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Original paper

EVALUATION OF QUALITY DETERIORATION OF MARINE SHELLFISH DURING STORAGE AT DIFFERENT TEMPERATURES

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ABSTRACT

Study on evaluation of fish freshness has been carried out using several parameters such as chemical, sensory, and physical parameter in which each has its own merits and demerits. Oxidation-reduction potential and K value are a physico-chemical and chemical methods available in assessing fish freshness which are both considered as objective methods. This study aimed to find out the effect of different temperatures storage on ORP and K value change of marine shellfish.

Material used in the study were black tiger shrimp (Penaeus monodon) and scallop (Amusium sp.). The experiment was laboratory experimental method. The samples were stored in room temperature (35±1^{o}C) and refrigerated temperature (11±1^{o}C). Analyses performed were ORP value (pH/ORP meter) and K-value (Ion-exchange chromatography method) and analysis were conducted in 4 replication. This study was carried out at laboratory of Fisheries Processing Technology, UNDIP Semarang and laboratory of PAU, UGM Yogyakarta.

The ORP of black tiger shrimp and scallop stored at refrigerated temperature initially were 0.23 Volts and 0.32 Volts. There were a maximum ORP of 0.3 Volts (shrimp) and 0.35 Volts (scallop) in the 2nd day of storage. These ORP then decreased to – 0.12 Volts and 0.01 Volts for shrimp and scallop, respectively. At room temperature storage, the ORP ranging from 0.26 to 0.33 Volts. This value consecutively decreased to – 0.17 Volts (shrimp) and – 0.16 Volts (scallop) after 32 hours storage. Initial K value of black tiger shrimp and scallop stored at room temperature were 1.32% and 1.51%, respectively and after 32 hours storage there were an increase in K value to 6.14% and 5.43%. Increase in K value was slower for samples stored at refrigerated temperature than that of room temperature.

Key words: redox potential (ORP), K value, black tiger shrimp (*Penaeus monodon*), scallop (*Amusium sp.*), storage temperature

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INTRODUCTION

Shrimp, scallop and other shellfishes are very often caught and consumed by people because of its nutrient, of high protein content. (Hadiwiyoto, 1993). Shrimp meat contains more enzyme cathepsin which causes protein deterioration proceeds faster compared to that of fish. In addition, shrimp meat contains high water content that can be a good media for pathogen bacterial to grow. (Clucas, 1981). All fish including shellfish are susceptible to deteriorate soon after death by physical, biological or chemical process that results to its freshness degradation. Freshness quality covers all things related to appearance, taste, odor, flavor, and texture that will affect people before buying the fish. Therefore, fish freshness is very important to evaluate before fish it is sold to the consumer.

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The evaluation of fish freshness has been an important issue in the fisheries industries and scientific field for long time since a big trading of fish has begun. Owing to the importance in determining the product quality of fish, many researches have conducted analysis. This resulted in introducing tremendous number of methods for analyzing fish freshness. For its application, however, simplicity, rapidity, reliability and economically low price will be of concern by people working in fish industries. Considering the essential of fish freshness quality, many method have been proposed to evaluate fish freshness including physical, chemical and sensory methods. The chemical methods has been considered as an objective method and therefore superior to methods involving sensory evaluation. Some chemical methods of fish freshness evaluation based on nucleotide degradation have been proposed as 95% or more of the non protein nitrogen of the muscle is accounted for by some compounds such as amino acids, TMAO, urea, nucleotide and compounds related to nucleotide. K value is widely used as fish freshness index to evaluate the quality change of raw fish. However, it is generally recognized that using of the K value as quality index for all fish species is limited since the K value fluctuates significantly depending on species, handling, temperature of storage and the measurement procedure, usually tedious, time consuming and cumbersome. Introduction of method of fish freshness evaluation based on physico-chemical properties such as oxidation-reduction potential (ORP) has been evaluated. This method is considered to be simpler, rapid and reliable for the quality of fish freshness.

This study is aimedat : 1) evaluate the changing of ORP and K value of shellfish stored at different temperatures; 2) observe any relationship between ORP and K value as freshness indices. Doing this study is expected to give more information on fish freshness evaluation methods especially using physico-chemical and chemical methods of analysis.

MATERIALS AND METHODS

Materials

Raw materials of shellfish used as sample in this study are shrimp (*Penaeus monodon*) and scallop (*Amusium sp*.). These samples were taken randomly and bought from fish market in Semarang and brought to the Laboratory by putting in sterofoam box with ice. Average weight for shrimp was 39 gram (range 25-50 gram) with average length of 13 cm (range 10-15 cm). The average weight of scallop was 12 gram (range 10-15 gram) with average diameter of shell was 8 cm (range 7,5 – 9,5 cm). All samples were found after 18 hours from catching in the sea (northern coast of Java). Chemical materials and equipment for analysis used in this study are shown in **Table 1**.

Table 1. Chemical and Equipment used

No.	Chemical Material	Equipment
1.	Dowex Resin 1-X4	Table top centrifuge (max.4000 rpm)
2.	Natrium hydroxide (Na OH) 1N	pH meter TPX-90i
3.	Ammonium hydroxide $(NH_4)_2$ SO_4 1N	Micro tube pump
4.	Chloride acid (HCl) 10%	Polypropylene mini column
5.	Perchloric acid ($HClO_4$) 10 and 5%	Spectrophotometer, UV Fis
6.	Pottasium hydroxide (KOH) 1 and 10N	Thermometer
7.	Ammonium hydroxide (NH_4OH) 0,5 & 0,05 N	Homogenizer/ mortar
	Chloride acid 0,01N containing NaCl 0,6N	Pippet
8.	Chloride acid (HCl) 0,002N	Filter paper
	Aquadest	Beaker glass
9.	Quinhydron	Vacuum pump
10..		Knife and chop board

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Methods

- Organoleptic / Sensory Analysis
  This analysis was done by Scoring Test using score sheet organoleptic for fish SNI-01-2729-1992 issued by Directorate General of Fisheries Jakarta (1994/1995). Scoring scale was ranged from 1 for the lowest and 9 for the highest value. The limit value of 5 was considered as rejected. Data obtained were then analyzed for its average value using 95% degree of confidence.
- K Value Analysis
  K value was measured by using Ion exchange chromatography method (Uchiyama *et.al*, 1970). The procedure is as follows:
  Pre-treatment Dowex resin 1-X4
  - Wash Dowex resin 1-X4 with 5 – 10 volume of 1N (4%) NaOH solution, left overnight. The final pH reaches 14
  - Wash the resin with aquadest till it reach neutral pH (depending on the pH of water used)
  - Wash the resin again with twice volume of 1N (7%) $(NH_4)_2\ SO_4$, leaved for 2 hours, the pH reach 9.5
  - Wash again the resin with aquadest till it reaches neutral pH
  - Wash with 5-10 volume of 10% HCl solution, left for 2 hours
  - Wash with aquadest until its pH reaches neutral, depending on the pH of water used

  Extraction of ATP-related compounds
  - Cut fish into small part and remove bones and scales, then cut the sample into fine pieces by knife and mix them
  - Take 2 grams of sample and add 2 ml of cold water then crush them in the mortar
  - Add 2 ml 20% $HClO_4$, cooled with ice and crush them. All of the solution is transferred into a centrifuge tube. Wash mortar inside and stick by 10% HClO4, cooled in ice. Do this three times.
  - Centrifuge at 4000 rpm for 10 minutes at 5oC
  - Take 10 – 20 mL of supernatant into a beaker
  - Drop 10N KOH. Insoluble KclO4 is yielded and solution becomes white. Initial pH is around 0, after approximately 20 drops of 10N KOH will reach pH 3-4, then drop 1N KOH into the solution (approximately 10 drops) while mixing until it reaches pH 6.5
  - Solution is transferred into centrifuge tube. It is not necessary to wash beaker
  - Centrifuge at 4000 rpm at 5oC for 10 minutes
  - Take 10 – 20 mL of supernatant into a beaker, then adjust pH to 9.4 with 0.5N Ammonium, add 0.05N Ammonium to reach pH 9.4 precisely

  Column Chromatography
  - Take dowex 1-X4 into the column made from plastic
  - Use pipette to take 2 mL extract of ATP related compounds and put into column carefully without making dirty, then silicon tube is connected to the end of column
  - To maintain ion charge constant, sample should be in the same level to dowex. The point of silicon tube is connected to syringe/beaker with 50 mL capacity
  - Add distilled water (pH 9.4) carefully, then suck it by syringe or pour it in the beaker. Solution which flew out of column becomes 20 mL and it is thrown away
  - Add 50 mL 0.002N HCl, then suck using a syringe / or pour in the beaker (this is for HxR and Hx $\rightarrow$ A)
  - Add 50 mL of 0.01N HCl containing 0.6M NaCl (0.6 mol/liter). This is for ATP, ADP, AMP and IMP $\rightarrow$ B

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- Read each sample using Absorptiometer OD 258 nm, then K value can be computed from the equation:
  K value = OD A : (OD A + OD B) x 100%

- ORP Analysis
  The oxidation-reduction potential is defined as the measurement of electrode potential to estimate the degree of oxidation or reduction of a particular foodstuff. The equation is expressed as follow:

$$ORPORP_0 + \frac{RT}{nF} \ln \frac{(oxidant)}{(reduc \tan t)}$$

  where ORPo is the potential at pH 7; R is gas constant (8.314 J/ K.mol); T is absolute temperature (K); F is Faraday quantity of electricity (96.496J/volt); n is the number of electrons transfer in the process; oxidant is the substance which acts as an electron acceptor; reductant is the substance that acts as an electron donor.
  The measurement of ORP was carried out using an electrometer (pH meter, TPX – 90i) as reported by Okouchi *et.al* (2001), in which platinum and glass electrodes were used to measure ORP and pH, respectively. The electrode was specially designed for measuring ORP and pH both for liquid and solid states and ORP/pH of the samples were measured by placing the electrode in a liquid mixture of ample meat and water (aquadest) in the ratio of 18 ml of aquadest : 2 grams of meat sample. Before use, the electrode was checked with quinhydron standard solution which has ORP of 0.26±0.02Volt. Between repetitive readings, the electrode was cleaned with pure water and soaked in it for several minutes before the next measurement. This equipment measures ORP and pH of the sample simultaneously.

RESULTS AND DISCUSSION

Organoleptic / Sensory Assessment

The most common methods for analyzing acceptance for fisheries products by consumer is by using sensory analysis of odor, appearance, texture and flavor (Farber, 1965). This method is considered the simplest, easy, cheap and can be carried out without any complicated apparatus. Shrimp was found after 12 hours from catching from Jepara. Scallop was found after 15 hours from catching and taken from Kendal area.
Freshness deterioration of shrimp was detected by assessing appearance, odor, and texture. Initial changed discoloration was detected on the body and abdomen of shrimp after 8 hours of storage (for room temperature) and 3 days of storage (for refrigerated temperature). Discoloration of shrimp occurs due to autolysis process and it is related to enzymatic activity which catalyzes reaction between sugar and amino substances resulted in discoloration (Trenggono, 1990). The same occurrence also happens on scallop, in which muscle color of scallop turns to reddish, not clear and dull. This color muscle will finally turn to black white pale during storage and samples stored in room temperature change faster than that of sample stored in refrigerated temperature. Organoleptic change occurs in shrimp and scallop depending on the presence of volatile components. The initial change of pH occurs in fish meat due to production of lactic acid during glicolysis process (Flick and Martin, 1992). This change of pH meat activates enzyme chatepsin to degrade protein into amino acids that causes tenderization of meat texture (Peranginangin, et.al, 1986).

Oxidation-reduction potential (ORP) of Samples Stored at Refrigerator (11±1°C)

Table 2 shows ORP value of shrimp and scallop during storage at refrigerated temperature for 9 days. Initial ORP of

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shrimp and scallop stored at refrigerator are 0.22 V and 0.32 V, respectively. The ORP value of shrimp and scallop increases in first day of storage and reach the maximum value of 0.3 V (shrimp) and 0.35 (scallop) on 2nd day of storage. The measurement after 3 days of storage and afterward, ORP tends to decrease even reaching minus value of –0.12 V (for shrimp) on 9 days of storage. Hebert *et.al* in Huss and Larsen (1977), stated that ORP of fresh fish initially shows positive value of 100mV – 300mV and then drastically decreases for staled fish (when fish is completely deteriorated).

Table2. ORP Value of Shrimp and Scallop Stored at Refrigerated Temperature

Storage time (day)	ORP of Shrimp (Volt)	ORP of Scallop (Volt)
0	± 0.004	0.321± 0.013
1	0.234 ± 0.007	0.331 ± 0.024
2	0.298 ± 0.016	0.354 ± 0.051
3	0.252 ± 0.007	0.338 ± 0.044
4	0.234 ± 0.009	0.264 ± 0.029
5	-0.035 ± 0.025	0.176 ± 0.037
6	-0.040 ± 0.022	0.069 ± 0.024
7	-0.043 ± 0.023	0.060 ± 0.014
8	-0.058 ± 0.072	0.046 ± 0.022
9	-0.120 ± 0.014	0.013 ± 0.048

There is a relationship between fish deterioration and ORP change, where ORP of fish increases initially and then decreases when deterioration process proceeds. Agustini *et.al*. (2001), stated that initial increase in ORP during fish deterioration is related to the presence of some redox couples such as NAD^+, NADH, $NADP^+$, NADPH, Fe^{2+} which can act as reductant or oxidant. In this study it is considered that oxygen has little effect on the change of ORP. Moreover, the ORP maximum of kuruma prawn (*Penaeus japonicus*) of0.27 V lower than tiger shrimp (*Penaeus monodon*) and scallop (*Amusium sp*.) were used in this study with ORP maximum of 0.3 V and 0.35 V, respectively. The difference in ORP value resulted was due to different species used as sample, and also different preparation of the sample. In case of kuruma prawn, the whole fish flesh was used to measure ORP directly. Whereas for shrimp, the solution of meat was used by dilution of shrimp meat (2 gram) in 18 mL of aquadest. The sample in the form of solution will have high moisture content. This moisture / water will be degraded into hydrogen and oxygen ion which can act as oxidant and reductant, apart from other chemical compounds within the shrimp meat.

ORP of scallop did not show negative value until the end of storage time (9 days). This was caused by the presence of TMAO-reducing bacteria not completely convert TMAO to TMA. Huss and Larsen (1977) showed that available TMAO on fish will finish after 6 days of storage and TMAO was considered as the main factor that affects the change on ORP of fish. They concluded that before TMAO was completely degraded to TMA by TMAO-reducing bacteria, ORP was considered as positive.

ORP of Sample Stored at Room Temperature (35±1^{o}C)

The ORP of samples stored at room temperature were analyzed for 32 hours and the results can be seen on **Table 3**.

Table 3. ORP Value of Shrimp and Scallop Stored at Room Temperature

Storage time (hour)	ORP of Shrimp (Volt)	ORP of Scallop (Volt)
0	0.260 ± 0.010	0.355 ± 0.015
8	0.100 ± 0.057	0.260 ± 0.026
16	-0.078 ± 0.036	-0.025 ± 0.005
24	-0.148 ± 0.037	-0.085 ± 0.072
32	-0.177 ± 0.057	-0.161 ± 0.030

The initial ORP of shrimp and scallop stored at room temperature was 0.26 V and 0.33 V, respectively. These values did not increase during storage but decrease up to the end of storage time (32 hours) and reached ORP value of – 0.177 V (for shrimp) and – 0.161 V (for scallop). In this case, the maximum ORP did not exist and this phenomenon can be explained by the presence of microorganism that grows faster at room temperature and convert TMAO to TMA. Huss and Larsen (1977) said that the presence of microorganism can affect decrease in ORP. Microorganism used TMAO as electron acceptor when oxygen depleted in which lactic acid and TMAO were converted to TMA, acetic acid and CO2 (Hadiwiyoto, 1993). In mammal, TMAO presence in very less amount therefore ORP take longer to attain negative value after death compared to that of fish (Huss 1988).

K-value determination of sample stored at refrigerated and room temperatures

K-value of shrimp and scallop during storage at refrigerated and room temperatures can be seen on **Table 4** and **Table 5**.

Table 4. K-value of shrimp and scallop during storage at refrigerated temperature

Storage time (days)	K-value of shrimp (%)	K-value of scallop (%)
0	1.44 ± 0.831	1.68 ± 0.811
1	2.19 ± 1.420	2.62 ± 0.901
2	2.28 ± 1.216	2.94 ± 1.743
3	2.53 ± 0.884	3.24 ± 0.403
4	2.70 ± 0.924	3.56 ± 1.771
5	3.43 ± 1.723	4.01 ± 1.180
6	3.88 ± 0.742	4.43 ± 2.381
7	4.72 ± 1.089	4.56 ± 1.213
8	7.03 ± 1.134	5.77 ± 1.205
9	7.50 ± 1.652	6.58 ± 1.869

Table 5. K-value of shrimp and scallop during storage at room temperature

Storage time (days)	K-value of shrimp (%)	K-value of scallop (%)
0	1.32 ± 0.471	1.51 ± 0.533
8	1.95 ± 0.852	1.79 ± 0.740
16	2.13 ± 0.433	2.08 ± 0.962
24	4.68 ± 0.692	4.85 ± 1.182
32	6.14 ± 1.600	5.43 ± 1.839

K-value of shrimp and scallop increases gradually during storage at both temperature, but at room temperature changes more rapidly compared to refrigerated temperature. The higher the temperature, the faster is the degradation of ATP proceeds. As it is considered that ATP degradation is enzymatic process,

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temperature has important effect to the rate of the reaction. (Flick and Martin, 1992). ATP degradation for invertebrate (shrimp and scallop) have slightly different pattern to that of fish. After death, degradation of ATP to its related compounds affect the freshness quality of the meat. Shrimp and scallop produce more dominant in Adenosine (AdR) rather than IMP. This different in pattern was considered as main cause for K-value of shrimp and scallop not significantly increase like fish in general. (Saito, et.al, 1959). Shrimp tends to increase faster compared to that of scallop. This was due to shrimp meat considered as better media for bacterial growth because contained more nitrogen compounds and enzyme cathepsin, so that protein can be degraded faster (Houwing, 1974)

CONCLUSION

From this study it can be concluded that:

1. ORP value of shrimp and scallop during storage at refrigerated temperature increases initially and reaches a maximum of 0.3 V and 0.35 V for shrimp and scallop, respectively. This ORP value decreased after 3 days of storage. While K-value of shrimp and scallop increases during storage and reaches the maximum K value of 7.5% and 6.58% for shrimp and scallop, respectively.
2. ORP value of shrimp and scallop during storage at room temperature did not increase initially, but decrease directly and reach minus value of – 0.177 V and – 0.161 V for shrimp and scallop, respectively. Whereas K-value for both samples increase along with storage time.

REFERENCES

Agustini TW, Suzuki M, Suzuki T, Hagiwara T, Okouchi S and Takai R.2001. The possibility of using oxidation-reduction potential to evaluate fish freshness. *J. Fisheries Science* (67): 2

Clucas I.J. 1981. *Fish handling, preservation, and processing in The tropics*. Part I. Tropical product Institute. London.

Farber, L. 1965. "Freshness Test" in: G. Borgstorm (ed). *Fish as Food* Vol.IV. Academic Press. New York.

Flick GJ. and Martin RE. 1992. *Advances in Seafood Biochemistry* : Composition and Quality. Technomic Publishing Co. Lancaster, USA

Hadiwiyoto, S. 1993. *Teknologi Pengolahan Hasil Perikanan*. Liberty, Yogyakarta

Houwing, H. 1974. *Technical, economic, and condition for an industrial plant for irradiation preservation of shrimp*. Commission of the European Communities. Euroisotop Office.

Huss, HH and A. Larsen. 1977. The post mortem changes in the oxidation-reduction potential of fish muscle and internal organ. Proceeding X Intern. *Symposium IAMS*. Polland. Pp: 265 – 279

Huss, HH. 1988. *Fresh fish quality and quality changes*. FAO. Danish International Development Agency, Rome – Italy.

Okouchi, S, Mizuno H, Kusatka k, Ishihara Y and Amaroji, Y. 1998. Evaluation of aging index of hot and cold spring water by ORP. *Onsen Kagaku* Vol 48:29-35

Peranginangin, R, TAR. Hanafiah, S.Putro, R. Mulyanto. 1986. Storage life of

ISSN: 1410-5217
Accredited: 23a/DIKTI/Kep/2004

fresh water fish at room temperature and crush ice. *Jurnal Penelitian Pasca Panen Prikanan* No.51

Saito K, Arai and M. Matsuyoshi. 1959. A new method for estimating the freshness of fish. *Nippon suisan gakkaishi* (24): 749-750.

Trenggono. 1990. *Analisa Hasil Perikanan*. PAU Pangan dan gizi, UGM-Yogyakarta

Uchiyama, H, Ehira S, Kobayashi H, Shimizu W. 1970. Significance in measuring volatile base and trimethiamine nitrogen and nucleotide in fish muscle as indeces of fish freshness. *Bull. Japan Soc.Science Fish* (36): 177-187