and H_2S producing bacteria of the sample group without ice (WI) was higher than the sample groups of ice and shrimp ratio 1:1, ice and shrimp ratio 1:3 and ice and shrimp ratio 1:5 with the lapse of storage days. It was happened because the sample group of WI was stored without ice so the cooling system was very slow and temperature was high. The TVC and H_2S producing bacteria were lowest of the sample ice and shrimp ratio 1:1 due to well surrounded by high amount of ice and maintain of low temperature. According to Capell *et al*. (1997) "the accepted spoilage level of a sample is $\log_{10}$ 7 cfu/g". The TVC sample group of without ice exceeded the spoilage level of $\log_{10}$ 7 cfu/g on 5th sampling day where as other sample exceeded the spoilage level at the end of 7th storage day. The microbial growth of the samples which were stored in ice were delayed. In case of chilled fish total viable counts is not so effective indicator for quality and shelf life but H_2S producing bacteria contributed important part in spoilage (Huss *et al*. 1974).

5.5 Shelf life of shrimp

The present study obtained that the shelf life of without ice sample was 4 days and ice and shrimp ratio 1:5 sample was 5 days. The shelf life of ice and shrimp ratio 1:1 and ice and shrimp ratio 1:3 samples were 7 days which were more or less similar to the result obtained by Haider *et al*. (2011). They reported that the shrimps which were collected from the farm and stored in ice were the acceptable condition up to seven days. The samples which were collected from the depots and stored in ice were the acceptable condition for four days. Martinsdottir *et al*. (2001) reported that the estimated shelf life of deep water shrimp was 6 days. The shelf life of shrimp (*Peneous merguiensis)* which were harvested in Singapore and stored in ice was four days (Yamagata and Low 1995). According to Jensen and Hansan (1973) "if fish are left without ice for a few hours, their shelf life will decrease sharply compare to the fish that are preserve in ice". Higher temperature reduces the shelf life of the products but chilling extends the shelf life of commodities (Huss 1995).

5.6 Correlation

The pH, TVB-N, TVC, H_2S producing bacteria and sensory scores were used as quality indicators for the cold water shrimp. The correlation coefficient of the estimated quality parameters showed that there were a good correlation between the parameters (Table 5). The TVC found excellent correlation with TVB-N (R^2 = 0.94), spoilage bacteria (R^2 = 0.93), and sensory scores (R^2 = 0.85). The TVB-N had good correlation with spoilage bacteria (R^2 = 0.76) and Sensory scores (R^2= 0.73) and sensory score had good correlation with spoilage bacteria (R^2= 0.89). According to Luten and Martinsdottir (1998) "There is a linear correlation between the sensory quality expressed as QI score and storage life on ice, which can help to make the decision about the storage life of fish in ice". The pH values showed good negative correlation because at 7th day the pH value slightly decrease whereas pH value gradually increase up to 5th storage day.

6 CONCLUSION

The study revealed that the quality index, TVB-N and TVC value increased during storage days. So chilling temperature and the ice had direct effect on the quality and shelf life of the shrimp. The sample of without ice had lost its quality faster and had a shorter shelf life than the samples which were stored with ice due to the highest microbial contamination and TVB-N value. H_2S producing bacteria contributed important part in spoilage. The shelf life of the sample ice and shrimp ratio 1:1 and ice and shrimp ratio 1:3 were similar based on chemical and microbial data due to rapid cooling and lower temperature. This indicated that ice and chilling temperature extends shelf life and delayed spoilage.

The study clearly showed that there were no significance difference between the groups of sample with lapse of storage days but the quality parameter of QI, TVB-N, TVC and H_2S producing bacteria had a strong liner correlation.

Based on this study we can recommended that the ice and shrimp ratio of 1:1 and ice and shrimp ratio 1:3 at chilling temperature were the best ratio to extend shelf life and maintain quality of shrimp. Ice and shrimp ratio 1:3 will be efficient and more practical because it will save cost of ice and give facilities of more space for shrimp preservation in box or board.

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APPENDICES

Appendix 1: Analysis of variance

1. ANOVA analysis for H_2S producing bacteria of shrimp at different storage days.

Anova

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	4	25.25019	6.312549	2.626551
Column 2	4	24.05279	6.013196	1.672876
Column 3	4	23.28813	5.822032	1.878767
Column 4	4	23.48494	5.871234	1.919515

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.584095	3	0.194698	0.096174	0.960672	3.490295
Within Groups	24.29313	12	2.024427			
Total	24.87722	15				

2. ANOVA analysis for Total Volatile Count (TVC) of shrimp at different storage days.

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	4	27.08866	6.772165	1.865573
Column 2	4	25.54794	6.386985	1.009236
Column 3	4	25.37627	6.344066	1.204744
Column 4	4	25.73144	6.432861	1.301594

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.45859	3	0.152863	0.113629	0.950437	3.490295
Within Groups	16.14344	12	1.345287			
Total	16.60203	15				

3. ANOVA analysis for pH of shrimp at different storage days.

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	5	39.36	7.872	0.04487
Column 2	5	39.39	7.878	0.04787
Column 3	5	39.375	7.875	0.046263
Column 4	5	39.56	7.912	0.05817

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.005224	3	0.001741	0.035324	0.990756	3.238872
Within Groups	0.78869	16	0.049293			
Total	0.793914	19				

4. ANOVA analysis for TVB-N for shrimp of different storage days

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	4	154.24	38.56	553.0682
Column 2	4	109.535	27.38375	84.03654
Column 3	4	116.305	29.07625	133.931
Column 4	4	126.275	31.56875	175.7687

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	290.319	3	96.773	0.408841	0.74954	3.490295
Within Groups	2840.413	12	236.7011			
Total	3130.732	15				

5. ANOVA analysis for odour of QI of shrimp at different storage days.

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	4	6.97	1.7425	0.786492
Column 2	4	5.77	1.4425	0.421825
Column 3	4	6.1	1.525	1.002433
Column 4	4	6.07	1.5175	0.473158

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.200419	3	0.066806	0.099566	0.958726	3.490295
Within Groups	8.051725	12	0.670977			
Total	8.252144	15				

6. ANOVA analysis for colour of QI of shrimp at different storage days.

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	4	7.56	1.89	0.4558
Column 2	4	6.56	1.64	0.172467
Column 3	4	6.53	1.6325	0.202225
Column 4	4	7.07	1.7675	0.297492

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.17835	3	0.05945	0.210819	0.886969	3.490295
Within Groups	3.38395	12	0.281996			
Total	3.5623	15				

7. ANOVA analysis for dark in head of shrimp at different storage days.

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	4	9.26	2.315	0.870967
Column 2	4	7.83	1.9575	0.610492
Column 3	4	7.89	1.9725	0.729225
Column 4	4	8.7	2.175	0.8097

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.35325	3	0.11775	0.15594	0.923837	3.490295
Within Groups	9.06115	12	0.755096			
Total	9.4144	15				

8. ANOVA analysis for Roe colour of shrimp at different storage days.

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	4	3.53	0.8825	0.181092
Column 2	4	3.71	0.9275	0.179225
Column 3	4	3.53	0.8825	0.181092
Column 4	4	4.1	1.025	0.308367

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.054169	3	0.018056	0.084993	0.966928	3.490295
Within Groups	2.549325	12	0.212444			
Total	2.603494	15				

9. ANOVA analysis for average QI factors for four sample groups.

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	4	27.08866	6.772165	1.865573
Column 2	4	25.54794	6.386985	1.009236
Column 3	4	25.37627	6.344066	1.204744
Column 4	4	25.73144	6.432861	1.301594

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.45859	3	0.152863	0.113629	0.950437	3.490295
Within Groups	16.14344	12	1.345287			
Total	16.60203	15				

Appendix 2: Average sensory evaluation data of different storage days.

10. Sensory evaluation Parameters for the sample groups

Dark in head				
Storage Days	WI	SIE	SIM	SIL
0	0.93	0.93	0.93	0.93
3	2.6	2	1.83	2.13
5	2.8	2.07	2.13	2.67
7	2.93	2.83	3	2.97

Colour				
Storage Days	WI	SIE	SIM	SIL
0	1.1	1.1	1.1	1.1
3	1.73	1.53	1.6	1.57
5	2	1.93	1.63	2.07
7	2.73	2	2.2	2.33

Odour				
Storage Days	WI	SIE	SIM	SIL
0	0.97	0.77	0.77	0.77
3	1.17	1.37	1.2	1.17
5	1.9	1.3	1.13	1.8

7	2.93	2.33	3	2.33

Roe Colour				
Storage Days	WI	SIC	SIMC	SILC
0	0.37	0.37	0.37	0.37
3	0.8	0.97	0.8	0.83
5	0.96	0.97	0.96	1.23
7	1.4	1.4	1.4	1.67

11. Average QI scores for four sample groups

Storage days	Total QI			
	WI	SIE	SIM	SIL
0	3.17	3.17	3.17	3.17
3	6.57	5.87	5.43	5.7
5	7.87	6.27	5.83	7.77
7	10.53	8.57	9.6	9.3

Appendix 3: Chemical evaluation data

12: pH measurement of the four sample groups with different sampling days

Storage Days	WI	SIE	SIM	SIL
0	7.50	7.50	7.50	7.50
3	7.98	7.97	7.93	8.00
5	8.03	8.13	8.12	8.07
7	7.90	7.93	7.92	8.02

13. TVB-N measurement of the four sample groups with different sampling days

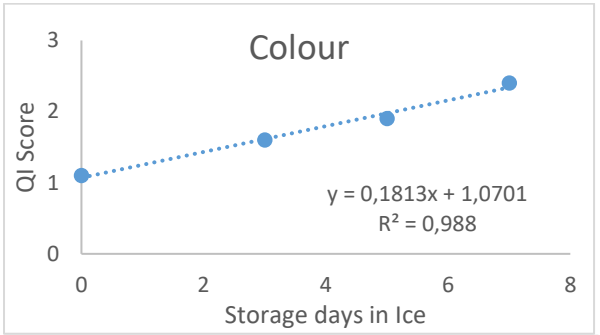
Storage days	WI	SIE	SIM	SIL
0	15	15	15	15
3	31	27	28	29
5	37	30	29	35
7	71	37	44	47

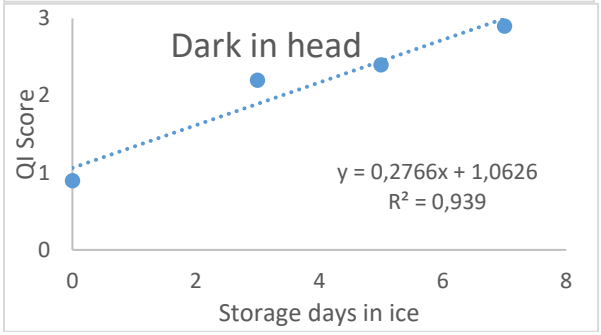
Appendix 4: Microbiological evaluation data

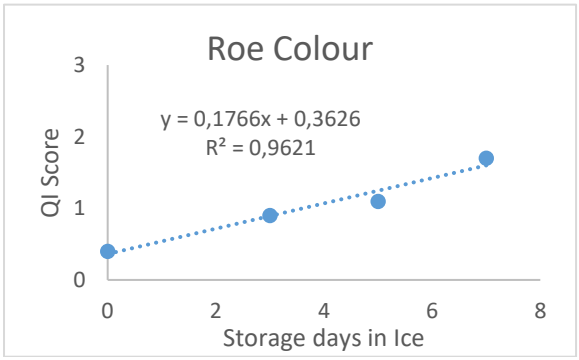
14. Microbiological result of the four sample groups with different sampling days

TVC (cfu/g)				
Storage days	WI	SIE	SIM	SIL
0	10^5	10^5	10^5	10^5
3	2.7×10^6	3.15×10^6	1.1×10^6	1.55×10^6
5	3.95×10^7	4.5×10^6	2.7×10^6	2.7×10^6
7	1.15×10^8	2.5×10^7	3.95×10^7	5.15×10^7
		H_2S producing bacteria		
0	1.4×10^4	$1.\times10^4$	1.4×10^4	1.4×10^4
3	1.02×10^6	1.6×10^6	2.45×10^5	3.2×10^5
5	2.45×10^7	4.15×10^6	2.55×10^6	2.65×10^6
7	5.05×10^7	1.21×10^7	2.2×10^7	2.55×10^7

Appendix 5: Linear Regression equation for Quality Index for sensory evaluation







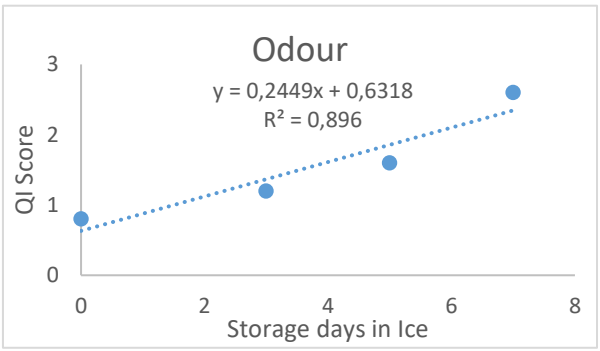


Figure 1: QI for Colour, dark in head, odour and Roe colour of shrimp with storage of all sample groups.

Appendix 6: Appearance of shrimp sample at different storage days.

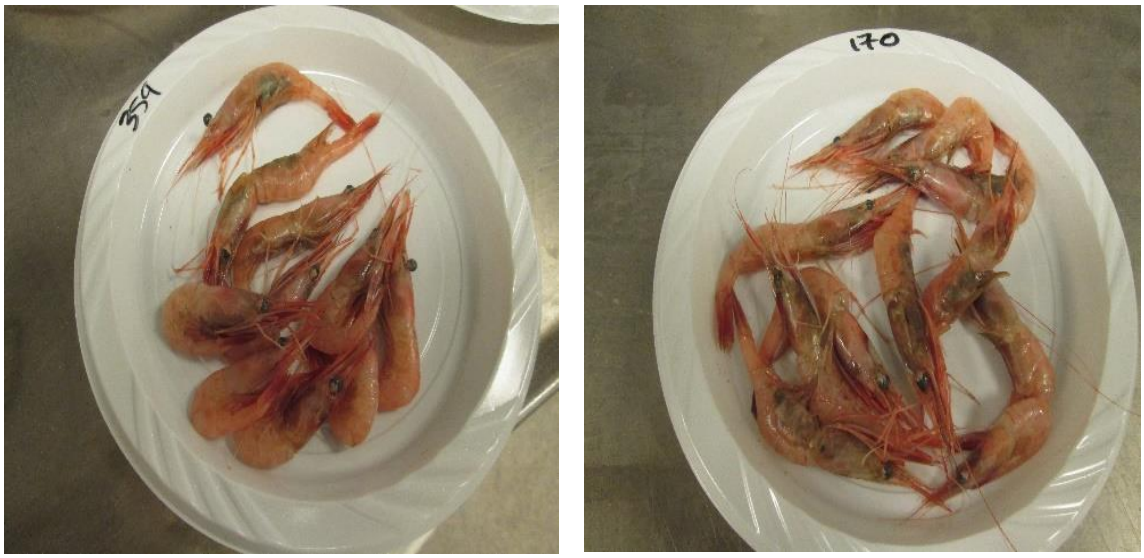


Figure 2: Appearance of shrimp sample stored at 0 day.

WI

SIL

SIE

SIM

Figure 3: Appearance of shrimp sample groups stored at 3rd day. Without Ice (WI), Ice and shrimp ratio = 1:5 (SIL), Ice and shrimp ratio = 1:3 (SIM), Ice and shrimp ratio = 1:1 (SIE).

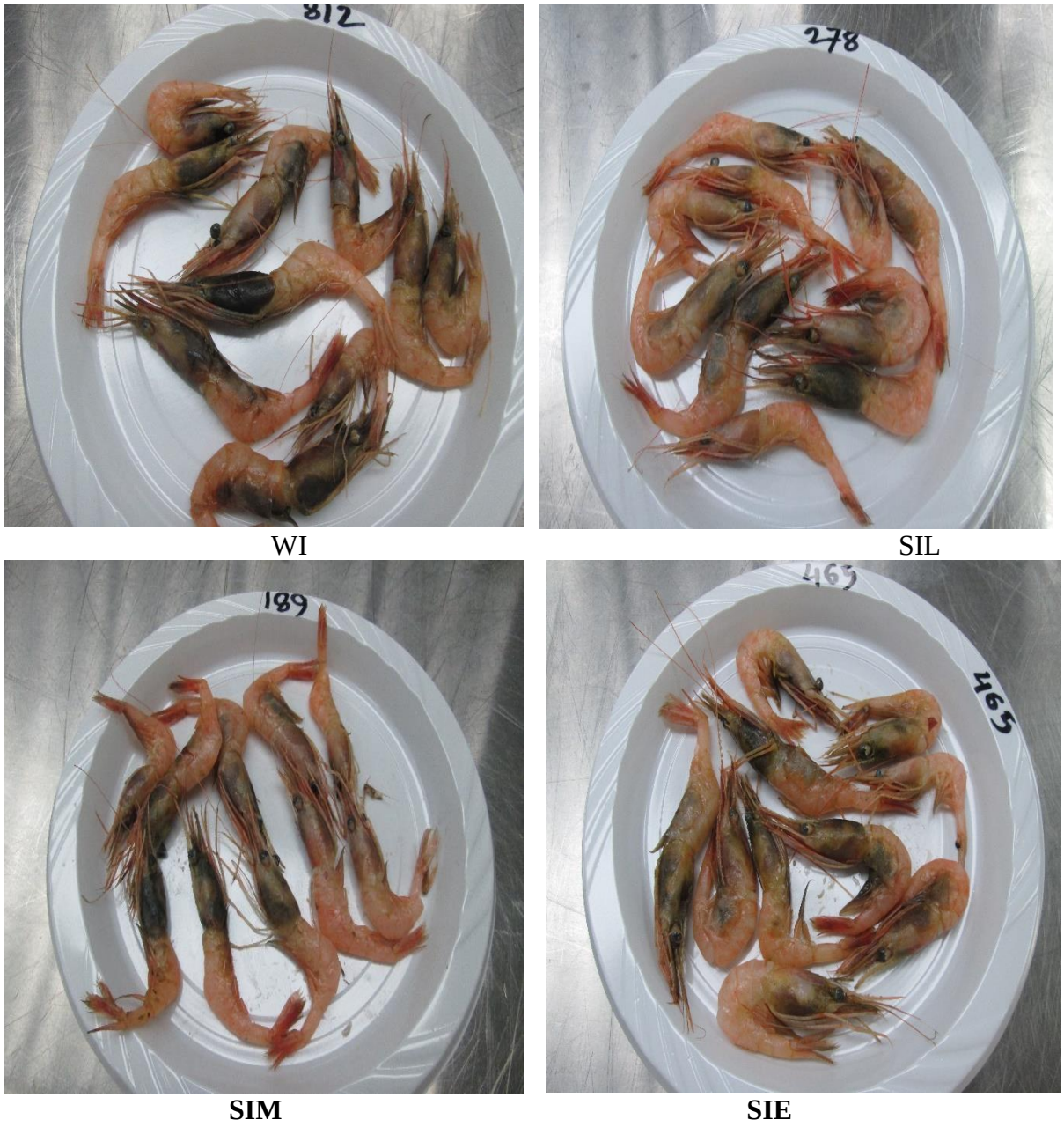


Figure 4: Appearance of shrimp sample groups stored at 5th day. Without Ice (WI), Ice and shrimp ratio = 1:5 (SIL), Ice and shrimp ratio = 1:3 (SIM), Ice and shrimp ratio = 1:1 (SIE).

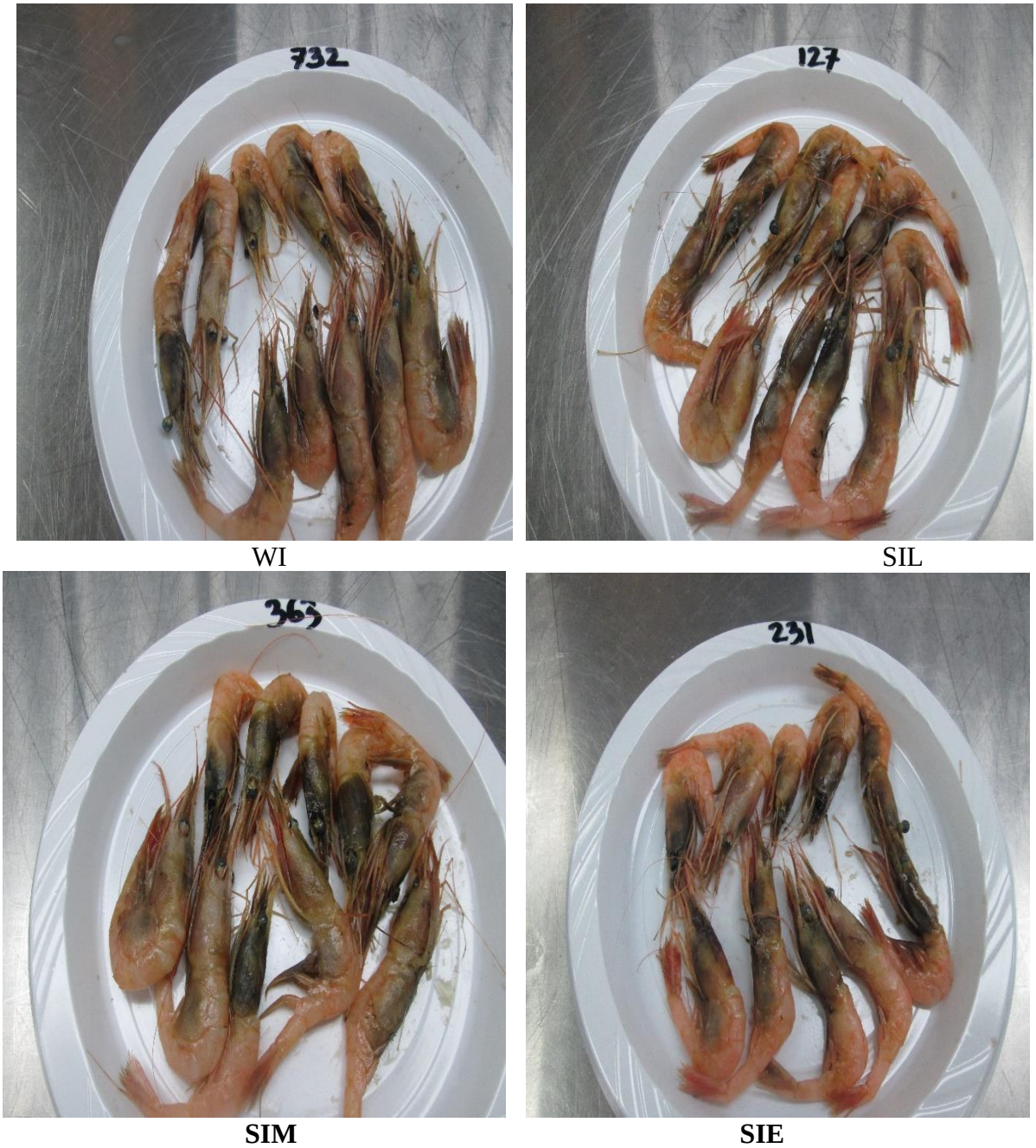


Figure 5: Appearance of shrimp sample groups stored at 7th day. Without Ice (WI), Ice and shrimp ratio = 1:5 (SIL), Ice and shrimp ratio = 1:3, (SIM) and Ice and shrimp ratio = 1:1 (SIE).