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Quality characteristics including formaldehyde content in selected Sea foods of Tuticorin, southeast coast of India

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Abstract

General quality characteristics including formaldehyde content of iced and un-iced sea foods were assessed in this study. Formation of formaldehyde in sea food is by enzymatic reaction and oxidation of lipids as a result of the activity of microbes. Great attention has been paid to volatile aldehydes like formaldehyde in aquatic products. Results showed that formaldehyde levels were significantly higher in un-iced fishes than that of the immediately iced fishes and it was in the range of 0.001 - 0.32 mg/kg in iced fishes and it was in the range of 10.64 - 18.75 mg/kg in un-iced fishes. Study showed that seafood contained high amount of formaldehyde because of natural production by postmortem enzymatic reaction. This study clarified that improperly stored seafood is a source of formaldehyde to human being. Sensory and microbiological qualities were good in fresh fishes than that of formaldehyde contained fishes. Normally sea foods were in good quality until the formaldehyde content and microbiological counts were below the permissible levels.

Introduction

Food safety has become an important issue and a widely talked word of modern age. Scientific discipline describes that proper handling, preparation and storage of food prevent food borne illness (Satin, 2004). This includes a number of routines that should be followed to avoid potentially severe health hazards in human. Nowadays people are more health conscious and emphasize that food should not only be nutritionally balanced but also safe for human consumption. Many national and international agencies are working in collaboration to ensure food safety, and in most of the industrialized countries the issue of food safety, quality and environmental concerns are more important than the product price and income changes (FAO, 2009). Seafood constitutes an important food component for a large section of world population.

Sea foods are the cheap source of animal protein which come after meat and poultry animal protein foods. Seafood is an important diet for many people due to their unique nutritional composition. As the world population is growing, the per capita consumption of seafood is also increasing rapidly. The low fat content of many sea foods and the effect on coronary heart disease of the n-3 polyunsaturated fatty acids in fatty pelagic fish species are extremely important aspect for health conscious people particularly in affluent countries where cardiovascular disease mortality is high (Adebayo *et al*., 2012a). Apart from providing nutrition,sea foods also cause harm to human health. The most significant sources of fish borne diseases are related to the microbiological and chemical hazards. Chemical contamination in fish may include natural toxicants such as mycotoxins (Melchert and Pabel, 2004) and marine toxin (Vale *et al*., 1999), environmental contaminants such as mercury and lead (Hui *et al*., 2005) and naturally occurring substances. Among the contaminants, attention has been paid to volatile toxic aldehydes such as formaldehyde classified by the International Agency for Research on Cancer (IARC) in the Group 1 as carcinogenic to humans (IARC, 2004).The amounts of formaldehyde formed depend mainly on the quality of fish, capturing time and temperature of storage, and it causes muscle toughening and water loss in fish species, leading to lower acceptability as well as functionality (Ang and Hultin, 1989; Badii and Howell, 2002). Formaldehyde is metabolized naturally in our bodies by normal metabolism and can also be found in the air, natural food, some skin-care products as well as preservatives in processed food, especially in dried and frozen food. Small amount of formaldehyde does not harm health however it can cause minor to serious problems such as pain,

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vomiting, coma and possible death when large doses of formaldehyde is taken.

Formaldehyde was produced by enzymatic decomposition of trimethylamine-oxide (TMAO) to equimolar amounts of formaldehyde (FA) and dimethylamine (DMA) in seafood during postmortem storage and the general reaction equation is TMAO → DMA + formaldehyde (Phillipy and Hultin, 1993; Kimura *et al.*, 2003). Formaldehyde also produced naturally and developed during postmortem in marine fish by an enzymatic reduction. The enzymatic reduction leads to breakdown of trimethylamine oxide to formaldehyde and dimethylamine. The formation of formaldehyde in fish can cause muscle toughness.

Formaldehyde A is a simple compound which is made of carbon, hydrogen and oxygen with the chemical formulae of CH_2O (Formaldehyde). It is found everywhere and produced naturally by plants, animals and humans as a part of normal life process. Other than that, formaldehyde is biodegradable and it can be destroyed through the photochemical processes which occur in the atmosphere, by biological actions in the body and natural processes in soil and water. It also categorized as the simplest aldehyde and its systematic name is methanol (Zhang *et al.*, 2009). Formaldehyde is a colourless, pungent and irritant compound. It is usually found as 37 - 56% aqueous solution which is known as formalin (Stoker *et al.*, 2004). Formaldehyde is used for various purposes, such as preservatives and disinfectant. It also can be used in the production of cosmetics and industrial processes as well as paper and pulp production (Mcnary and Jackson, 2007). Ingestion of as little as 30 ml of formalin has been reported to cause death in an adult human being. Ingestion may cause corrosive injury to the gastrointestinal mucosa with nausea, vomiting, pain, bleeding and perforation. Corrosive injuries are usually most pronounced in the pharyngeal mucosa, epiglottis and esophagus. Systemic effects include metabolic acidosis, Central Nervous System depression and coma, respiratory distress, renal failure and associated cancer and tumor development (Wooster *et al.*, 2005).

In 2002, WHO reported that high concentration of formaldehyde was found in marine fish, when they are exposed longer time to the environment without proper preservations. Formaldehyde was naturally produced in fish, however, fishmonger and fish vendors tend to carelessly add the formaldehyde in to the fish by injecting or by keeping the fishes in ice made of water mixed with formalin in Asian and European countries to prolong shelf life and to make the fish look fresh. Although the regulation has been enforced, but still there are reports about the illegal addition of this compound by fishmonger without concerning the consumer safety.

Formaldehyde is known to be carcinogenic to human. Therefore, it is important to investigate the formaldehyde content in the fish since it is claimed to be the major contaminant in seafood in order to understand the risks of fish consumption and to provide additional information in food safety. In this study, natural formaldehyde content of the sea food was determined for consumer safety. Also the qualities of iced and un- iced fishes with formaldehyde were determined.

Materials and Methods

Raw materials

Seven types of commercial fishes such as *Chirocentrus dorab* (Mulluvaalai), *Arius jella* (Keluthi), *Trachinocephalus myops* (Thannipanna), *Cephalopholis forosa* (Kalava), *Sillago sihama* (Kelekka), *Lates calcarifer* (koduva) and *Lutjanus malabaricus* (seppili) were selected as target sample based on more consumption in the short survey. Each of fish sample weighing 250 to 500 g were purchased from Tuticorin fish landing center. The collected samples were stored in sterile bags and kept in an ice box and brought to the laboratory. Total fishes were divided in to two lots and one lot was immediately iced and second lot was kept without ice for 7 hours and the sensory, microbial qualities and spoilage indicators including formaldehyde content were analyzed for both batches of fish samples.

Determination of pH

pH of the samples was determined by the method of Goulas and Kontominas (2007). 10 g of the sample was homogenized with 50 ml of distilled water and the pH value of the homogenate was measured by means of a glass electrode pH meter (HANNA pH213) that was previously standardized.

Determination of Total Volatile Base Nitrogen (TVB-N)

TVB-N was determined according to the procedure of Siang and Kim (1992) by using Conway micro diffusion unit. The extract was prepared by mixing 2 g of the sample with 4% Trichloroacetic acid in a 50 ml beaker and was homogenized properly. It was left for 30 min at room temperature with occasional shaking and then filtered. The filtrate was labeled and stored. Three Conway units were taken which had been thoroughly cleaned and the edge of the outer ring of each unit was applied with sealing agent (Vaseline). Using a micropipette, 1 ml of boric acid solution was added into the inner ring of each unit. In to the outer ring of each unit, 1ml of the sample extract was added. 1ml of saturated potassium carbonate solution was carefully pipetted into the outer ring of each unit and closed with clip. The solutions in the units were then mixed gently, to prevent any solution mixing from one ring to the other. The units were placed in an incubator at 37°C for 60 min. After that, the unit's covers were removed and the inner ring solution, now green in colour was titrated with 0.02N Hydrochloric acid using a burette until green colour solution turned into pink. An average titrate volume of Hydrochloric acid was found from the results of three titrations for each sample. For each value, the Total Volatile Base-Nitrogen values were calculated. A blank test was also carried out using 1ml of 1%Trichloroacetic acid, instead of sample extract.

Sensory quality assessment

Sensory methods were used to assess the degree of freshness based on odor, colour, general appearance, condition of eyes, slime and consistency of flesh. The difference in the sensory characteristics between iced and un-iced fish were judged by a trained panel of expert members. The grading of fish using score on the characteristics has been followed by Multilingual Guide to European Commission Freshness Grades for Fishery Products (Hawgate *et al*., 1992) to judge the quality of the fish.

Microbiological quality assessment

Total plate count was determined by standard plate count methods. About 10 - 15 g of whole fish sample was blended with appropriate volume of 0.2% peptone water in a sterilized blender for a few minutes until homogenous slurry was obtained. 0.1 ml aliquots was spread onto plate count agar (Hi-media, Mumbai, India) for total aerobic counts according to Collins and Lyne (1976) and colony counts were expressed as colony forming units per gram of muscle (CFU/g).

Determination of formaldehyde

Nash's Reagent (Nash, 1953) was used as an indicator by diluting 15 g of ammonium acetate with an addition of 0.3 ml of acetyl acetone and 0.2 ml of acetic acid. Nash's reagent was kept in amber glass reagent bottle at all times because the reagent is light sensitive. Trichloroacetic acid was used to adjust the pH of fish was appropriately 0.1 N potassium hydroxide (KOH) and 0.1 N hydrochloric acid (HCl) were used to adjust the pH of the distillate in order to be in the range from 6.0 to 6.5. The working standard solution was ranged in 0 - 5 mg/l and it was prepared from the intermediate standard solution (10µg/g) for the graph calibration.

The fish samples were cut into small pieces and 30g of the sample was homogenized with 60 ml of 6% w/w Trichloroacetic acid. The mixture was filtered and the pH of the filtrate was adjusted to 7.0 with 30% w/w Potassium hydroxide (KOH) and stored in ice for one hour. The test was performed by mixing 5 ml of the standard solution, Trichloroacetic acid, fish extract, 2 ml of Nash's reagent and then heated in the water bath at 60˚C for 30 min. The absorbance was measured immediately at 415 nm by UV spectrophotometer.

Results

The selected experimental sea foods were presented in Table 1. The formaldehyde content in selected iced and un-iced seafood from Tuticorin was summarized in Table 2. The highest amount of formaldehyde content was observed in un-iced sample of *Lutjanus malabaricus* and it was 18.75 mg/kg while freshly iced fishes contained the lowest amount of formaldehyde in the range of 0.001 - 0.32 mg/kg. The pH values of the iced fresh and un-iced fishes are presented in Table 3. The highest pH of 8.0 was recorded in un-iced sample of *Trachinocephalus myops*. The lowest range of pH 7.0 - 7.02 was found in the immediately iced fresh sample.

The volatile amine contents of iced and un-iced samples are shown in Table 3. The highest Total Volatile Base - Nitrogen value noted in the un-iced fish samples was 41.5 mg/100g where as for the iced samples it was 3.55 mg/100g. By comparison with iced samples the un-iced samples had higher amount of volatile amine production.

Studies were conducted to detect the changes in sensory quality of iced and un-iced fishes and the results are presented in Table 4. The sensory characteristics of fresh fish gills were red and the eyes were in full bloom and bright in appearance. The eyes were bulging with protruding lens and transparent. There were no slime on the surface of the body and flesh was firm and elastic. The results revealed that the sensory qualities of the iced fresh fishes were in excellent condition. In comparison with iced fresh fish, the gills of un-iced fish were found slightly blackish.Also, there was slight loss of brightness and natural colour. They were found emitting moderate to strong sour odor.

The amount of bacterial load in iced fish samples and un-iced samples were shown in Table 5. The

Table 1. Experimental sea food samples

Name of the sea foods	Nature	Vernacular name	Common name
Chirocentrus dorab	Finfish	Mulluvaalai	Dorab wolf herring
Arius jella	Finfish	Keluthi	Black fin sea catfish
Trachinocephalus myops	Fin fish	Thannipanna	Snack fish
Cephalopholis formosa	Finfish	Kalava	Blue lined hind
Sillago sihama	Finfish	Kelakka	Silver sihago
Lates calcarifer	Finfish	Koduva	Barramundi
Lutjanus malabaricus	Finfish	Seppili	Malabar blood snapper

Table 2. Formaldehyde content in selected sea foods

Fish samples	Amount of formaldehyde (mg/kg) in iced fresh fishes	Amount of formaldehyde (mg/kg) in un iced fishes
Chirocentrus dorab	0.2	10.8
Arius jella	0.06	16.4
Trachinocephalus myops	0.002	10.64
Cephalopholis formosa	0.2	15.70
Sillago sihama	0.001	17.14
Lates calcarifer	0.01	12.44
Lutjanus malabaricus	0.32	18.75

result showed that there was considerable decrease of bacterial load in iced samples and all the fishes showed only less than 30 colonies that is too low to count. But in the case of un-iced samples higher number of bacterial colonies were noted and it was very high of about 108 in *Arius jella*, *Lutjanus malabaricus, Lates calcarifer* and *Cephalopholis formosa.*

Discussion

Formaldehyde is naturally produced and developed by an enzymatic reduction during postmortem of marine fish and crustaceans. The enzymatic reduction leads to the breakdown of trimethylamine oxide to formaldehyde and dimethylamine (Badii and Howell, 2002). This compound accumulates during improper storage of fish reacts with protein and subsequently causes protein denaturing and muscle toughness (Sotelo *et al.*, 1995). Fish containing the highest levels of formaldehyde between 10 - 20 mg/kg may not be considered palatable as a human food source (Yasuhara and Shibamoto, 1995). Earlier studies have shown that Trimethylamine oxide (TMAO) in fish breakdowns into dimethylamine (DMA) and formaldehyde more rapidly which are usually high in visceral organs (Ali *et al.*, 2008). Recently, there is an increasing demand on food safety research for global food production, processing, distribution and preparation in order to ensure a safer global food supply (Scott, 2003). However, the chemical contamination presence in food can cause the major sources of food borne diseases. For example the presence of formaldehyde, great attention has been paid towards this volatile toxic compound because it is classified in the Group 1 as carcinogenic to human (IARC, 2004).

The formaldehyde content in selected sea food sample shows differences among the iced and un-iced fishes. The highest amount of formaldehyde was observed in un-iced fishes and it was high in *Lutjanus malabaricus* (18.75 mg/kg). During the ageing and deterioration of fish flesh, formaldehyde is formed (Nordiana *et al.*, 2011). Formaldehyde is chemically reactive and crosslink with myofibrillar proteins of fish which causes the flesh texture become tougher and also reduces the water holding capacity (Haard and Simpson, 2000) leading to lower acceptability as well as functionality (Li *et al.*, 2007). Formaldehyde reacts with protein molecules and link together contributing to the hardening of protein (Stoker *et al.*, 2004). Yeasmin *et al.* (2010) found that the muscle texture of the Rohu Fish *(Labeo rohita)* became slightly hard after dipping the fish in 5% of formalin for 5 minutes.

Present study showed that there is formaldehyde content in fish samples. However, formaldehyde content among all iced and un-iced samples were higher than the limits set by Malaysian food regulation act (1885) that the maximum limit value for formaldehyde in fish and fishery products are 5 mg/kg. Xuang *et al.* (2009) reported the United States Environmental protection agency has fixed the maximum limit of 2 mg/kg, and the Ministry of Agriculture of China it was 10 mg/kg, the Italian ministry of health fixed the same as China, where

Table 3. pH and volatile amines of the selected sea food samples

Fish samples	pH in iced fresh fishes	pH in un-iced fishes	TVB-N (mg/100g) in iced fresh fishes	TVB-N (mg/100g) in un- iced fishes
Chirocentrus dorab	7.02	7.89	3.3	38.5
Arius jella	7.0	7.94	3.55	40.6
Trachinocephalus myops	7.01	8.0	2.56	35.5
Cephalopholis formosa	7.01	6.25	1.49	38.6
Sillago sihama	7.02	7.30	1.23	32.9
Lates calcarifer	7.0	7.90	1.0	39.2
Lutjanus malabaricus	7.0	6.11	3.12	41.5

Table 4. Comparison of sensory quality of iced fresh sea foods and uniced fishes

Storage condition	Sensory quality	Overall quality
Iced fresh fishes	Bright appearance. The texture was soft, firm and elastic. The odour of the neck and gill were natural and gills were slightly pinkish. The surface of the fish was shining, iridescent and there is a no incidence of any slime in the fish body.	Excellent
Un iced fishes	Slight loss of natural odour and strong pungent odour. The gill becomes slightly blackish and slight loss of brightness. The eye became sunken and the texture is slightly hard with moderate suppleness.	Good

Table 5. Microbial properties of selected sea food samples

Fish samples	TPC (No. of colonies) in iced fresh fishes	TPC (cfu/g) in un iced fishes
Chirocentrus dorab	<30	3.2×10^7
Arius jella	<30	1.0×10^8
Trachinocephalus myops	<30	9.7×10^5
Cephalopholis formosa	<30	3.7×10^8
Sillago sihama	<30	5.5×10^7
Lates calcarifer	<30	1.7×10^8
Lutjanus malabaricus	<30	1.3×10^8

as Yasuhara and Shibamoto (1995) has fixed as 10 - 20 mg/kg. Nash's reagent method was widely used to determine the formaldehyde content in fish (Abu Samah *et al.*,2006) and in the present study also Nash reagent method was followed. Formaldehyde content in varies with types of fish have been detected in tissues of yellow banded scad (Low *et al.*, 1990), cod (Bianchi *et al.*, 2007), yellow tail scad (Noordiana *et al.*, 2011), kawakawa *(Enthynnus affinis)* and mackerel species *(Rastrelliger faughni)* (Abu Samah *et al.*, 2006).

According to Malaysian Food Regulation act (1985), Regulation acts 148 and 159, only smoked fish and meat are permitted to incidentally absorb formaldehyde during processing in a proportion not exceeding 5 mg/kg. In 2002, WHO reported that high concentration of formaldehyde was found in improperly preserved fish. Formaldehyde can be also observed in fishery products sold by fishmonger and fish vendors who tend to carelessly add the formaldehyde to the fish in order to prolong the shelf life and to make the fish look fresh. But in the present study formaldehyde production in fish was only naturally due to the enzymatic reaction not from the external source. The formaldehyde from fish body to the human beings through the consumption of spoiled fish causes inflammation of the linings of the mouth, throat and gastrointestinal tract and eventual ulceration and necrosis of the mucous lining of the gastrointestinal tract (Owen *et al.*, 1990; Sindhu and Sidhu, 1999; Yanagawa *et al.*, 2007) and in case of chronic exposure, formaldehyde has the potential to cause cancer and a variety of unknown pathology (Wippermann *et al.*, 1999;Vaughan *et al.*, 2000; Hildesheim *et al.*, 2001). Thus, if the fish with high formaldehyde is consumed by humans for a long period, they may encounter a host of biochemical as well as pathological abnormalities and subsequent health hazards remain unclear.

According to Jaafar *et al.* (2013), formaldehyde content in all marine fish are influenced by various factors such as the amount of dark muscle, the amount of substrate, cofactors, temperature, storage time and the degree of combination of the flesh. There are various amounts of formaldehyde in fish which contain dark muscle were reported. For example, in mackerel species, yellow banded scad and Tongkol species the range of formaldehyde values were 0 - 2.2

mg/kg (Low *et al.*,1990), yellow tail scad had a range of 0.44 - 0.94 mg/kg. In mackerel the formaldehyde values ranged between 1.12 - 1.74 mg/kg from different wet market (Noordianna *et al.*, 2011). Kawakawa fish *(Enthynnus affinis)* had 0.443 mg/kg while in fresh and it was 2.5 mg/kg in the samples of wet market (Jaafar *et al*., 2013). In the present study fresh fishes having formaldehyde content was more or less similar to the above citations, but in the un-iced fishes the formation of formaldehyde was more because of microorganism's activity. In the present study all the experimental fishes are coming under the fatty fish category, so more lipid in the fishes oxidizes to produce Trimethylamine oxide compound and the enzyme breaks down the component into trimethylamine (TMA), dimethylamine (DMA) and formaldehyde, where the formation of trimethylamine and formaldehyde are responsible for the fishy odour (Cui *et al*., 2007).

Formaldehyde is also sold in solid form polymer. Formalin is usually added to seafood after caught and for storage during transportation in order to preserve them from pathogens. It was recently used as preservatives in fish and seafood due to its anti-microbial property (Norliana *et al*., 2009). Other than that, some countries use formaldehyde in processed seafood as food additive such as in herring and caviar (WHO, 2002). But in the present study the raw materials were taken from the landing centre itself and externally there was no formaldehyde addition for preservation. In addition to that formaldehyde content was only observed in un-iced fishes, it is evident exposure to ambient temperature without icing is responsible for formaldehyde formation in the experimental fishes. The observed formaldehyde in all the fishes is produced naturally through enzymatic degradation of nutrients by microorganisms.

The pH was higher in *Trachinocephalus myops*(8) than *Arius jella* (7.94), *Lates calcarifer* (7.90), *Chirocentrus dorab* (7.89) and *Sillago sihama* (7.30). The lowest pH was found in *Cephalopholis formosa* (6.25) and *Lutjanus malabaricus* (6.11). There were significant differences among the highest and lowest pH values of iced and un-iced fish samples. The typical pH of the live fish muscle was 7.0 whereas due to the glycogen in the muscle of the fish being metabolized into lactic acid which account for the low pH recorded. The production of alkaline bacterial metabolites in spoiled fish was coincided with the increase of the pH of the samples (Kyrana *et al*., 1997). Kyrana *et al.* (2002) reported that the increase in pH in fish muscle may be due to the storage period which was also associated with the state of rapid spoilage of fish. The fish stored for a long period in ambient temperature in un-iced condition is the reason for the increasein the pH values which leads to higher amount of volatile amines with formaldehyde production.

Formaldehyde develops after postmortem in marine fish through the enzymatic reduction of trimethylamine Oxide (TMAO) to equimolar amounts of formaldehyde and dimethylamine (Sotelo *et al*., 1995). Different amount of formaldehyde has been observed among the species as well as between iced and un-iced fishes. This condition can be explained by the different level of trimethylamine - Nitrogen from species to species and also enzymatic reaction to reduce trimethylamine oxide to formaldehyde and dimethylamine that occurs in sea food. Different result was observed in fresh sea food because of the reduction of trimethylamine oxide depends on the bacterial activity (Binachi *et al*., 2007). Changes in total volatile base - nitrogen values in both iced and un-iced fishes were observed in the present study. In iced fresh fish, the initial total volatile base - nitrogen values were 1.0 - 3.55 mg/100g which increased considerably to 32.9 - 41.5 mg/100g in un-iced fishes. The deteriorative changes in fish muscle are possibly associated with the hydrolysis of cellular compounds by intracellular enzyme and bacterial enzyme during post-mortem period. Hydrolysis of protein and other nitrogenous compounds by autolytic enzyme together with bacterial action leads to an increased total volatile base - nitrogen value (Baldwin, 1961). The results of the present study indicated that the low bacterial load in fresh fish may be the main reason for low total volatile base - nitrogen value. There is a direct relationship between the bacterial load and TVB-N value and total volatile base - nitrogen value increases with the higher bacterial load. Leitao and Rios (2000) reported that total volatile base - nitrogen content equal to 8.7 mg/100g in fresh *M.rosenbergii* and 26 mg/100g in un-iced fishes. Acceptable limit of total volatile base - nitrogen for fish is 30 mg/100g g muscle was reported by Connel (1995). Thus, the present result reveals that the total volatile base - nitrogen value exceeded the acceptable limit in un-iced fishes. The present study result indicated that the accumulation of free formaldehyde in fish flesh during un-iced condition is based on the storage time dependent decay of substrate saturated fish muscle Trimethylamine-nitrogen activity by trimethylamine oxidase.

The sensory characteristics of fresh fish gills were red and the eyes were in full bloom and bright in appearance. The eyes were bulging with protruding lens and transparent. There was no slime on the surface of the body, and flesh was firm and elastic. In the present study above all the characters were observed in the iced fresh fishes and are sensorially in excellent condition. Compared to iced fresh fish, the un-iced fish gills became slightly blackish and there was slight loss of brightness. There was loss in natural colour and change in odour. Fishes were found emitting moderate to strong sour odour. Discolouration of the flesh with definite dullness and loss of brightness in general appearance was evident among the samples under investigation. The eye became sunken and the muscle texture became slightly hard compared to fresh fish. This is an indication of the formalin produced fishes can be easily identified by the sensory quality. Fishes with less amount of formaldehyde cannot be detected through any objectionable sensory quality. Formaldehyde was produced through the enzymatic changes in fish, but it was palatable to the tongue only in higher concentration above 20 mg/kg. So that consumers cannot feel the palatability of formaldehyde in un-iced fishes.

In entire transportation chain and in markets preservation of fish without ice might be the major cause of quality deterioration as the sign of spoilage was evident. These findings were in agreement with the number of earlier studies, where irreversible quality related problem of fin and shellfishes were reported due to un-iced and improper post-harvest handling (Barlie *et al*., 1985 and Reilly *et al*., 1985). In some other studies carried out on oraganoleptic quality assessment suggest that the shelf-life of sea food during ice storage vary with species to species and some factors like chemical composition, ambient temperature, post harvest handling and icing method (Dawood *et al*., 1986; Kodoria and Rojas, 1996; Rahman *et al*., 2001). It was found by Shamshad *et al*., (1990) that shelf-life of sea food ranged from 7 hours at 35°C to 13 days at 0°C. Fonseka and Ranjini (1994) also reported similar results where, under ambient temperature, sea food reached beyond acceptable condition just after 12 hours of storage but it attained an acceptable shelf life of 15 days under iced condition. But in the present study, storing in ambient temperature for 7 hours have high formaldehyde production and it affects the sensory quality of fishes. Sotelo *et al.* (1995) reported formaldehyde production in fish muscle caused by chemical and physical changes during un-iced condition leads to loss of quality, reflected mainly by an unacceptable texture as well as an undesirable flavor, odour and colour.

The quantitative changes in bacterial load in iced and un-iced fishes were also assessed. Initial bacterial load of iced fresh fish was <30 for all the fishes. But in un-iced fishes the total plate count was increased in all the seven experimental fishes and it ranges from 10^5 - 10^8 and it may be due to enzymatic reduction of bacteria responsible for higher production of trimethylamine oxide by improper handling and un-iced storage of fishes. Premaratne *et al.* (1986) reported an initial total plate count of *M. rosenbergii* was 1.6×10^2 which was increased to 8.6×10^7 after exposed to ambient temperature for 8 hours. Angel *et al.* (1981) also found the fish samples are in acceptable condition only if the fishes were immediately iced.

According to Huss (1995) during the aerobic storage, specific spoilage bacteria should be around 10^7 to 10^8CFU/g^{-1} to produce significant amount of chemical compounds especially volatile amines (TMA-N, TVB-N, FA) associated with spoilage. Volatile amines producing bacteria were indicated by the activity of amino decarboxylation shown by their corresponding amino acids. Numerous bacteria have been reported to possess trimethylamine oxide activities. The result showed that biogenic amine producing bacteria grew between 5 to 8 log CFU/g^{-1} during delayed icing of fishes. Our results are in agreement with Pons-Sánchez-Cascado *et al.* (2005), who recorded volatile and biogenic amine forming bacteria in anchovies stored without ice. Ababouch *et al.* (2007) also found volatile amine producing bacteria in sardine stored in ambient temperature of 25 - 28°C. Microorganisms are present on the external surface (including slime) and in the gut of fish. After death they gradually invade the flesh while producing an ideal media for their growth and multiplication and produced volatile amines especially formaldehyde.

There are a few suggestions that can be considered to overcome the formaldehyde content in fish. Several methods have been taken and proposed in order to control or reduces formaldehyde content in fish. Consumers choose the fish that are fresh and avoid with those of unusual smell and also avoid time delayed preserved fishes. Freshness is a property of fish that has a considerable influence on its quality. Besides, public also advised to immediate icing is essential for keeping the sea food from enzymatic deterioration. After icing proper cooking is essential for the removal of formaldehyde while formed during icing storage. The EPA'S Exposure Factors Hand book (1997) has reported cooking the fish will result in weight loss due to moisture and fat loss which subsequently decreases the formaldehyde concentration in cooked fish. The formaldehyde concentration was decreased after roasting and boiling. The decrease of formaldehyde content was due to the evaporation of the sample during the cooking process (Bianchi *et al*., 2007).

Conclusion

This study showed that there was high formaldehyde content in un-iced fishes. However, formaldehyde content of all the iced fresh fish species was still lower than 5 mg/kg where as the un-iced fishes had higher formaldehyde content. However, there were some limitations in this study such as the temperature change, time of storage and handling could possibly influence the concentrations of formaldehyde since it is a volatile compound. There is no adverse health effects on human due to the (<5 mg/kg) formaldehyde contaminated fish consumption based on the risk assessment calculation. Thus the study proves fishes from landing center can be considered safe for consumption because of fresh condition. Further more immediately proper icing can potentially reduce the formation of formaldehyde.

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