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Formaldehyde content and quality characteristics of selected fish and seafood from wet markets

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Abstract: Formaldehyde was used by fishermen and fish vendors to preserve the freshness and quality of fish and seafood. The study was undertaken to determine the formaldehyde content and quality characteristics of fish and seafood from wet markets. Formaldehyde content was in the range of 0.38 to 15.75 µg g^{-1}. Three types of biogenic amines (histamine, putrescine and cadaverine) were detected from all samples in which histamine content ranged from 0.25 to 1.97 µg g^{-1}, putrescine from 0.33 to 9.09 µg g^{-1} and cadaverine from 0.34 to 5.81 µg g^{-1}. Amino acids as biogenic amines precursor were also determined with lysine ranged from 12.75 to 28.80 mg g^{-1}, arginine from 8.17 to 27.83 mg g^{-1} and histidine from 1.93 to 10.14 mg g^{-1}. As for the microbiological analyses, total plate counts for all fish types ranged from 5.68 to 7.13 log cfu g^{-1} and the proteolytic counts from 5.12 to 6.91 log cfu g^{-1}. Samples were also analyzed for the presence of putrescine/ cadavarine/ histamine producing bacteria where the counts ranged from 3.50 to 6.52 log cfu g^{-1}. The pH of all selected fish ranged from 6.25 to 7.28. There was no significant difference ($p>0.05$) among fish purchased from different wet markets. Hence this study suggested that fish and seafood from wet markets can be considered in good quality since the formaldehyde content and microbiological counts were still below the permissible limits.

Keywords: fomaldehyde, fish, seafood, quality characteristic, wet market

Introduction

Fish and seafood are an important part of a healthy diet and are considered as the biggest source of protein (Ashie et al., 1996). By composition, fish contain fat, free amino acids and water which is susceptible to spoilage by microorganisms and biochemical reactions during post mortem process (Fernandes and Venkatraman, 1993; Ismail, 2005). Thus, fish and seafood are very perishable and can only be kept fresh in ice for 8 to 14 days depending on the species. In order to keep the freshness of fish and seafood, fishermen and fish vendors tend to carelessly use formaldehyde as preservation agent.

Formaldehyde is the simplest member of aldehyde family but a very reactive chemical, where the gaseous form is known as formaldehyde and the liquid form as formalin. Characteristically, formaldehyde is a colorless, strong-smelling, irritating, poisonous, and flammable gas and its chemical formula is CH_2O which is also known as methanal, commonly produced by the oxidation of methanol (WHO, 2002). Formaldehyde is used as disinfectant and preservative, and also widely used in textiles, plywood, papers, insulators, plastics and paint industries. Recently, International Agency for Research on Cancer (IARC) has classified formaldehyde as a Group 1 carcinogenic to humans (2004). According to the United States Environmental Protection Agency (EPA), maximum daily dose reference (RfD) for formaldehyde is 0.2 µg g^{-1} body weight per day (Wang et al., 2007). In 1985, Italian Ministry of Health has proposed formaldehyde values of 60 µg g^{-1} and 10 µg g^{-1} for *Gadidae* and crustaceans, respectively (Bianchi et al., 2007).

As formaldehyde is carcinogenic to human, it is important to investigate the content of formaldehyde in fish and seafood since they are claimed to be the major source of protein, and therefore providing more information to the production of safe and hygienic food. According to Malaysian Food Regulations 1985, Regulation 148 and 159 (2006), only smoked fish and meat are permitted to incidentally absorb formaldehyde during processing in a proportion not exceeding 5 µg g^{-1}. However, for fresh fish, the permitted amount of formaldehyde present in fish is not specified.

Formaldehyde may be formed during the ageing and deterioration of fish flesh. However, high levels

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of formaldehyde do not accumulate in the fish tissues due to subsequent conversion of formaldehyde to other chemical compounds (Tsuda et al., 1988). Besides natural formation of formaldehyde in fish and seafood by enzymatic reaction, other biochemical reactions can also occur such as oxidation of lipids as a result of microorganism activities. This will eventually result in physical damage of fish or production of chemical metabolites such as biogenic amines or other unpleasant compounds (Gram et al., 2002; Arashisar et al., 2004).

Changes in pH, microbial numbers, and free amino acids have been proposed and/or used as indices of the freshness of iced aquatic species (Fatima and Qadri, 1985). It is not known however, which chemical indicators are applicable for the detection of decomposition in fish and seafood although a number of compounds or groups of compounds have been suggested. Arginine, cadaverine, putrescine and histamine (biogenic amines) formed by the decarboxylation of free amino acids have also been suggested as chemical indicators of decomposition (Hollingworth et al., 1990; Özogul et al., 2002). The importance of these biogenic amines is related to food intoxication and their potential use in the assessment of food freshness and quality (Lehane and Olley, 2000).

Freshness is a property of fish that has a considerable influence on its quality (Connel, 1995). Loss of freshness followed by spoilage is the result of a complex combination of microbiological, chemical and physical processes (Pedrosa-Menabrito and Regenstein, 1990). The shelf life of freshly harvested fish depends on the bacterial flora, storage temperature, handling and physiological condition of fish. However, very few findings on chemical decomposition indicators have been established and thus leads to lack of information and documentation on quality changes in fish and seafood. The purpose of this research was to determine the formaldehyde content and quality characteristics of selected fish and seafood from wet markets.

Materials and Methods

Samples preparation

Fish and seafood samples weighed between 500 to 800 g were purchased from three different wet markets. The ten species selected were mackerel (*Rastrelliger* spp.), bombay duck (*Harpodon nehereus*), jewfish (*Pennahia* spp.), threadfin bream (*Nemipterus virgatus*), hardtail scad (*Megalaspis cordyla*), black pomfret (*Parastromateus niger*), yellowtail scad (*Atule mate*), bridshrimp (*Metapenaeus lysianassa*), white prawn (*Penaeus merguiensis*) and squid *(Loligo* spp.). Each sample was deposited into sterile plastic bag and stored in ice boxes. Flesh was separated from skin and bones without damaging the gut by using sterile scalpel. A 400 g of samples were packed in sterile polystyrene boxes and stored at -20°C until further analyses.

Chemicals

Nash's Reagent (Nash, 1953) was used as an indicator by diluting 15 g ammonium acetate in a 100 ml Erlenmeyer flask with an addition of 0.3 ml of acetylacetone and 0.2 ml of acetic acid. Nash's Reagent is light sensitive and was kept in dark-glass reagent bottle at all time. Trichloroacetic acid, TCA was used to adjust the pH of fish flesh (2.32, 3, 4, 5 and 5.68) appropriately. A 0.1 N potassium hydroxide, KOH and 0.1 N hydrochloric acid, HCl have been used to adjust the pH of the distillate to be in range of 6.0 to 6.5. Three ranges of used working standard which were 0-1 mg l^{-1}, 0-5 mg l^{-1} and 0-30 mg l^{-1} were prepared from intermediate standard solution of 10 µg g^{-1} to calibrate the graph.

Formaldehyde determination

The fish samples were thawed and cut into small pieces and 30 g samples were homogenised with 60 ml of 6% w/w TCA. The mixture was filtered through a Whatman No. 1 filter paper (Whatman, Maidstone, England) and the filtrate was adjusted to pH 7.0 with 30% w/w KOH and stored in ice for 1 h. The test was performed by mixing 5 ml of the standard solution, TCA, fish extracts, 2 ml Nash's Reagent and was heated in water bath at 60°C for 30 min. The absorption at 415 nm was measured immediately by UV/vis spectrophotometer (Thermo Fisher Scientific, Waltham, MA).

pH determination

A 10 gram of fish flesh was weighed and homogenized thoroughly with 100 ml distilled water for 5 minutes. The pH of supernatant was recorded.

Amino acid determination

Samples and standard amino acids solution were subjected to hydrolysis by mixing 0.3-0.5 g of samples with 15 ml of 6 N HCL before ultrasonically submerged for few minutes to remove dissolved oxygen which was later flushed with nitrogen gas. The samples were heated in oven for 24 h at 110°C. The samples were left to cool at room temperature and transferred into 50 ml volumetric flask. Sample solution was filtered through filter paper, discarding the first 2 ml initial filtrate. For standard amino acid, 10 ml internal standard (1 ml = 2.5 mole AABA) was

added before being transferred into 50 ml volumetric flask. Sample filtrate was stored for four weeks at -20°C. Analyses was performed by Shidmadzu high performance liquid chromatography (Shimadzu, Kyoto, Japan) consisting of a Model SPD-6A with UV detector set to 254 nm, and a Model C-R6A chromatopac integrator. A LiChrosopher 100 RP-18 reverse phase column (5 µm, 125 x 4 mm, E. Merck, Damstadt, Germany) was used for the analysis of amine standard solution samples. For HPLC analysis, a small volume of the above filtrate was filtered through 0.2 µm aqueous syringe filter, with 25 mm diameter. A 10 µl sample filtrate was pipetted into tubes (measuring 6 x 50 mm). Tubes were placed into reaction vial and vacuumed to 100 millitorr before adding 20 µl of fresh redrying solution (consisting of methanol: water: ethylamine at a ratio of 2:2:1 v/v) and was then mixed thoroughly. Samples were vacuumed to 70 millitorr before adding fresh 20 µl of derivation reagent (consisting of methanol: water: triethylamine: PITC at a ratio of 7:1:1:1), mixed and tubes returned to reaction vial and valve closed. They were left to stand for 20 mins at room temperature and vacuumed again to 50 millitorr. For immediate analysis of sample, 100 ml sample diluents (consisting Na_2HPO_4) was added and mixed. Limited volume of 20 µl was used as injection volume into HPLC for samples whilst injection volume for standard solution was 8 µl. These samples were left to stabilize for a few weeks at temperature between -10 to -20°C.

Biogenic amine determination

A slightly modified method by Yen and Hsieh (1991) was used to determine the biogenic amines (histamine, cadavarine, and putrescine) in fish and seafood samples as described below:

Mixture of amines standard solution consisting of putrescine dihydrochloride (182.9 mg), cadaverine dihydrochloride (171.4 mg), and histamine dihydrochloride (165.7 mg), were dissolved in 10 ml deionised water until final concentration of each amine is 10 mg l^{-1} solution. The amine standard solutions were freshly prepared every time prior to each experiment and were injected accordingly for samples comparison. Each stock solution of standard amine was freshly prepared in deionised water in duplicates for two levels to cater for different levels of amines found in seafood in following concentration: (0, 20, 40, 60, 80, 100 µg g^{-1}) for all cadaverine, histamine and putrescine. The amines solution was analyzed using HPLC and the average peak area of chromatography from two injections of each duplicates standard were recorded. Linear regression was determined by the least squares method and the correlation coefficient was calculated. A 20 g of seafood flesh were transferred into a 250 ml centrifuge tube and homogenized with Polytron type homogenizer (LH, Ltd England) with 50 ml 6% trichloroacetic acids (TCA) for 3 min. The homogenate was centrifuged at 11000 rpm for 10 min at 4°C to allow precipitation and was filtered through Whitman No.1 filter paper. The filtrate was placed in a volumetric flask and made up to 100 ml with 6% trichloroacetic acid. Each extract (2 ml) was derivatized with benzoyl chloride as described below.

The benzoyl derivative of amines was prepared according to Redmond and Tseng (1979) with some modification. A volume of 1 ml of 2 M sodium hydroxide was added to 50 µl of standard amine solution, followed by 10 µl benzoyl chloride. The mixture was mixed on a vortex mixer and left standing for 20 min. Two ml of saturated sodium chloride was added and followed by extraction with 4 ml diethyl ether. After centrifugation at 10000 rpm for 10 min at 4°C and separation by washing twice with deionised water using a separation funnel, the upper organic layer was transferred into clean tube and evaporated to dryness in stream of nitrogen gas. The residue was dissolved in 500 µl and 5 µl aliquots were injected for HPLC analysis.

Each derivatized amine standard solution consisting of histamine dihydrochloride, putrescine dihydrochloride and cadaverine dihydrochloride was injected and analyzed individually and finally run simultaneously to determine their retention times. Isocratic elution system was used for the analyses of these amines. The mobile phase of acetonitrile-water (60:40 v/v) at flow rate of 1.1 per min resulted in clear separations and resolution for all of the three biogenic amines tested.

Microbiological analysis

Two replicated pooled samples for both types of storage were blended using stomacher bags for 60 s. One gram from the pool sample was removed and diluted with 9 ml sterile peptone water (0.1% peptone water +0.9% saline). A 0.1 ml aliquots was spread onto different selective agars namely plate count agar for total aerobic counts, arginine decarboxylase agar and ornithine decarboxylase agar for putrescine producing bacteria, lysine decarboxylase agar for cadaverine producing bacteria, and modified Niven's media for histamine producing bacteria. Proteolytic counts were determined by count on the skim milk agar. All agar plates were incubated for 48 h at 37°C for mesophilic counts before the colony was counted using a colony counter and reported as colony forming units per gram (cfu g^{-1}).

Statistical analysis

All experiments were done in triplicate. The data were recorded as means ± standard deviations and were analyzed with SPSS (version 11.0 for Windows, SPSS Inc., Chicago, IL, USA), and the statistical significance was determined at $P<0.05$.

Results and Discussions

Formaldehyde content in selected fish and seafood

The formaldehyde content in selected fish and seafood from different wet markets were summarized in Table 1. The highest amount of formaldehyde content was in bombay duck at 15.75 μg g^{-1} while threadfin bream contained the lowest amount of formaldehyde at 0.38 μg g^{-1}. There were significant differences ($p<0.05$) among the highest and lowest amount of formaldehyde in the samples. White prawn, bridshrimp, mackerel, yellowtail scad, jewfish, hardtail scad, black pomfret and squid, contained 0.69, 1.08, 1.37, 0.72, 0.87, 0.87, 0.68 and 0.49 μg g^{-1} of formaldehyde respectively. There was no significant difference ($p>0.05$) among threadfin bream with squid, yellowtail scad and jewfish with black pomfret, and jewfish with hardtail scad. Meanwhile the formaldehyde content in fish and seafood sold in wet market P2 was the highest, at 2.62 μg g^{-1}. These results might indicate that fish and seafood sold in wet market P2 have been stored frozen for a longer period of time compared to P1 and P3. Sotelo et al. (1995) found that formaldehyde accumulated during frozen storage, reacted with protein and subsequently caused protein denaturation of the muscle.

pH

Table 2 shows the results for pH of the selected fish and seafood from different wet markets. The highest pH recorded was in black pomfret sample, at 7.28. The lowest pH was found in the mackerel sample, at 6.25. There were significant differences ($p<0.05$) among the highest and lowest pH values and among different wet markets. However, there was no significant difference ($p>0.05$) between pH of the white prawn and that of jewfish. Meanwhile the pH value in fish and seafood sold in wet market P2 was the lowest, at 6.73. The lowest pH of the fish muscle was due to the glycogen in the muscle which would have been metabolized to lactic acid by then and would account for the low pH recorded. The typical pH of live fish muscle was ≈7.0. The production of alkaline bacterial metabolites in spoiled fish which coincided with the increase in Total Volatile Basic Nitrogen (TVB-N) might increase the pH level of samples (Kyrana et al., 1997). Kyrana and Lougovois (2002) also found that the increase in pH in fish muscle occurred due to the storage period which was also associated with the state of rapid spoilage of fish. Thus the fish might have been stored for a long period before being distributed to wet markets.

Amino acids

Table 3 shows amino acids content in the selected fish and seafood from different wet markets. Three types of free amino acids determined were precursors for biogenic amines, namely lysine, histidine and arginine. Arginine usually decomposes into ornithine and agmatine (Halász et al., 1994). Ornithine can be converted into putrescine and lysine into cadaverine while histamine is also formed mainly through the decarboxylation of histidine by exogenous decarboxylase released by many bacterial species known to possess histidine decarboxylase (Tsai et al., 2007).

From the results, bridshrimp contained the highest amount of lysine (28.80 mg g^{-1}) and arginine (27.83 mg g^{-1}). On the other hand, white prawn showed the lowest amount of lysine (12.75 mg g^{-1}), arginine (8.17 mg g^{-1}) and histidine (1.93 mg g^{-1}). There was significant difference ($p<0.05$) in the amount of histidine among samples. The highest histidine content was found in the mackerel sample which is 10.14 mg g^{-1}. Most of the samples contained low amount of histidine which was below 10 mg g^{-1} as compared to other two types of amino acids. This indicated that the decarboxylation of this amino acid into histamine by microbe was less. The amount of free amino acids in fish muscle varied between each species much similar to the research conducted by Bramstedt (1962). Amino acids are broken down during bacterial spoilage to malodorous compounds such as putrescine and cadaverine (Ingram and Dainty, 1971; Pivarnik et al., 1998).

Biogenic amines

Three types of biogenic amines were determined in this study which were histamine, cadaverine and putrescine. Table 4 shows the content of biogenic amines among different types of sample from three wet markets. Variation was also observed in the formation of amines among the species. Earlier reports stated that the histamine:cadaverine ratio varies among species (Middlebrooks et al., 1988). Bridshrimp showed the highest content of putrescine at 9.09 μg g^{-1}. This level correlated with the higher amount of arginine in bridshrimp where arginine is a precursor for the formation of putrescine. The yellowtail scad sample showed the highest cadaverine content at 5.81 μg g^{-1}.

Histamine content was highest in white prawn, at

Table 1. Formaldehyde content in selected fish and seafood from different wet markets

FISH AND SEAFOOD TYPE	AMOUNT OF FORMALDEHYDE (μg g^{-1})			
	P1*	P2	P3	Mean
Mackerel	1.26±0.12	1.74±0.07	1.12±0.09	1.37±0.09^{e}
Threadfin bream	0.40±0.02	0.42±0.08	0.34±0.05	0.38±0.06^{a}
Bombay duck	18.36±0.80	18.35±0.66	10.53±0.29	15.75±0.58^{f}
Yellowtail scad	0.44±0.06	0.78±0.12	0.94±0.19	0.72±0.12^{b}
Jewfish	0.48±0.11	1.35±0.25	0.78±0.11	0.87±0.16^{bc}
Hardtail scad	0.68±0.07	0.88±0.08	1.05±0.04	0.87±0.06^{c}
Black pomfret	0.53±0.06	0.67±0.06	0.85±0.03	0.68±0.05^{b}
Squid	0.43±0.05	0.56±0.03	0.47±0.03	0.49±0.04^{a}
White prawn	0.72±0.06	0.70±0.09	0.66±0.05	0.69±0.07^{d}
Bridshrimp	1.58±0.12	0.80±0.09	0.87±0.12	1.08±0.11^{d}
Mean	2.49±0.15^{A}	2.62±0.15^{B}	1.76±0.10^{C}	

*P: Different wet market

t Mean±SD

^{A,B,C} means with different superscripts within column differed significantly between market ($p<0.05$)
^{a,b,c,d,e,f} means values with different superscripts within row differed significantly among fish and seafood types ($p<0.05$)

Table 2. pH of fish and seafood from different wet markets

FISH AND SEAFOOD TYPE	pH			
	P1*	P2	P3	Mean
Mackerel	6.24±0.02	6.27±0.02	6.23±0.01	6.25±0.02[a]
Threadfin bream	6.96±0.01	6.87±0.00	6.88±0.01	6.90±0.01[e]
Bombay duck	6.40±0.02	6.40±0.02	6.06±0.04	6.29±0.03[a]
Yellowtail scad	6.44±0.03	6.88±0.00	6.28±0.13	6.53±0.05[b]
Jewfish	6.69±0.02	6.56±0.01	7.27±0.01	6.84±0.01[cd]
Hardtail scad	6.43±0.03	6.47±0.06	6.65±0.02	6.65±0.03[b]
Black pomfret	7.31±0.03	7.36±0.04	7.17±0.03	7.28±0.03[g]
Squid	6.71±0.00	6.62±0.03	7.33±0.03	6.80±0.02[c]
White prawn	6.71±0.00	6.91±0.04	6.92±0.05	6.84±0.03[d]
Bridshrimp	7.25±0.03	7.14±0.12	7.26±0.03	7.22±0.06[f]
Mean	6.75±0.02[A]	6.73±0.03[B]	6.81±0.04[C]	

*P: Different wet market

t Mean±SD

[A,B,C] means values with different superscripts within column differed significantly between market ($p<0.05$)

[a,b,c,d,e,f] means values with different superscripts within row differed significantly among fish and seafood types ($p<0.05$)

Table 3. Amino acids content in fish and seafood from wet markets

FISH AND SEAFOOD TYPES	AMINO ACIDS CONTENT, mg g^{-1}				
		P1*	P2	P3	Mean
Mackerel	HTD	10.20±0.04	10.27±0.01	9.96±0.08	10.14±0.04^{i}
Threadfin bream		2.89±0.00	2.90±0.03	3.49±0.05	3.09±0.03^{b}
Hardtail scad		3.98±0.10	4.42±0.04	3.93±0.02	4.11±0.05^{e}
Jewfish		3.74±0.06	3.89±0.14	3.06±0.01	3.56±0.07^{c}
Black pomfret		4.63±0.01	2.46±0.02	3.86±0.03	3.65±0.02^{d}
Yellowtail scad		6.48±0.02	6.95±0.15	6.55±0.06	6.66±0.08^{h}
Bombay duck		4.19±0.01	4.71±0.25	4.22±0.01	4.38±0.09^{f}
Squid		5.87±0.04	5.67±0.09	5.70±0.03	5.75±0.06^{j}
White prawn		1.64±0.01	2.60±0.04	1.56±0.00	1.93±0.02^{a}
Bridshrimp		6.86±0.02	5.61±0.17	6.84±0.03	6.44±0.07^{g}
Mackerel	ARG	12.28±0.03	12.38±0.03	12.06±0.04	12.24±0.03^{e}
Threadfin bream		7.80±0.05	8.85±0.08	8.87±0.12	8.51±0.09^{b}
Hardtail scad		9.45±0.07	10.81±0.97	9.13±0.06	9.80±0.36^{c}
Jewfish		11.77±0.03	12.56±0.13	10.99±0.01	11.77±0.06^{d}
Black pomfret		13.32±0.11	10.51±0.11	13.02±0.01	12.28±0.08^{e}
Yellowtail scad		11.66±0.13	11.62±0.14	11.68±0.04	11.65±0.10^{d}
Bombay duck		14.49±0.04	15.82±0.77	15.70±0.24	15.34±0.35^{f}
Squid		23.06±0.08	23.44±0.33	22.97±0.12	23.16±0.18^{g}
White prawn		5.83±0.03	11.63±0.04	7.06±0.27	8.17±0.12^{a}
Bridshrimp		30.97±0.07	21.48±0.13	31.05±0.30	27.83±0.17^{h}
Mackerel	LYS	20.04±0.09	19.15±0.02	19.81±0.12	19.67±0.08^{f}
Threadfin bream		12.97±0.12	14.52±0.09	16.33±0.15	14.61±0.12^{b}
Hardtail scad		14.85±0.07	18.90±0.18	17.92±0.01	17.22±0.09^{c}
Jewfish		18.20±0.05	19.02±0.15	14.35±0.09	17.19±0.10^{c}
Black pomfret		20.55±0.70	15.59±0.16	18.96±0.25	18.37±0.37^{d}
Yellowtail scad		18.71±0.15	19.41±0.29	19.53±0.13	19.22±0.19^{e}
Bombay duck		20.18±0.01	21.59±0.06	21.25±0.06	21.01±0.04^{i}
Squid		21.54±0.04	23.43±0.07	21.98±0.01	22.31±0.04^{j}
White prawn		18.55±0.24	11.61±0.18	8.09±0.04	12.75±0.15^{a}
Bridshrimp		30.55±0.29	25.22±0.21	30.64±0.25	28.80±0.25^{k}

*^{P}: Different wet market

t Mean±SD

^{a,b,c,d,e,f,g,h,i,j,k} means values with different superscripts within row differed significantly among fish and seafood types ($p<0.05$)

^{HTD}: Histidine

^{ARG}: Arginine

^{LYS}: Lysine

Table 4. Biogenic amines content in fish and seafood from wet markets

FISH AND SEAFOOD TYPES		BIOGENIC AMINES CONTENT, µg g^{-1}			
		P1*	P2	P3	Mean
Mackerel	**PUT**	ND	ND	ND	ND
Threadfin bream		2.43±0.02	0.81±0.01	0.81±0.41	1.35±0.15^{f}
Hardtail scad		0.33±0.01	0.33±0.01	0.31±0.01	0.33±0.01^{a}
Jewfish		0.44±0.06	0.44±0.06	0.46±0.00	0.44±0.04^{b}
Black pomfret		3.12±0.02	0.41±0.01	0.40±0.01	1.31±0.01^{e}
Yellowtail scad		1.52±0.01	1.56±0.00	1.50±0.00	1.53±0.01^{g}
Bombay duck		0.84±0.01	0.81±0.04	0.83±0.01	0.83±0.02^{d}
Squid		0.24±0.01	0.51±0.01	0.52±0.01	0.42±0.01^{b}
White prawn		0.69±0.01	0.62±0.05	0.65±0.02	0.65±0.03^{c}
Bridshrimp		9.69±0.02	9.16±0.01	8.42±0.09	9.09±0.04^{h}
Mackerel	**HIS**	ND	ND	ND	ND
Threadfin bream		ND	ND	ND	ND
Hardtail scad		0.61±0.01	0.60±0.01	0.57±0.02	0.60±0.01^{c}
Jewfish		ND	ND	ND	ND
Black pomfret		0.81±0.06	ND	0.71±0.01	0.51±0.03^{b}
Yellowtail scad		ND	ND	ND	ND
Bombay duck		ND	ND	ND	ND
Squid		0.75±0.08	ND	ND	0.25±0.03^{a}
White prawn		2.34±0.06	1.79±0.02	1.76±0.02	1.97±0.03^{d}
Bridshrimp		ND	ND	ND	ND
Mackerel	**CAD**	0.33±0.02	0.34±0.02	0.34±0.01	0.34±0.02^{a}
Threadfin bream		2.64±0.02	1.00±0.01	0.98±0.03	1.54±0.02^{d}
Hardtail scad		0.88±0.09	0.88±0.09	0.79±0.06	0.85±0.08^{b}
Jewfish		1.37±0.01	1.37±0.01	1.37±0.00	1.37±0.01^{c}
Black pomfret		6.92±0.11	1.77±0.02	1.73±0.02	3.47±0.05^{h}
Yellowtail scad		5.85±0.02	5.82±0.04	5.76±0.02	5.81±0.02^{i}
Bombay duck		1.70±0.02	0.46±0.01	0.47±0.01	0.87±0.01^{b}
Squid		2.87±0.03	2.86±0.01	2.86±0.01	2.86±0.02^{f}
White prawn		1.55±0.01	3.55±0.39	3.58±0.10	2.89±0.17^{g}
Bridshrimp		1.44±0.01	2.57±0.02	2.53±0.01	2.18±0.01^{f}

*P: Different wet market
t Mean±SD
ND: not detected
a,b,c,d,e,f,g,h,i means values with different superscripts within row differed significantly among fish and seafood types ($p<0.05$)
PUT: Putrescine
HIS: Histamine
CAD: Cadaverine

Table 5. Mesophilic count (at 37°C) of selected fish and seafood from wet market

FISH AND SEAFOOD TYPES		BACTERIOLOGICAL COUNT (log CFU g^{-1})			
		P1*	P2	P3	Mean
Mackerel	TPC	7.79±0.14	6.80±0.04	6.79±0.06	7.13±0.08^{g}
Threadfin bream		7.10±0.02	6.75±0.26	7.04±0.06	6.96±0.11^{ef}
Hardtail scad		6.93±0.07	6.93±0.03	6.89±0.03	6.92±0.04^{def}
Jewfish		6.86±0.04	6.87±0.07	6.84±0.02	6.86±0.04^{cde}
Black pomfret		5.67±0.11	5.68±0.16	5.69±0.18	5.68±0.15^{a}
Yellowtail scad		6.30±0.30	6.38±0.10	6.31±0.14	6.33±0.18^{b}
Bombay duck		7.09±0.16	6.10±0.03	6.09±0.16	6.43±0.12^{b}
Squid		7.05±0.05	6.83±0.30	7.28±0.04	7.05±0.13^{fg}
White prawn		6.81±0.13	6.78±0.07	6.64±0.21	6.74±0.14^{c}
Bridshrimp		6.85±0.22	6.83±0.15	6.66±0.23	6.78±0.20^{cd}
Mackerel	SM	6.01±0.38	6.04±0.36	6.13±0.50	6.06±0.41^{b}
Threadfin bream		5.96±0.45	5.96±0.45	6.51±0.20	6.14±0.37^{b}
Hardtail scad		6.69±0.09	6.25±0.16	5.86±0.87	6.27±0.37^{bc}
Jewfish		6.59±0.13	6.55±0.12	6.61±0.07	6.58±0.11^{bc}
Black pomfret		5.09±0.49	5.09±0.24	5.19±0.42	5.12±0.38^{a}
Yellowtail scad		5.17±0.60	5.32±0.73	5.40±0.80	5.30±0.71^{a}
Bombay duck		6.49±0.05	5.69±0.02	5.64±0.07	5.94±0.05^{b}
Squid		6.99±0.11	6.65±0.34	7.08±0.22	6.91±0.22^{c}
White prawn		6.55±0.21	6.55±0.20	6.54±0.18	6.55±0.20^{bc}
Bridshrimp		6.52±0.28	6.52±0.28	6.68±0.05	6.57±0.20^{bc}
Mackerel	HDA	4.58±1.89	5.00±1.73	4.63±1.96	4.74±1.86^{b}
Threadfin bream		4.63±1.93	4.72±1.87	4.74±1.89	4.70±1.90^{b}
Hardtail scad		5.10±1.69	4.86±1.94	5.08±1.69	5.01±1.77^{c}
Jewfish		5.32±1.61	4.95±1.77	5.23±1.57	5.17±1.65^{c}
Black pomfret		3.62±2.50	3.36±2.71	3.53±2.63	3.50±2.61^{a}
Yellowtail scad		4.76±1.91	4.60±1.88	4.60±2.03	4.65±1.94^{b}
Bombay duck		6.46±0.89	5.50±1.46	5.43±1.49	5.80±1.28^{d}
Squid		6.19±1.01	6.24±1.08	6.07±1.21	6.17±1.10^{e}
White prawn		5.69±1.35	5.56±1.54	5.64±1.52	5.63±1.47^{d}
Bridshrimp		5.73±1.33	5.73±1.33	5.75±1.32	5.74±1.33^{d}
Mackerel	ODA	5.72±0.20	5.79±0.10	5.86±0.24	5.79±0.18^{c}
Threadfin bream		6.30±0.19	6.03±0.33	5.94±1.01	6.09±0.51^{cd}
Hardtail scad		5.91±0.24	6.51±0.02	6.51±0.06	6.31±0.11^{de}
Jewfish		6.47±0.07	6.27±0.13	6.36±0.09	6.37±0.10^{de}
Black pomfret		4.53±0.47	4.54±0.47	4.56±0.49	4.54±0.48^{a}
Yellowtail scad		5.10±0.13	4.80±0.17	4.98±0.20	4.96±0.17^{b}
Bombay duck		6.34±0.23	5.68±0.24	5.54±0.31	5.85±0.26^{c}
Squid		6.55±0.03	6.47±0.15	6.54±0.15	6.52±0.11^{e}
White prawn		5.95±0.83	6.07±0.33	6.08±0.28	6.03±0.48^{cd}
Bridshrimp		6.11±0.38	6.11±0.38	5.82±0.72	6.01±0.49^{cd}
Mackerel	LDA	5.72±0.12	5.81±0.13	5.84±0.21	5.79±0.15^{b}
Threadfin bream		6.41±0.21	5.77±0.12	5.91±0.19	6.03±0.17^{bc}
Hardtail scad		6.13±0.05	5.84±0.50	6.38±0.17	6.12±0.24^{bc}
Jewfish		6.48±0.26	6.43±0.28	6.41±0.16	6.44±0.23^{d}
Black pomfret		5.24±0.08	5.15±0.01	4.96±0.41	5.12±0.17^{a}
Yellowtail scad		5.21±0.26	5.27±0.03	5.32±0.21	5.27±0.17^{a}
Bombay duck		6.82±0.04	5.65±0.37	5.67±0.29	6.05±0.23^{bc}
Squid		6.53±0.22	6.39±0.05	6.65±0.15	6.52±0.14^{d}
White prawn		6.01±0.27	6.16±0.41	5.91±0.79	6.03±0.49^{bc}
Bridshrimp		6.14±0.14	6.14±0.14	6.12±0.24	6.13±0.17^{c}

*P: Different wet market

t Mean±SD

TPC: Total plate count

SM: Skim milk

HDA: Histidine DA

ODA: Ornithine DA

LDA: Lysine DA

a,b,c,d,e,f,g means values with different superscripts within row differed significantly among fish and seafood types ($p<0.05$)

1.97 µg g^{-1}. There was significant difference ($p<0.05$) among different sample types and among different wet markets. There was no detection of histamine in the mackerel, threadfin bream, jewfish, yellowtail scad, bombay duck and bridshrimp samples. This might due to the fact that putrescine and cadaverine inhibited histamine metabolizing enzyme (Taylor and Sumner, 1987). Marks (Rupp) and Anderson (2005) also reported that histamine is not always found in spoiled fish, and that putrescine and cadaverine may be better markers for decomposition. It is also worth noting that the amount of biogenic amines in each fish types correlated with amount of amino acid and growth of biogenic amine producing bacteria.

There were significant differences ($p<0.05$) in the level of putrescine, cadaverine and histamine in selected fish from different wet markets. This indicated that the formation of biogenic amines varied among different types of fish and the different amount between each market might due to different biochemical and microbial activities that brought about the formation of biogenic amines in each type of fish. The amount of biogenic amine among all types of fish was less than the allowable limit of biogenic amine stated by several countries, where 50 µg g^{-1} is proposed by the US Food and Drug Administration (FDA); while the European Community, South Africa and Italy ruled it at 100 µg g^{-1}; and Australia and Germany, 200 µg g^{-1} (Auerswald et al., 2006; Carelli et al., 2007).

Microbiological profile

Mesophilic count for different agar namely total plate count, proteolytic, and biogenic amine producing bacteria for all fish types from three different wet markets were shown in Table 5. The highest total plate count was found in the mackerel sample, at 7.13 log cfu g^{-1} while the lowest count was in the black pomfret at 5.68 log cfu g^{-1}. There were significant differences ($p<0.05$) among the lowest and highest total plate count. There were no significant differences ($p>0.05$) among hardtail scad, jewfish, bridshrimp, white prawn, threadfin bream, yellowtail scad and bombay duck. The higher value for total plate count that ranged from 5 to 7 log cycles might due to improper handling of fish in wet markets and cross contamination might have occurred. Based on the data collected, these samples are still not considered as spoilt. According to Huss (1995) during the aerobic storage, specific spoilage bacteria should be around 8 to 9 log cfu g^{-1} to produce significant amount of chemical compounds associated with spoilage.

For almost all samples, results of the proteolytic counts showed higher reading than the biogenic producing bacterial counts. Most of the samples showed similar proteolytic count. Squid had the highest proteolytic count at 6.91 log cfu g^{-1}. The proteolytic bacteria associated with spoilage in seafood might be from the genera *Pseudomonas* and *Aeromonas* spp. *Pseudomonas* and H_2S producing bacterial population have been reported to be the specific spoilage bacteria in fish from temperate and tropical waters (Gram and Huss, 1996).

Biogenic amines producing bacteria were indicated by the activity of amino decarboxylation shown by their corresponding amino acids. Numerous bacteria have been reported to possess histidine decarboxylase activities. The result showed that three types of biogenic amine producing bacteria grew between 3 to 6 log cfu g^{-1}. Our results are in agreement with those of Pons-Sánchez-Cascado et al., (2005), who recorded putrescine- and cadaverine-forming bacteria in anchovies stored in ice. Ababouch et al., (2007) also found histamine-forming bacteria in sardine stored in ambient temperature (25-28°C) and in ice.

Conclusion

The amount of formaldehyde among all fish and seafood was still lower than the suggested amount by the Italian Ministry of Health, which is 60 µg g^{-1} and 10 µg g^{-1} for *Gadidae* and crustaceans, respectively. Overall microbiological analysis showed that the presence of microbe was still lower than the limit amount stated in previous research which was less than 8 log cfu g^{-1}. Thus, fish and seafood from wet markets can be considered in good quality since seafood spoilage generally involves growth of microorganisms at high numbers and the interaction between different groups of microorganisms may influence their growth and metabolism.

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