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RESEARCH ARTICLE

Changes in Freshness of Steak and Loin Tuna (*Thunnus albacares*) during 15 Day-chilled Storage

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ABSTRACT

The present study was undertaken to assess the effect of chilled storage on the quality of steak and loin tuna by studying the changes in chemistry, microbiology and sensory attributes. Six fresh tuna unloaded in Pelabuhan Ratu, West Java, Indonesia were transported to Jakarta with proper icing. Upon arrival at the factory, the fish were carefully cut into steak and loin, followed by storage at chilled temperature (0-4°C) for 15 days in the laboratory. Changes in freshness were monitored by 3 days interval, including K-value, histamine, heavy metals, microbiology and sensory test. The initial values of K-value, histamine contents, total microbial count and histamine producing bacteria were 2.01-13.74%, 1.28-1.61 mg/100 g, 2.68-5.10 log CFU g^{-1} and 1.35-2.20 log CFU g^{-1}, respectively, whereas mercury and cadmium concentrations (markers of environmental pollution) were around the Indonesian standards. During chilled storage at 0-4°C for 15 days, the K-value of tuna steak constantly increased from 2.85-29.88% and the histamine content of it also increased from 1.41-5.60 mg/100 g while total microbial count and histamine producing bacteria fluctuated within the acceptable levels, i.e., 2.68-5.10 log CFU g^{-1}; and 1.35-2.20 log CFU g^{-1}, respectively. However, the sensory scores by 7 trained panelists gradually decreased with storage time and both steak and loin tuna were judged as not fresh after 15 days of chilled storage.

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INTRODUCTION

Indonesia is one of the leading exporters of tuna in the world and 94,221 tons of tuna (US $ 243,937 million value) were exported in 2004 (MMAF, 2011). Chilled tuna, however, have problems regarding freshness due to increasing histamine and others with time or heavy metals. The Food and Drug Administration (FDA) reported that there were 350 reject cases of tuna in the US during 2001-2005 due to the problems mentioned above. Likewise, in 2005, the export of tuna from Indonesia to the European Union member countries was subjected to the Rapid Alert System for Food and Feed (RASFF) which led to export suspension by the European Communities (2006). It is obvious therefore that efforts to improve postharvest handling methods are highly imperative to maintain the quality of fish.

Thus, the freshness of fish is the most important attribute when assessing the quality (Ozogul *et al*., 2005) but loss of freshness followed by spoilage is a complex combination of microbiological, chemical and physical processes (Pedrosa-Menabrito and Regenstein, 1990). At present, the K-value mentioned below is used for the indicator of freshness of fish by measuring the degradation of adenine nucleotides. The endogenous enzyme in fish degraded adenine nucleotides on the beginning of the storage period. The microbial metabolism also contributes to muscle's degradation in the later stages of storage. In post-mortem fish muscle, degradation of Adenosine Triphosphate (ATP) takes place according to the following sequence: ATP→ADP→AMP →IMP→HxR→Hx (ADP: adenosine diphosphate, AMP: adenosine monophosphate, IMP: inosine monophosphate, HxR: inosine, Hx: hypoxanthine). IMP imparts the fresh flavour of fish whereas Hx effect on the loss of fresh fish flavor. The hypoxanthine concentration has been recommended as an indicator of fresh fish quality. The K-value is defined as the ratio of the amount of inosine (HxR) and hypoxanthine (Hx) to the amount of ATP and degraded compounds (ADP, AMP, IMP, HxR and Hx) shown as a percentage (Ozogul and Ozogul, 2002).

Along with the K-value, decreasing freshness of fish can also be measured by sensory tests using trained panelists. The declining quality of tuna can also be measured through the analysis of histamine. Major Scombridae fish, including tuna has a high content of the **amino acid** histidine which can be transformed into decarboxylated scombrotoxin (histamine). Food poisoning occurs when fish containing high histamine levels is consumed. The presence of histamine in fish is an indication of spoilage which is dependent upon the availability of free amino acids, the presence of decarboxylase positive microorganisms (bacteria containing enzymes which can decarboxylate free amino acids) and conditions favoring the bacterial growth (Halasz *et al*., 1994; Brinker *et al*., 2002). FDA (1998) set the maximum level of histamine on fish at 5 mg/100 g to ensure the safety. In addition to a decrease in freshness, histamine poisoning and increased levels of microbes, tuna also contains a lot of harmful heavy metals, including mercury and cadmium as a result of bioaccumulation during the tuna life cycle.

Although, there are a number of studies on the freshness of tuna or other fish types (Kim *et al*., 1999; Du *et al*., 2002; Staruszkiewicz *et al*., 2004; Guizani *et al*., 2005; Patange *et al*., 2005; Ko, 2006; Tahmouzi *et al*., 2012), the information on the evaluation of tuna post-captured in Indonesia and the quality change of tuna steak and loin at different storage times in chilling temperatures is still limited. This study was designed to investigate the quality of post-captured and quality changes of tuna (steak and loin) stored in a chilling room, by using chemical (K-value, histamine, heavy metals), microbiological and sensory assessment.

MATERIALS AND METHODS

Fish samples: Fishes were caught off in the coast of Pelabuhan Ratu, Indonesia on Mei 2007 during a single trip. Six Tuna were gutted immediately after catching. Newly caught tuna were unloaded and transported to Jakarta with proper icing. Upon arrival at the factory, the fish was carefully cut into loin (800 g) and steak (200 g) and stored in a cold room (0-4°C)

Chemical analysis: The K-value is defined as the ratio of the sum of inosine (HxR) and hypoxanthine (Hx) to the sum of ATP and related catabolites (ADP, AMP, IMP, HxR, Hx) expressed as a percentage. Nucleotide analysis was carried out according to Yunizal *et al*. (1998), using UV spectrophotometer (Lambda 25 UV-Vis spectrometer, ParkinElmer) at 250 nm wavelength:

The K-value was calculated according to the following concentration ratio:

$$\text{K}-\text{value} = \frac{[\text{Ino}]+[\text{Hx}]}{[\text{ATP}]+[\text{ADP}]+[\text{AMP}]+[\text{IMP}]+[\text{Ino}]+[\text{Hx}]}\times 100$$

where, [Ino] and [Hx] are the amount of inosine and hypoxanthine; [ATP], [ADP], [AMP], [IMP], [HxR] and [Hx] are the amount of ATP and degraded compounds.

The histamine content of the fish was determined by the Association of Official Analytical Chemists standard (AOAC, 1995); fluorometric method. The eluate sample was derivated and then analyzed immediately in a spectrofluorometer (LS 45 Luminescence Spectrometer, ParkinElmer) with 350 nm excitation and 444 nm emission wavelengths.

The heavy-metal content of fish was determined by the Indonesian National Standard SNI 01-2354[1].5-2006 (Cd) and SNI 01-2354[1].6-2006 (Hg) (Badan Standar Indonesia, 2006). Cadmium was released from the fish muscle by dry digestion at 450°C. The ashes were dissolved in hydrochloric acid (HCl) 6 M and nitric acid (HNO_3) 0.1 M. The solution was atomized by graphite furnace-argon. Cadmium interacts with the light of a hallow cathode lamp. Cadmium absorption was measured by atomic absorption spectrophotometry. For analyses of total Hg, homogenized samples from the tissue (1-3 g) were digested to a transparent solution with 10 mL of the mixture H_2SO_4-HNO_3 (1:1) under reflux. The resultant solutions were measured by atomic absorption spectrophotometry (Analyst 800 AAS, ParkinElmer).

Sensory analysis: Seven trained panelists were asked to evaluate the sensory quality of tuna samples using the descriptive terms listed in Table 1 according to the assessment scheme of the Research Center for Marine and Fisheries Product Processing and Biotechnology Jakarta with slight modifications. The panelists were selected from the research center. The acceptability scores were measured and the mean score of each sample was calculated. The panel was repeated on different days until all samples were scored.

Microbiology analysis: In every sampling, a 10 g portion was cut from each tuna steak and loin and homogenized with 9 mL (1:9 w/v) of sterile buffer (NaCl 0.85%). Homogenates were serially diluted with the same sterile buffer and then 0.1 mL out of each dilution series was transferred to an agar plate. Bacterial colonies were counted after 24-48 h incubation at 30-32°C.

Table 1: The sensory assessment scheme for steak and loin tuna

Score	Appearance	Color	Odor	Texture
1	Dry and mature (pale)	Dark (bleak)	Ammonia, stink, putrid odor	Disintegrating, very soft
2	Dry and thick rainbow	Dark brown	Sour, rancid, slightly ammonia	Soft flash, flaccid
3	A little oily and a thick rainbow	Bright brown	No odor (neutral) or slightly sour	A little elastic mushy
4	Oily and thin rainbow	Hazel muscle	Fishy or typical shellfish odor	Slightly compact,
5	Oily and clear transparent	Bright red muscle	Fresh "seaweedy" odor	Elastic, firm, compact

Niven's differential agar medium (0.5% trypton, 0.5% yeast extract, 2.7% L-Histidine.2HCl, 0.5% NaCl, 0.1% $CaCO_3$, 0.2% agar and 0.006% bromocresol purple, with acidity 5.3) was used to detect histamine-producing bacteria in tuna samples (Niven *et al*., 1981). This media was sterilized for 10 min at 121°C. Inoculated plates were incubated at 25°C for 3 days and visually examined for purple colonies with purple halo on the yellow background.

Statistical analysis: The data were subjected to analysis of variance (ANOVA) and the means were compared using the Duncan's test. Significance of differences was defined at $p<0.05$.

RESULTS AND DISCUSSION

of 60 kg m^{-2}. The surface of boxes and hatches were coated by fiberglass. The fishing spot was carried out on a rumpon (fishing ground) at 08°11'10.9" south latitude and 106°21'52.6" east longitude.

Immediately after being captured, the tuna was stunned by puncturing the cranium causing instantaneous insensibility. Most of the captured tuna were immediately gutted on the vessels while gutting on the rest was delayed due to stormy weather with the inevitable risk of degrading quality. After gutting and thorough washing, the tuna were placed in boxes and covered with ice with the ratio of 1:1. Once the fish landed on the seaport, the visual inspection was done by a trained grader and each fish was classified based on the standard. The best-quality tuna were graded as A (TR1 and TR2) and the rests were graded as B (TR3, TR4, TR5 and TR6) (Table 2).

The tuna were transported to Jakarta with proper icing. Upon arrival at the factory, tuna were handled quickly and carefully. It was cut into steak and loin, the most desirable form of exported tuna. The average edible portion after filleting was 57.68%, thus meeting the general edible portion of major tuna species at 60% as had been investigated by Korsmeyer and Dewar (2001).

The mean score for overall acceptances of tuna is shown in Table 3. Generally, all tuna samples were sensorial accepted, despite significant difference in some attributes, mainly between TR1, TR2 and other samples due to different post-capture handling on vessels. Based on the Duncan's test, the most significant differences of TR1 and TR2 with the others were in appearance, texture and aroma attribute. This is thought to be caused by differences in the handling. Table 1 shows that the quality of TR1 and TR2 samples which were gutted immediately were notably better than the others.

Table 2: Tuna handling of fishing vessel

Arrest date	Time	Handling	Fish code	Weight (kg)	Grade
29-Apr.-07	14:30	Stored in the box without gutting; gutted on 30th April	TR3	23	B
29-Apr.-07	17:00	Stored in the box without gutting; gutted on 30th April	TR4	20	B
30-Apr.-07	07:10	Gutted and stored in the box immediately	TR1	23	A
30-Apr.-07	08:00	Gutted and stored in the box immediately	TR2	27	A
1-May-07	19:10	Stored in the box without gutting; gutted on 4th May	TR5	18	B
1-May-07	20:00	Stored in the box without gutting; gutted on 4th May	TR6	26	B

Table 3: Quality of fresh tuna

Fish code	pH	K-value (%)	Histamine (mg/100 g)	TPC (Log CFU g^{-1})	HPB (log CFU g^{-1})	Sensory scores: Appearance	Color	Aroma	Texture
TR1	6.01±0.01	4.10±0.01	1.52±0.04	2.0±0.10	1.0±0.00	4.43±0.53^{c}	4.43±0.79^{c}	4.71±0.49^{b}	4.57±0.79^{c}
TR2	5.94±0.01	3.66±0.02	1.37±0.01	4.4±0.21	1.0±0.00	3.86±0.38^{bc}	3.57±0.53^{b}	4.29±0.49^{ab}	4.14±0.38^{bc}
TR3	5.97±0.01	2.43±0.02	1.29±0.02	2.0±0.17	1.0±0.00	3.86±0.69^{bc}	4.14±0.38^{c}	4.14±0.38^{a}	4.00±0^{b}
TR4	5.93±0.01	13.74±0.04	1.61±0.03	2.0±0.10	1.0±0.00	2.86±0.53^{a}	3.43±0.49^{b}	4.00±0^{a}	3.14±0.49^{a}
TR5	5.85±0.01	2.01±0.01	1.28±0.01	3.9±0.10	1.8±0.15	3.14±0.38^{ab}	2.71±0.49^{a}	3.86±0.38^{a}	3.00±0^{a}
TR6	5.91±0.01	5.68±0.01	1.41±0.02	3.5±0.12	2.3±0.10	3.43±0.53^{ab}	3.43±0.53^{b}	3.86±0.69^{a}	3.71±0^{b}

Values are Mean±SE, Fish code: TR (Tuna range); TPC: Total plate count, HPB: Histamine producing bacteria. Values with different letters within the same column are statistically different at $p<0.05$

Quality of fresh tuna: The quality of fresh tuna was determined by chemical analysis (pH value, K-value, histamine and heavy metals represented by mercury and cadmium content), microbiology and sensory evaluation. Chemical, microbiological and the **sensory evaluation** result of each sample is presented in Table 3.

The pH values of fresh tuna ranged from 5.85-6.0. Shortly after the fish die, **lactic acid** which decreasing muscle pH is formed through some anaerobic biochemical processes. The pH of muscle tissues generally range from 7-7.5 but can fall down to 6-5 after rigor mortis (Robb, 2002). The pH drop is different for each fish species; below 5.5 for tuna but ranged between 6.2-6.6 for other fish species (Haard, 2002).

al., 2005). The freshness index was comparable with sensory analysis results which indicated that tuna samples were of good quality. This result was in accordance with (Guizani *et al*., 2005), who reported a linear relationship between sensory attributes and K-values of fresh fish.

Histamine concentration of fresh tuna was 1.28-1.61 mg/100 g which was below FDA standard. Raw albacores with histamine below 1.5 mg/100 g are considered excellent quality and can only be achieved by appropriate handling and chilling on board (Craven *et al*., 2001). The amount of histamines produced by the fish is strongly influenced by temperature, time, storage conditions and fish species (Lehane and Olley, 1999).

The total microbial counts in fresh tuna ranged from 1.8-4.4 log CFU g^{-1}. This number is smaller than the standard of total bacteria for fresh fish which is 5.7 log CFU g^{-1} (Badan Standar Indonesia, 2006). The amount of producing bacteria in fresh tuna ranged from 1-2.3 log CFU g^{-1}. The amount of histamine-producing bacteria effects the level of histamine in fish. Most histidine decarboxylase-producing bacteria that play an important role on histamine formation are classified in Enterobacteriaceae family (Tahmouzi *et al*., 2012); they all had the ability to decarboxylate histidine to histamine (Ndaw *et al*., 2007) and most of them are mesophiles. Mesophilic bacteria can grow well in moderate temperature (20-45°C) and it is the most important factor to support histamine formation (Kim *et al*., 2002). Storage at 4°C can inhibit the growth of most histamine-producing bacteria.

Table 4: **Heavy metals** (mercury and cadmium) content of tuna

Fish code	Mercury (ppb)	Cadmium (ppb)
TR1	0.076±0.002	0.091±0.004
TR2	0.341±0.011	0.398±0.012
TR3	0.170±0.009	0.052±0.002
TR4	0.443±0.012	0.117±0.010
TR5	0.392±0.010	0.101±0.009
TR6	0.501±0.012	0.092±0.004
Standard*	0.5 ppm	0.3 ppm

Values are Mean±SE, *European Communities (2006)

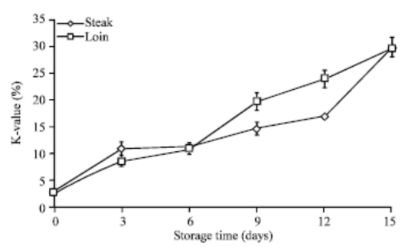


Fig. 1: Freshness (K-value) of steak and loin tuna during storage time

Mercury levels in tuna ranged from 0.076-0.501 ppb whereas the cadmium levels ranged from 0.052-0.398 ppb in (Table 4). The amounts of mercury and cadmium were well below the maximum permitted concentrations by EU legislation for human consumption which are 0.5 and 0.3 ppm for mercury and cadmium, respectively. The level of heavy metals in fish is linear with the weight. Levels of mercury and cadmium are diverse depending on the accumulation process in fish and the interaction of several parameters both abiotic (water and sediments) or biotic (size, sex, age, average growth, eating habits, the position of tropical, habitat). Moreover, the effect of body size on total mercury loading is widely recognized in marine organisms (Storelli *et al*., 2005).

Quality changes during storage time: Results of the freshness (K-value) of steak and loin tuna during storage time are presented in Fig. 1. The initial K-value for fresh tuna was 2.85%. The K-value increased linearly during storage and after 15 days reached the value of 29.88% and 29.63% for steak and loin, respectively. The K-values in tuna during storage time showed statistically significant ($p<0.05$) increases at the end of the storage period. This value is much lower when al. (1997), final fitness value is determined by the initial value of fish freshness associated with fish condition at the time of death and the handling process.

The average of histamine concentrations of steak and loin tuna during storage time are shown in Fig. 2. As shown, a slight increase in the histamine value was noticed with time.

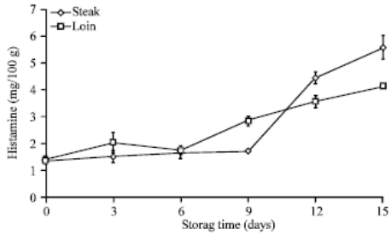


Fig. 2: Histamine of steak and loin tuna during storage time

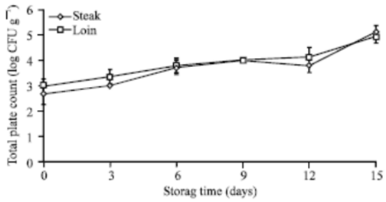


Fig. 3: Total plate count of steak and loin tuna during storage time

The increases varied from as low as 1.41 mg/100 g to as high as more than 5.6 mg/100 g. The histamine concentrations showed significant difference ($p<0.05$) in relation to the storage time and a non-significant difference ($p<0.05$) in relation to the form of preparation (steak or loin). A significant increase in the histamine value is shown until 15 days which was above FDA standard on the last day (>5 mg/100 g). Time and temperature have been reported as the key factors in controlling histamine development, as these factors influence the growth of histamine-producing bacteria and the formation of their histidine decarboxylases (FDA, 2001). Once the enzyme has been formed before the storing of fish, it can continue to produce histamine (Kim *et al*., 2002).

Counts of total microbial on steak and loin tuna as related to storage time are summarized in Fig. 3. At the start of incubation, total microbial counts were very low (2.68 and 2.98 log CFU g^{-1}). There was a steady increase over the period of storage to 5.10 and 4.93 log CFU g^{-1}. There were significant increases in the total microbial count of fish stored in chilled storage on days 15 and the other which was above the standard of total microbial for fresh fish of 5.70 log CFU g^{-1} (Badan Standar Indonesia, 2006). This shows that the processes of handling and storage were appropriate, so that contaminants can be minimized. Several kinds of microbial growth in fish can be inhibited at a temperature of 0-4°C but there were psychrophilic bacterial species that are still able to grow in small amounts (Huss, 1995).

The histamine-forming bacteria on steak and loin tuna as related to storage time are given in Fig. 4. Histamine-forming bacteria were low and undetectable at the beginning of the experiment (1.35 log CFU g^{-1}). However, during subsequent incubation, counts of histamine-forming bacteria increased slightly, reaching 2.2 log CFU g^{-1}. This count was not comparable with the concentrations of histamine formed.

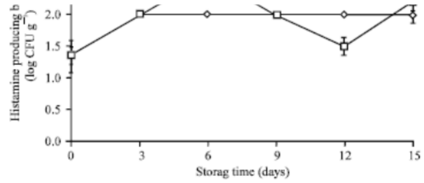


Fig. 4: Histamine producing bacteria of steak and loin tuna during storage time

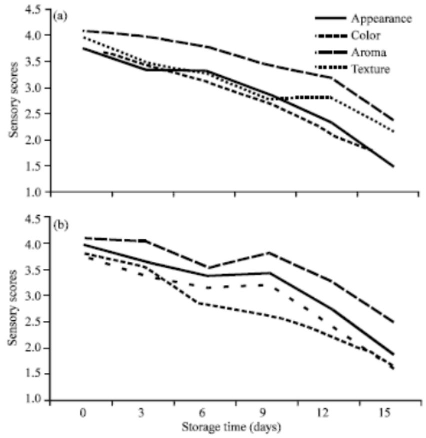
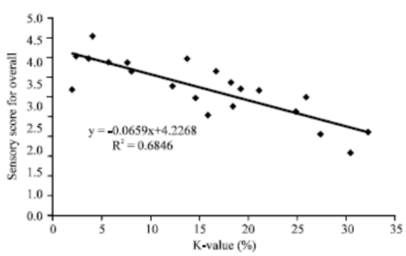


Fig. 5(a-b): Sensory scores of (a) Steak and (b) Loin tuna during storage time

As revealed by Kim *et al*. (1999), the number of bacteria are not directly related to the histamine production but more related to its ability to provide the histidine decarboxylase.

Bacteria can be found in almost all fish, especially those that have been contaminated post-catch. They grow well at temperatures of 10°C but at 5°C their growth can be inhibited. According to Kim *et al*. (2002), the growth of histamine producing bacteria *Morganella morganii* can be controlled with cold temperatures (0-4°C) but histamine formation can be controlled only in frozen storage.

The average of sensory analysis is presented in Fig. 5. On the whole, the sensory score decreased with storage time, indicating the progressive loss of freshness in storage condition. The overall acceptance showed significant differences ($p<0.05$) in relation to the storage time and a non-significant difference ($p<0.05$) in relation to the form of preparation. The panelist rejected fish on 15 days at chilling storage (0-4°C). The sensory acceptance decrease found in steak and loin tuna over storage time corresponded with an increase in K-value.



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storage time

Figure 6 shows a linear relationship of the overall acceptance of the tuna by the panelists with the K-value during storage time. The correlation between sensory value and K-value was $r^2 = 0.68$. The sensory score decreased with the increase of the K-value. The results found in this study agreed with those reported by Guizani *et al*. (2005). Who reported linear relationships between sensory attributes and chemical components on K-value during storage of fresh fish.

CONCLUSION

According to the variance analysis, there were significant differences in some attributes between tuna TR1 and TR2 with TR3, TR4, TR5 and TR6. This could be caused by differences in the handling after catch. When the K-value is low the fish are grouped into `sashimi grade`. The histamine concentrations were under the allowable limit (5 mg/100 g) based on FDA regulation. Mercury and cadmium contents in tuna increase with fish weight. During storage time at chilling temperature (0-4°C), histamine, K-value, sensory and microbiology increased linearly. There were significant differences ($p<0.05$) on K-value, histamine and sensory result in relation to the storage time and a non-significant differences ($p<0.05$) in relation to the form of preparation (steak or loin). Bacteria found in the tuna included histamine-producing bacteria that can grow at chilling temperature. The sensory scores by the trained panelists gradually decreased with storage time and both steak and loin tuna were judged as not fresh after 15 days of chilled storage.

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