

Original Paper

## CHANGES IN OXIDATION AND REDUCTION POTENTIAL (Eh) AND pH OF TROPICAL FISH DURING STORAGE

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### ABSTRACT

Four tropical fish species, *Thunnus albacares* (Yellowfin tuna), *Ephinephelus striatus* (Nassau Grouper), *Cyprinus carpio* (Carp), and *Osphronemus gourami* (Gouramy), were assayed for oxidation reduction potential (Eh) and pH in different temperature, i.e. ambient and chilled temperature. Every species has different pattern of Eh and pH values. Eh values of tropical freshwater fish were higher than tropical marine fish, however pH values four tropical fish have same trend. The rates of the Eh and pH changing in four tropical fish were faster at ambient storage and they were slower at chilled storage. The present study also demonstrated the relationship between Eh and pH.

**Key words:** Eh ; fish freshness ; pH ; tropical fish

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### INTRODUCTION

Fresh fish is more perishable products than other foodsuff (Gram and Huss, 1996; Lu *et al.*, 2010). Fish from different environment have different chemical compositions (Huss, 1988). Marine and freshwater fish contain several chemical compounds that affect to biochemical reaction after death. Directly after death, a series of biochemical reactions start, which is paramount importance for the quality and shelf life of products. These reactions depend on several factors: fish species, the physiological of fish condition, environment factors, catching and harvesting methods, and killing procedures (Oehlenschläger and Rehbein, 2009). During storage, fish characteristics will change until spoilage occurring due to biochemical, chemical, physical and microbial that are affected by both time and temperature (Ashie, *et al.*, 1960 in Nilsen, *et al.*, 2002).

The analytical methods used for fish freshness can be divided into objective methods

and sensory methods. The objective methods are chemical and biochemical methods, physical methods, and microbiological methods (Oehlenschläger, 2010). The chemical methods for measuring fish freshness are K value (Saito, *et al.*, 1959, Ehira and Uchiyama, 1987, Agustini, *et al.*, 2001), analysis of trimethyl amine (Dyer, 1945, Bullard and Collins, 1980; Sadok, *et al.*, 1996), analysis of total volatile basic nitrogen (TVB-N) (Conway, 1950; Okoro, *et al.*, 2010; Howgate, 2010), and biogenic amines (Blonz and Olcott, 1977; Takagi and Shikata, 2004). Physical methods comprise texture analysis (Herrero and Careche, 2006; Olafsdottir, *et al.*, 2004), analysis of electrical resistance or conductivity by Torrymeter, Intellectron Fischtester VI, RT Freshness grader, and time domain spectroscopy (TDR) (Kent, *et al.*, 2005). Further, microbiological methods are total viable count (TVC), determination of specific

spoilage organisms (SSO), polymerase chain reaction (PCR), oligonucleotide probes, antibody techniques, and bacterial sensors (Oehlenschläger, 2010).

Later, many researchers have been investigated fish freshness based on physico-chemical properties in fish. They used several methods such as VIS/NIR spectroscopics (Niu and Lee, 2000; Nielsen, *et al.*, 2002; Nielsen and Hieya, 2009), Eh and K value (Agustini, *et al.*, 2001; Wijayanti *et al.*, 2007), pH and Eh (Agustini, 2000; Agustini, *et al.*, 2001; Wijayanti, *et al.*, 2007 and Susanto, *et al.*, 2009).

Eh is a fish freshness measurement based on dielectric properties on fish meat which shows the relationship between occurrence of O<sub>2</sub> and microorganism (Brown and Finksiger, 1980). The redox or oxidation-reduction (O-R) potential (Eh) measures the potential difference in a system generated by a coupled reaction in which one substance is oxidized and a second substance is reduced simultaneously. A result by Huss and Larsen experiments indicated that ORP (Eh) had correlation with fish deterioration. Their result showed that Eh of fish increased initially and subsequently decrease until negative in spoiled fish (Huss and Larsen, 1979). According to Agustini, *et al.*, (2001), ORP cannot be used as single index for characterizing fish freshness because in fish sample ORP gave two same values in different freshness phase. Redox potential values measured depends on pH, each measurement of redox potential should be accompanied by a statement on pH (Brown and Finksiger, 1980).

pH is commonly used to measure of fish deterioration, it has been common to measure the pH of the muscle tissue (Howgate, 2009). Eh may vary from different fish due to the different concentration of various redox couples in fish meat, chemical composition, specific processing treatment given, and its storage condition (in relation to air) (Brown and Finksiger, 1980; Ray and Bhunia, 2008). In addition, Eh is more sensitive than pH for evaluating the change of fish freshness (Agustini, *et al.*, 2001).

Relation between Eh and pH has been observed by several reseachers namely on fresh water fish (Susanto, *et al.*, 2009), dark meat

marine fish (Wijayanti, *et al.*, 2007), and sub-tropical fish (Agustini, 2000).

This paper describes the determination of Eh and pH in different fish species, marine fish and freshwater fish, which were stored at different temperature, ambient and chilled, and their relationship.

## MATERIALS AND METHODS

### Fish

Yellowfin tuna (*Thunnus albacares*) (weight 12,000 g) was purchased from fish auction in Cilacap Regency located 300 km away from the laboratory. Time interval between Yellowfin Tuna harvesting and arrival of fish at the Cilacap fish landing was 1 month and during this period it was frozen. Fish were immediately brought to the laboratory in sterfoam covered with ice (1:1). Live Nassau grouper (*Epinephelus striatus*) ( weight range from 200-300 g) were collected from Jepara marine water, located 80 km away from laboratory. After collection, the Nassau grouper were brough in life condition inside the sterfoam which contained sea water to the laboratory.

Live cultured freshwater fish, Carp (*Cyprinus carpio*) (weight range from 400-500 g) and Gouramy (*Osphronemus gourami*) (weight range from 700-800 g) were taken from fish ponds in Semarang, 15 km far away from the laboratory. The live carp and gouramy were immediately brought to the laboratory in the sterfoam which contained fresh water. After arriving at laboratory, live fish were killed using cool water.

### Equipments and chemical standard

Electrometer type PCM308 S-SR (pH meter and Eh meter) (Toko Chemical Laboratory Co.Tokyo, Japan), mortar and pounder, Beaker Glass 25 mL from Pyrex Ltd. were used. Quinhidron standard solution for Eh analysis was purchased from Tokogawa Inc. Japan.

### Sample preparation for analysis

The randomly fish samples were used in Eh and pH analysis. Fish muscle samples were taken according to Ryder (1985). Fish muscle tissue (20–30 g) was collected from a dorsal part of fish after death (0 days of storage). These samples were prepared on cube form (3x2x1 cm) and wrapped in polyethylene bags being stored at 29°C and 10°C. Randomly fish meat samples divided into two groups, storing at ambient temperature (29°C) and chilled temperature (10°C). At ambient temperature, all samples were stored for 3 days and at chilled temperature, they were stored for 12 days.

### Assessing of Eh on fish meat

Before Eh analysis, the electrodes of Eh meter was checked with quinhidron standard. Eh electrode is made from platinum material. Before use, the electrode was checked with quinhidron standard solution which has Eh of  $260 \pm 2$  mV. Between repetitive Eh reading, the electrode was cleaned and soaked in distilled water for several minutes until Eh value close on initial value before next measurement.

Eh analysis was carried out based on method that used by Okouchi, *et al.*, (1998) and Agustini (2000) with modification. The Eh of fish cube samples were measured on homogenized samples diluted in distilled water (1:10) with Eh meter. The third fourth part of Eh electrode was putted in the solution.

The principle of Eh measurement based on the following formula:

$$ORP = ORPo + \frac{R.T}{n.F} \ln \frac{(oxidant)}{(reductant)}$$

Eh is redox potential at pH 7.0, R is gas constant (8.314 J/K mol), T is absolute temperature (K), F is Faraday number (96.496 J/V), n is amount of moving electron on process, reductant is material that release electron, oxidant is material that accept electron.

After got Eh values, those values were entered on the following equation:

$$Eh_{total} = (219.36 - 0.74 \times \text{temperature of sample}) + Eh_{of\ sample}$$

### Assesing pH on fish meat

pH on fish meat were evaluated based on Agustini research (2000) with modification. The pH of fish cube samples were measured on homogenized samples diluted in distilled water (1:10; w:v) with a pH meter (Toko Chemical Laboratory Co.Tokyo, Japan). The measurement of pH and Eh were did in the same time.

### Data analysis

Each analysis was carried out in triplicate. Data were obtained as the mean and standard deviation (SD). Data analysis was performed by microsoft excel 2003 (Microsoft Crop., USA).

## RESULTS AND DISCUSSION

### Eh

Eh was analysis in two different storage temperature storage, (ambient and chilled temperature). Eh values of four tropical fish are shown in **Table 1** and **Table 2**. The rate of Eh changing of four tropical fish were faster at ambient storage (**Table 1**) compare to the chilled storage (**Table 2**).

At early of storage, Eh values of freshwater fish (carp and gouramy) on both storage were higher than Eh values of marine fish (yellowfin tuna and nassau grouper). The greater values may caused by different treatment of samples. The different redox potential in food depends on O<sub>2</sub> concentration in the environment of foods, density of food structure, concentration of reduction substrate, and the pH of foods (Garbutt, 1997).

**Table 1.** The Eh value changes during storage at ambient temperature

Storage Time (hours)	Eh of Yellowfin Tuna (mV)	Eh of Nassau Grouper (mV)	Eh of Carp (mV)	Eh of Gouramy (mV)
0	350 ± 0.71	341 ± 18.46	386 ± 5.00	369 ± 16.78
8	353 ± 0.71	339 ± 7.35	371 ± 10.28	353 ± 30.95
16	263 ± 9.19	212 ± 10.26	293 ± 24.39	254 ± 33.29
24	219 ± 4.95	186 ± 4.35	128 ± 37.29	140 ± 12.58
32	204 ± 4.24	167 ± 21.04	115 ± 18.49	113 ± 24.66

Note: Mean ± SD of three samples

**Table 2.** The Eh values changes during storage at chilled temperature

Storage Time (hours)	Eh of Yellowfin Tuna (mV)	Eh of Nassau Grouper (mV)	Eh of Carp (mV)	Eh of Gouramy (mV)
0	342 ± 0.71	332 ± 9.12	373 ± 12.12	343 ± 5.48
24	366 ± 4.24	229 ± 11.00	356 ± 9.42	305 ± 11.12
48	372 ± 4.95	291 ± 15.87	352 ± 14.15	307 ± 16.87
72	362 ± 7.78	333 ± 18.49	355 ± 49.00	340 ± 17.31
96	331 ± 5.66	358 ± 20.11	367 ± 12.03	335 ± 7.63
120	312 ± 2.83	357 ± 16.92	350 ± 14.84	327 ± 27.72
144	338 ± 12.02	281 ± 26.66	339 ± 8.98	286 ± 20.30
168	336 ± 16.97	256 ± 38.81	318 ± 35.00	284 ± 64.08
192	303 ± 11.31	256 ± 24.52	275 ± 6.66	285 ± 24.07
216	274 ± 31.11	235 ± 9.18	240 ± 11.59	182 ± 78.00

Note: Mean ± SD of three samples

Our results shown, Eh values in all samples range from 386 mV to 113 mV. According to Garbutt (1997), bacterial may grow on that redox range namely, *Pseudomonas fluorescens*, *Staphylococcus aureus*, *Proteus vulgaris*, and *Clostridium perfringens*. Those bacteria are included as obligate aerobe, facultative anaerobe and aerotolerant anaerobic groups. Those microorganism grow best when O<sub>2</sub> is available.

Our result on Eh pattern in yellowfin tuna was in agreement with previous report by Agustini, *et al.* (2001). At the initial storage, Eh value increased to maximum value after 48 h of chilled storage, then decrease until the end of storage on chilled temperature (216 h). In the contrary, the pattern of Eh values in other fish samples (white meat) were different from yellowfin tuna (dark meat) pattern. At initial storage, Eh values of white meat fish were decreased to the minimum value after 24 - 48 h at chilled storage. Then these values increased to the maximum Eh values afer 72 - 96 h storage then decreased until the end of storage (216 h). Eh would be positive in fish meat at early of storage and would be negative when

fish meat deteriorate at the end of storage or decay (Huss and Larsen, 1979; Agustini, *et al.*, 2001).

The difference of storage temperatures may cause the different rates of Eh changing. Chilled storage is able to retard the changes of Eh value in fish. Chilling is able to retards fish deterioration during storage (Sarmono, 1998). Chilled temperatures can prolong the fish and fish products shelf life up to weeks or months (Oehlschläger, 2010).

In marine fish samples, Eh values of yellowfin tuna were higher than Eh values of nassau grouper. It is may caused the occurence of different concentration on redox potential pair compounds in fish meat such as NADH, NADP<sup>+</sup>, and TMAO. NADH and NADP<sup>+</sup> compounds in red fish meat are about twice as large as that of white fish muscle. The total amount of NAD and reduced NAD in ordinary muscle of various fish range from 4 to 38 mg/100 g and that of NADP and reduced NADP from 0.3 to 1 mg/100g (Shimizu *et al.*, 1969). The dissappearance of these compounds may affect the rates of various oxidation and reduction reaction in fish muscle (Ikeda, 1979).

TMAO is a main factor that caused on redox potential changing in post mortem of marine fishes. TMAO is known to cause a high of Eh in flesh fish (Huss and Larsen, 1979, 1980). TMAO is a part of NPN fraction and its presence in all marine fish (Hebard, *et al.*, 1982) and some fresh water fish (Gram, *et al.*, 1989) is well established.

Decreasing Eh values in fish samples might caused by reducing of TMAO in fish muscle. It was caused the increasing amount of specific microorganism reducing TMAO to TMA (Huss, 1988). The different observed in the rates of Eh values may be due to the differences of bacteria reducing TMAO and in the content of TMAO in fish (Hebard, *et al.*, 1982). TMAO reduction is related to specific fish spoilage organisms such as *Micrococcus*, *Achromobacter*, *Flavobacterium*, *Pseudomonas*, *S. Putrefaciens*, *P. pusphureum*, *Vibrionaceae*, *Enterobacteriaceae* (Rab, 1997; Gram and Huss, 1996). These bacteria cause decreasing of Eh value (Huss and Larsen, 1979). The spoilage of fresh fish is certainly influenced by the presence of TMAO, particularly under conditions where oxygen is excluded. A number of well defined spoilage bacteria (*Shewanella putrefaciens*, *Aeromonas* spp, *Photobacterium phosphoreum*, *Vibrionaceae*) are able to utilize TMAO as the terminal electron acceptor in an anaerobic respiration resulting in off-odours and off flavours due to formation of trimethylamine (TMA) (Gram, *et al.*, 1987, 1990; Dalgaard, *et al.*, 1993; Gram and Dalgaard, 2002). The organism causing this type spoilage is a strict anaerobe requiring a low Eh for growth (Knochel and Huss, 1984a,b). The reduction of TMAO to TMA by a coupled reaction involving oxidation probably of lactate to acetit acid, CO<sub>2</sub> and H<sub>2</sub>O through an activation step involving a “triamine-oxidase” (Liston, 1979). In addition, rapid drop of Eh is causing by increasing of oxygen usage because the bacteria enter the log phase of growth (Banwart, 1989).

In addition, the changing of Eh also caused by the presence of bacteria in fish flesh. Fresh grouper contain a bacteria flora of *Moraxella*, *Staphylococcus*, *Micrococcus*, *Aeromonas*, *Acinetobacter*, *Pseudomonas*, *Alteromonas*, *Bacillus*, *Alcaligenes*, and *Streptococcus* (Mahmoud, *et al.*, 2004;

Jeyasekaran, *et al.*, 2008). In the carp and gouramy, *Aeromonas* is the predominant bacteria flora. Enterbacteriaceae, *Vibrio* and *Corynebacter* are overgrowth on their spoilage (Anggawati, *et al.*, 1992). Those status of bacteria are related to environmental conditions and the microbiological quality of the water, including temperature, salt content, natural bacterial flora in the water, ingestion of food by fish, methods of catch and chilling, and post harvest handling (Feldhusen, 2000; Lyhs, 2009).

### pH

The changes in pH of four fish stored in different temperature storage are shown in **Table 3 and Table 4**. Fish samples had pH range from 5.57 to 7.30. During storage period, the pH show clear trend. At ambient storage, pH of four different fish have same trend. At preceeding time storage, the pH of the samples decreased to the lowest value after rigor mortis, then pH increased until alkalie at the end of time storage. The decreased of pH may caused by the amount of lactic acid in fish meat. The increase of pH may be attributed to the accumulation of alkaline compounds such as ammonia and trimethylamine derived from microbial action during fish muscle spoilage (Ruiz-Capillas & Moral, 2005; Özyurt, *et al.*, 2009).

The result of pH measurements during spoilage invariably show that after the resolution of rigor mortis the pH increases, usually after few hours up to few days, depend on the condition of storage. The variability of different pH changes depend on species, harvesting procedures, biological condition, variation of season, and methods of killing (Howgate, 2009; Ozogul, 2010).

On both storage treatments, at early of storage period, initial pH of yellowfin tuna showed the acid condition. It was caused the initial condition of yellowfin tuna. We investigated pH in yellowfin tuna 30 days after it was caught. The lowest pH in yellowfin tuna was 5.4. According to Huss (1998), the lowest pH on tuna is between 5.4 – 5.6. In addition, on tuna the post mortem pH is below 6.0 because of high initial of glycogen. The minimum pH in

the fish muscle due to the accumulation of lactic acid formed as end product of glycolysis. In fish with high amounts of glycogen red muscle pH is lower than in fish with a high amounts of white muscle (Huss, 1995 in Tejada, 2009).

Dark-fleshed fish generally contain much more glycogen than white fleshed. The post mortem decrease of pH in fish meat depends on lactic acid produced by the decomposition of glycogen (Ikeda, 1979).

**Table 3.** The changes pH in four tropical fish during storage ambient at temperature

Storage Time (hours)	pH of Yellowfin Tuna	pH of Nassau Grouper	pH of Carp	pH of Gouramy
0	6.00 ± 0.10	7.30 ± 0.07	6.45 ± 0.09	6.85 ± 0.17
8	5.40 ± 0.00	7.05 ± 0.06	6.35 ± 0.05	6.75 ± 0.21
16	6.25 ± 0.05	7.70 ± 0.10	6.60 ± 0.12	6.70 ± 0.14
24	6.60 ± 0.07	7.97 ± 0.05	7.20 ± 0.08	7.38 ± 0.38
32	6.70 ± 0.07	8.15 ± 0.02	7.73 ± 0.26	7.58 ± 0.53

Note: Mean ± SD of three samples

**Table 4.** The changes pH in four tropical fish during storage at chilled temperature

Storage time (hours)	pH of Yellowfin Tuna	pH of Nassau Grouper	pH of Carp	pH of Gouramy
0	5.57 ± 0.35	7.05 ± 0.06	6.43 ± 0.13	6.83 ± 0.24
24	5.75 ± 0.05	6.85 ± 0.15	6.55 ± 0.11	6.85 ± 0.17
48	5.95 ± 0.05	6.80 ± 0.15	6.73 ± 0.21	6.85 ± 0.21
72	5.90 ± 0.01	6.90 ± 0.09	6.68 ± 0.08	6.83 ± 0.28
96	5.95 ± 0.05	7.05 ± 0.07	6.68 ± 0.15	6.95 ± 0.36
120	6.05 ± 0.05	7.40 ± 0.10	6.83 ± 0.29	6.93 ± 0.29
144	6.20 ± 0.00	7.38 ± 0.09	6.88 ± 0.43	7.38 ± 0.08
168	6.25 ± 0.05	7.60 ± 0.20	7.03 ± 0.19	7.29 ± 0.12
192	6.50 ± 0.07	7.65 ± 0.06	6.93 ± 0.13	7.18 ± 0.29
216	6.60 ± 0.00	7.65 ± 0.12	7.50 ± 0.29	7.29 ± 0.29

Note: Mean ± SD of three samples

The other three fish, at early of storage have neutral pH in different value. The pH on three fish were range from 6.45 to 7.30. The pH natural of live fish is just about 7.0, typically about 7.3 but this falls markedly after death as the fish goes through rigor mortis and glycogen is converted to lactic acid (Howgate, 2009). In addition, Huss and Larsen (1979) stated that at the initial of storage, pH of fish meat decrease to the lowest value, in rigor condition pH increase up to base condition at the end of storage. Fish bacteria were shown to be sensitive to low pH (Liston, 1979). At preceding storage, pH in carp fish was the lowest among fish samples. Low initial pH may associated with higher stress before slaughtering. This is caused by depletion of energy reserves, mainly glycogen. Low pH also promotes of oxidation of myoglobin and pH (Ozogul, 2010).

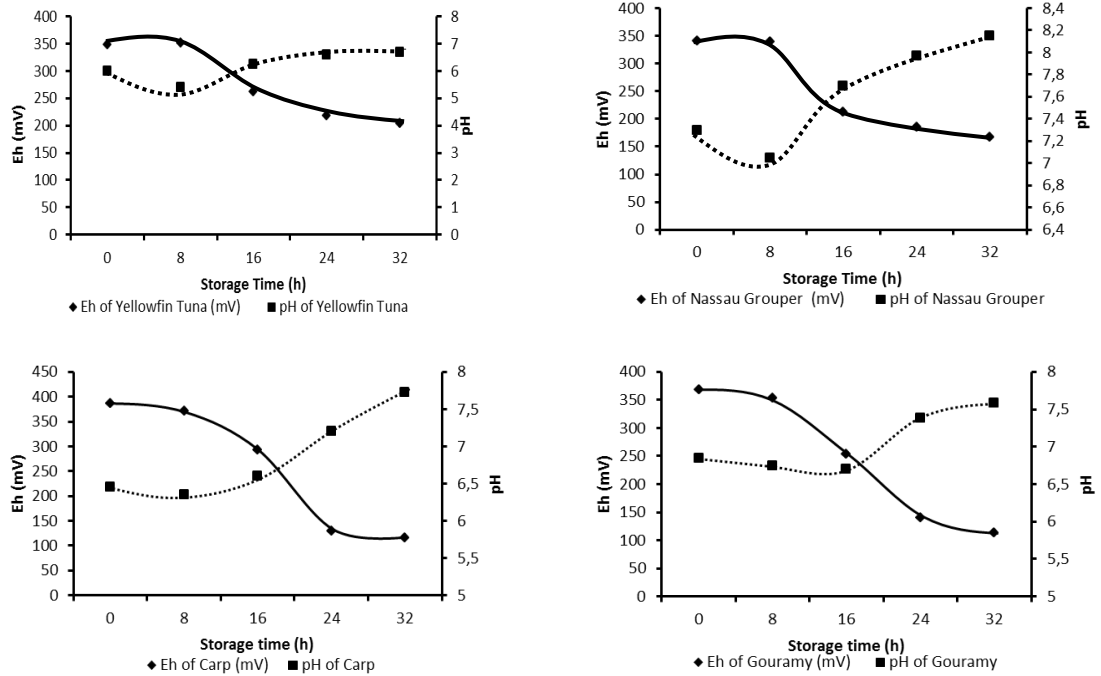
The kinds of bacteria on freshwater fish are quite variable and are undoubtedly influenced by the microflora of the water. Tropical water carry mostly *Micrococci*, *Coryneforms* and *Bacillus*. The composition of the microfloras of the different species of fish were mostly to be dominated by Gram-negative bacteria such as *Achromobacter*, *Flavobacterium*, *Pseudomonas* or less frequently *Vibrio* or *Enterobacter* genera (Liston, 1979). The rate of pH changing in fish might cause by glycogen content on fish meat. According to Ikeda (1979), the amount of glycogen on red meat is higher than on white meat.

At chilled storage, changes in pH of four tropical fish were slow down because at chilled storage, fish spoilage is retarded. According to Nychas and Drosinos (2010), at chilled storage microbial activity is retarded,

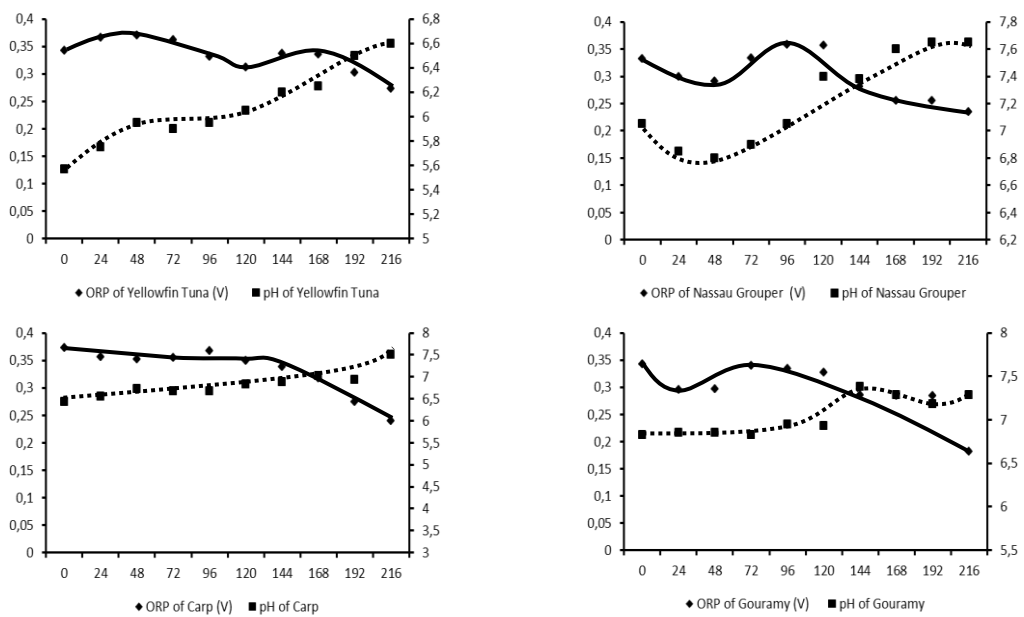
delaying but not inhibiting the spoilage of fish during storage.

*The relationships between Eh and pH*

The relationship between Eh and pH in four different fish on different temperature storage are shown in **Fig. 1** and **Fig. 2**.



**Fig 1.** The relationship between Eh and pH of tropical fish during storage at ambient storage. (♦) Oxidation reduction potential (Eh), (■) pH. Each symbol is represented as the mean.



**Fig. 2.** The relationship between Eh and pH of tropical fish during storage at chilled storage. (♦) Oxidation reduction potential (Eh), (■) pH. Each symbol is represented as the mean.



**Fig. 1 and 2** show the relations between Eh and pH, these patterns in four tropical fish were different. In yellowfin tuna, in which the Eh increased contrastly to pH and started to decrease when pH increasing. On the contrary, in the other three fish, Eh decreased proportionally to pH and then after pH reached the lowest value, pH increased until alkalie at the end of storage.

Based on afforementation above, pH and Eh patterns in yellowin tuna on both storage were contrary, but in the other three fish, these pattern were propotionally at preceeding time, then after mean time these pattern were contrary until the end of storage. The pattern of yellowfin tuna was agreement with Agustini (2000) research, in red meat which were stored at 0°C, 5°C, and 10°C, Eh and pH in different fish meat were contrary. This result also in agreement with Wijayanti, *et al.*, (2007), they investigated red meat marine fish, skipjack tuna.

Agustini (2000), stated that yellowfin tuna with tetracycline 100 ppm treatment, showed the slower decrease of Eh value than untreated sample. It showed bacteria activity causing Eh decrasing in fish samples. Alkalie pH in four tropical fish samples are suitable for bacteria growth. The spoilage of marine temperate of offensive fishy, rotten, H<sub>2</sub>S-off-odour and -flavour (Gram, *et al.*, 1989). The spoilage association with growth of gram negative psychrotropic non-fermenting rods. These bacteria are composed by *Pseudomonas* and *Shewanella putrefaciens* and at ambient temperature are predominated by mesophilic vibriionaceae (Gram and Huss, 1996). Microbial degradation of fish components, mainly amino acids and non-protein nitrogenous the combination and amount of microbial metabolites in the headspace of chilled products varies depending on which bacterium is the dominating specific spoilage organism (Ólafsdóttir, *et al.*, 2005). *Shewanella* is a predominant bacteria in marine fish and *Pseudomonas* is a dominant in tropical freshwater fish (Gram and Huss, 1996). *Shewanella*, *Pseudomonass*, and *Vibriionaceae* produce H<sub>2</sub>S from the sulphur containing amino acids (cystein). *Pseudomonass* produce CH<sub>3</sub>SH, (CH<sub>3</sub>)<sub>2</sub>S, ketones, esters, aldehydes, NH<sub>3</sub>, and hypoxanthin (Gram and Huss, 1996). Late

spoilage changes, development of spoilage odor of cod are explained by occurring of TMA, ester, acids, and sulphur are produced by microbial (Ólafsdóttir and Jóhndóttir, 2010). Gram negative bacteria *Pseudomonas* sp, *Shewanella putrefaciens*, and *Photobacterium phosphoreum* grow dominant on fish that store on chill temperature. The prominent characteristics of fish spoilage bacteria are an ability to reduce TMAO and to produce H<sub>2</sub>S (Nychas and Drosinos, 2010). These compounds are used by bacteria to grow.

## CONCLUSION

Based on the results of the changes of physicochemical properties in four tropical fish, it was concluded that every species has different pattern of Eh and pH. The rate of Eh and pH changing in four tropical fish were faster at ambient temperature storage and they were slower at chilled temperature storage. In addition, the relationship between Eh and pH were different for different fish.

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