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Original Paper

DETERIORATION QUALITY OBSERVATIONS OF MILK FISH (*Chanos chanos* FORSK) AND SHORT-BODIED MACKEREL (*Rastrelliger neglectus*) AT VARIOUS STORAGE TEMPERATURES USING FRESHNESS TESTING PAPER (FTP III)

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ABSTRACT

Freshness of fish is crucial in determining the nutritional value and sale value of fishery products. Assessment of fish freshness can be done with various methods based on several principles of chemistry, physics, microbiology, and organoleptic. K value is one of freshness test based on ATP degradation. Analysis of K values can be done with Freshness Testing Paper (FTP III). This method is relatively practical, easy, fast and the results can be accountable. The material that used in this study are fish milk fish (Chanos chanos Forsk) and short-bodied mackerel (Rastrelliger neglectus) with total 90 fish with an average weight of 99 grams / fish (milk fish) and 85 g / fish (short-bodied mackerel). The fish raw material purchased from Rejomulyo fish market (Semarang) and taken to the laboratory in a Styrofoam box that was given ice on the inside. This research used an experimental method called descriptive exploration. Storage of samples was handled at different temperatures namely $30^oC \pm 2^oC$, $15^oC \pm 2^oC$ and $1^oC \pm 0^oC$. The observed parameters include organoleptic test and analysis of K Value (FTP III). The results showed that K values for all samples increased during storage at different temperatures. The highest K values of milk fish and short-bodied mackerel fish during storage at a temperature of 0°C was on 96-hours storage time. The highest K values of milk fish and short-bodied mackerel fish during storage at a temperature of 15 ° C was on 72-hours storage time. Whereas the highest K values in milk fish and short-bodied mackerel fish during storage at 30°C temperatures, was on the 24-hours storage time. The fastest increase in K value was observed at a temperature of 30°C compared to storage at a temperature of 15°C and 0°C for all the fish samples. This means that the deterioration quality of fish will be faster at high temperatures. K values of mackerel increased more rapidly than milk fish.

Keyword : *K value*, FTP III, milk fish (*Chanos-chanos*, Forsk), short-bodied mackerel (*Rastrelliger neglectus*), storage temperature

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INTRODUCTION

Fish are included in fishery which is the most widely known than the other fisheries. As food, the fish position becomes very important, because it have many contain the components that needed by the body. Besides being the source of very high animal protein, fish meat

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also contain compounds that are potential for the human body.

The availability of a growing fishery must be balanced with the knowledge of appropriate handling, preservation and processing of fishery products. Moreover, fishery products are including one of foodstuffs that is very easy to damage (perishable food) caused by the autolysis process that are bacteriolytic and enzymatic which can decrease the quality of fish. In the fishery industries, the perfection handling of fresh fish plays an important role, toward for determines the quality and the value of fish as food or quality of products in further processing.

If temperature is lowered faster, the growth rate of microorganism and enzymatic activity will be retarded in fish meat. As it is known that the temperature is very important in the process of fish deterioration and proper fish handling is very important in keeping the freshness of fish, so we need the practical test equipment evaluation of fish freshness.

Various methods for measuring the level of fish freshness are available now, whether it is objective or subjective. An objective assessment can be classified as a physical assessment, physico-chemical, chemical and biological. K value is a chemical method that most often used to measure the freshness of fish. K value provides a relative freshness value, especially in autolytic changes that occur during storage. The main purpose of using the equation K value is to achieve a special value that quantitatively show the level of fish freshness with a completed value of the fish freshness limit (Henehan *et al*., 1997).

Method of determining a quantitative K value some studies was done by the HPLC method (Agustini, *et.al*, 2001 and Ryder, 1985) and ion exchange chromatography. Research conducted by Agustini, *et al*., (2001) using the method of High Performance Liquid Chromatography (HPLC) according to Ryder (1985) was used sample of tuna (*Thunnus albacares*) which was stored at a temperature of 5°C, 0°C, and - 3°C gave K values 30% for each storage after 92 hours, 173 hours and 372 hours. The study concluded that the higher the storage temperature the faster the reaction that causes fish putrefy and get the higher K value.

Research of K value that conducted by Nanda (2004) using Milk fish as sample with ion exchange chromatography using the method of Uchiyama *et al*., (1972) indicated that the K values at room temperature reached 26.78% after 2 days of storage, whereas at refrigerator temperature reached 24.11% after 7 days of storage.

Method of K value analysis grows up until EAC Corporation Japan (1991), found the more practical method measurement by using paper (Freshness Testing Paper) III. In Indonesia, it is still very rare research using FTP III, as compared to other methods. The advantage of FTP is easy to use procedures, practical, fast and relatively inexpensive. If it studied more, then the use of FTP is compatible in Indonesia with tropical climate. FTP III can quickly analyze the freshness of the fish so that the value of fresh fish can be known before the further quality deterioration. Procedure using practical FTP III can also support the analysis process, which can be done in the field without having to laboratories.

The purpose of this research is to determine the influence of different storage temperatures on the pattern of changes K values in milk fish and short-bodied mackerel fish and to assess the difference quickness changes of K value in milk fish and short-bodied mackerel fish.

With this research, we expected to provide information to the general public about the value of fresh milk fish and short-bodied mackerel during storage at different temperatures. Other information that can be obtained is about the use of new device in chemical methods, which measure the K value withFTPIII.

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MATERIALS AND METHODS

Raw Materials

Raw materials used in this research were milk fish (*Chanos chanos* Forsk) and short-bodied mackerel (*Rastrelliger neglectus*) which had been purchased at Rejomulyo Fish Market, Semarang. Fish samples taken from the market to the laboratory by using a Styrofoam box with ice added with the comparison of ice:fish is 1:1. Length and the average weight of fish samples were 25.5 cm and 99 g (milk fish); and 19 cm and 85 grams (short-bodied mackerel). As for besides materials used in this study is the ice.

Fish Storage

Fish prepared at three different temperatures namely temperature of 0ºC using styrofoam boxes with flake ice (ice replaced every 8 hours to keep the constant temperature), temperature of 15ºC using a refrigerator and temperature of 30ºC with storage at room temperature .

K value Analysis of milk fish and short-bodied mackerel fish at the storage temperature of 0°C intervals of 24 hours with 5 observations. At the storage temperature of 15°C intervals of 18 hours with 5 observations, while at the storage temperature of 30°C intervals of 6 hours with 5 observations.

Organoleptic test

Organoleptic tests were conducted based on SNI 01-2345-1991 using the score sheet with 10 panelists. The steps are as follows:
The sample of fresh fish was prepared as much as 10 fish. Panelists rate based on the score sheet with fresh fish organoleptic characteristics observed: eyes, gills, mucus, meat and stomach, smell and consistency. Each of these characteristics has a range of values between 1 - 9.
The data obtained were processed by calculating the average and standard deviation of 95% confidence intervals to determine the level of freshness.
Requirements for quality of acceptance fish freshness as fresh fish is at least 7.

K Value Analysis

K value measurements were done with FTP III (EAC, 1991) with several stages as follows:
White meat was taken from the dorsal part of the fish samples, separated the flesh from skin and mashed with a mortar. The soft fish meat samples was taken as much as 0.5 g and put into a test tube. Next, 5 mL reagent of F III was added in a test tube and mix reagents use a glass stirrer. FTP III paper (**Fig. 1**) was soaked in a test tube containing a sample until the two lines A and B had been immersed all. FTP III Paper was taken and wrapped in transparent plastic for ± 10 minutes and then covered with black fabric to avoid direct contact with light. Color on line A and B matched in FTP III paper to standard color chart (figure 2) which showed K values, and the results recorded.

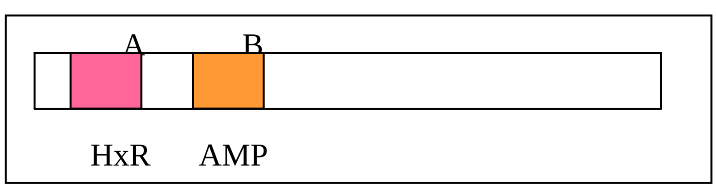

Fig. 1. FTP III paper

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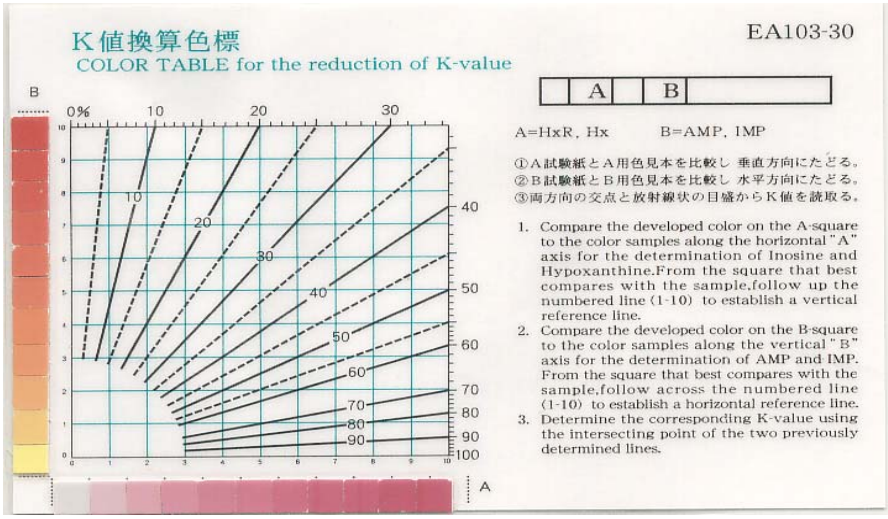

Fig 2. Color Table for the reduction of K value

The steps to read a standard color chart FTP III:

To determine inosine and hypoxanthine, changing color that occurred on line A of the FTP III was compared to samples color along a horizontal A line in the table.

A straight line was pulled if the color of the sample resembles the color of the line A in the table.

To determine the AMP and IMP, the next change of color that occurs in the line B of the FTP III was compared with samples color along a vertical line B in the table.

A straight line was pulled to form horizontal line, if the color that resembles the color of the line B was found in the table.

The K value was determined by finding the intersection of two lines. The final step was sought from the meeting point of two lines that had been predetermined. That point was considered as K value of the sample.

K Value Analysis

Analysis approach for the calculation and estimates regarding the decline of food quality kinetic equation based on the speed of the decline of quality. In addition to K values, also used the value of K 'which can indicate the ratio of the amount of ATP, ADP, AMP was left with a total of ATP and component degradation, which was the method of Miki and Nishimoto (Agustini, *et al*., 2001a). The K' is used in regression analysis to determine the quality of the reaction rate of deterioration quality sample. Equation the value of K' by Miki and Nishimoto (1984) was as follows:

K' (=100- *K value*) = a. Exp ($-k_f$.t)

Description:

K '(= 100-K value) = reaction rate,

kf = constants rate of reaction

a = constant t = storage time (hours)

On the research, the end of this equation is converted into:

$$Y = A - k_f.X.$$

Description:

Y = K value or reaction rate X = storage time (hours)

A = constant k_f = constants rate of reaction

The negative sign in this equation only shows the direction of decreasing linear lines, there is no effect on the constant rate of reaction value.

Experimental Design

This research was an experimental laboratories that called descriptive exploratory. Independent variables observed in this study were storage temperatures. These independent

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variables were used to predict the values of the dependent variable in this study, the K value.

Data Analysis

Data obtained from treatment in this study were recorded for later analysis. Statistical tests of F should be done first because it can indicate whether all the independent variables have an influence on the dependent variable. Statistical tests of T were performed to determine the significance of the influence of each independent variable and its coefficient. Calculation of data was done by using SPSS version 13.0.

RESULTS AND DISCUSSION

Organoleptic Test of Milk Fish (*Chanos chanos* Forsk) and Short-Bodied Mackerel (*Rastrelliger neglectus*)

Based on organoleptic tests using score sheets of the SNI No. 01-2345-1991 by 10 panelists found that the sample results have a confidence interval value between 7.84 $< \mu <$ 8.30 (milk fish) and 8.22 $< \mu <$ 8.42. This means the fish were still suitable for consumption or it still in the fresh condition. This value meets the minimum requirements of fresh fish according to the Indonesian National Standard (SNI 01-2729-1992), which is $\geq$ 7.00 (Dirjen Perikanan, 1995).

K Value Analysis

One method analysis of fish freshness widely used is K value. This has been proven by the number of literatures which state that the K value is an effective method. K value is expressed as a percentage of the ratio number of inosine (HxR) and hipoxanthine (HX) with total of ATP and degradation components results. Low K values indicate that the fish was fresh, the higher of K value the freshness of the fish diminished. That is because the amount of content inosine (HxR) and hipoxanthine (HX) that more and more.

In principle, the ice that added had to make the storage temperature lower until 0°C and then maintaining the temperature during storage. Analysis results can be seen in table 1 below.

Table 1. K value of milk fish and short-bodied mackerel during storage at 0^{o}C

Observation order -	Storage time (hour)	*K value* (%) ± SD Milk fish	*K value* (%)± SD Short-bodied mackerel
1	0	9,0 ± 0,00	12,7±0,58
2	24	9,5 ± 0,00	17,2±1,61
3	48	11,0 ± 2,00	17,5±0,87
4	72	12,0 ± 0,00	20,0±3,46
5	96	13,0 ± 0,00	22,5±0,50

The average K value obtained in the first observation of milk fish and short-bodied mackerel were small enough due to the condition of fish freshness at the beginning of measurement were in fresh conditions, where the solution components and low adenosine accumulation inosine-hipoxanthine still little. From the research shows that K initial values for short-bodied mackerel relatively higher than the milk fish, although by organoleptic test short-bodied mackerel fish better than milk fish. This indicates that the K value depends on the species and red meat fish species are usually higher than white meat fish because of some contain enzymes that play a role in the deterioration of fish quality. Actually the quickness change of K values in each species of fish are different, so it can be said that each species has a velocity change K value of each. It was reported that a K value could not be used as a standard value for the freshness of the all fish species (Agustini., *et. al.*, 2001).

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There are major differences in degradation rate between species, and even there are differences between fish in a single species due to poor sampling, fish size and environmental factors (Hiltz *et. al.*, in Nanda, 2004). K value based on the degradation of ATP is influenced by several factors such as fish movements before death, ways of handling, how to bleeding, storage temperature and how the fish died.

K value of the sample at the storage temperature 0°C showed sustainable changes that increase until the end of the observation, there were 13% of K value for milk fish, and 22.5% for short-bodied mackerel. K value improvement occurs gradually due to the temperature factor is used. According to Soewedo (1993) the temperature factor has a huge influence on the process deterioration of fish and it is the main factor among other factors. Putrid can still occur at low temperatures, although slow. This statement is consistent with the opinion of Henehan *et. al.* (1997), that the fresh fish where inosine and hipoxanthine still very few have a small K value, and K value rises along with increasing accumulation of inosine-hipoxanthine, mentioned also that the K value rises more slowly when the fish are stored in low temperatures.

Results from observations are presented in Table 2 below:

Table 2. K value of milk fish and short-bodied mackerel during storage at 15°C

Observation order -	Storage time (hour)	*K value* (%) ± SD Milk fish	*K value* (%)± SD Short-bodied mackerel
1	0	9,0 ± 0,00	12,3 ±1,15
2	18	9,5 ± 0,50	18,0 ± 0,89
3	36	13,3 ± 0,29	18,2 ± 1,75
4	54	13,7 ± 1,53	20,0 ± 3,46
5	72	14,0 ± 1,00	25,8 ± 1,76

K value at the storage temperature of 15 ° C was continued linearly with increasing temperature of the refrigerator. Temperature factors are the most influential factor to deterioration of fish quality, so the K value in the treatment of storage at a temperature of 15°C was higher when compared to storage at a temperature of 0°C. This was shown at the end of the observation, K values in milk fish and short-bodied mackerel fish were higher in temperature 15°C than at a temperature of 0°C.

Increasing K value at a temperature of 15°C is still small because the temperature 15°C is also still a low temperature / cold. After death, the fish will experience the process of decreasing the level of freshness and the first phase of what happened is autolysis. According to Tranggono (1990), autolysis occur more quickly in the fish that are not treated at low temperatures. Therefore, the effect of autolysis can be inhibited by providing ice or keep the fish in the refrigerator. Results from observations are presented in **Table 3**.

Table 3. K value of milk fish and short-bodied mackerel during storage at 15°C

Observation order -	Storage time (hour)	*K value* (%) ± SD Milk fish	*K value* (%) ± SD Short-bodied mackerel
1	0	9,0 ± 0,00	11,0 ± 0,00
2	6	13,0 ± 1,00	20,7 ± 2,93
3	12	13,0 ± 1,00	22,5 ± 3,61
4	18	18,5 ± 3,50	24,0 ± 1,00
5	24	23,7 ± 1,53	33,2 ± 4,04

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There was a big difference of K values at the 18 hours observation in 15°C storage temperature with 30°C storage temperature. K value at the 15°C temperature and 30°C temperature respectively about 9.5% and 18.5%. The difference of K values was also

found in the 0°C storage temperature with 30°C storage temperature, in the 24th hour. K value at the 24th hour storage at a 15°C temperature and 30°C temperature respectively about 9.5% and 23.67%. This supports the statement that the higher storage temperature the reaction changes are faster and the higher storage temperature so fish putridity characterized that will be known by increasing K value are becoming faster.

The change pattern of K value in temperature 30°C tends to increase until the end of the observation. The change pattern of this K value was equal to the pattern change of K value in temperature 0°C and temperature of 15°C.

K value of milk fish obtained at the end of this research was about 23.67%. In the study by Nanda (2004), K value of milk fish that stored at room temperature (32 ± 1°C) for 3 days was about 26.78%. In another study, by Miki and Nishimoto (1984) to obtain the 30% K value of tuna fish samples that stored at a temperature 5°C, 0°C and -3°C, the fish should be stored for 3, 85 days, 7.2 days and 15.5 days for each samples.

In accordance with research done by Agustini *et.al.* (2001) and theories that have been discovered by previous studies (Miki and Nishimoto, 1984; C Alasalvar, *et al*, 2001), it was found that the K value increased linearly with fish quality deterioration.

Statistical calculations of the temperature influence on K values produces the following equation: Y = 10.478 + 0.151 X. Value of constants was 10.478 explain that if the temperature variable constant, the average K value are 10.478%. Temperature regression coefficient was 0.151 which explain that the storage temperature on the 1°C will increase the K value about 0.151%.

In addition to K value, the value of K' are often used to determine the reaction rate or speed reaction of the deterioration quality of the sample. Equation K' is as follows: K' (= 100 - K value) = a. Exp (-kf.t) (Miki and Nishimoto in Agustini, *et al*., 2001a). Table 4 shows the data that needed to determine the value and the rate of reaction.

Table 4. Reaction rate of milk fish quality deterioration during storage

Temperature (°C)	Time (hour)	*K value* (%)	(100-*K value*)	Log (100-*K value*)
0	0	9	91	1,9590
	24	9,5	90,5	1,95664
	48	11	89	1,9494
	72	12	88	1,9445
	96	13	87	1,9395
15	0	9	91	1,9590
	18	9,5	90,5	1,9566
	36	13,33	86,67	1,9379
	54	13,67	86,33	1,9362
	72	14	86	1,9345
30	0	9	91	1,9590
	6	13	87	1,9395
	12	13	87	1,9395
	18	18,5	81,5	1,9112
	24	23,67	76,33	1,8827

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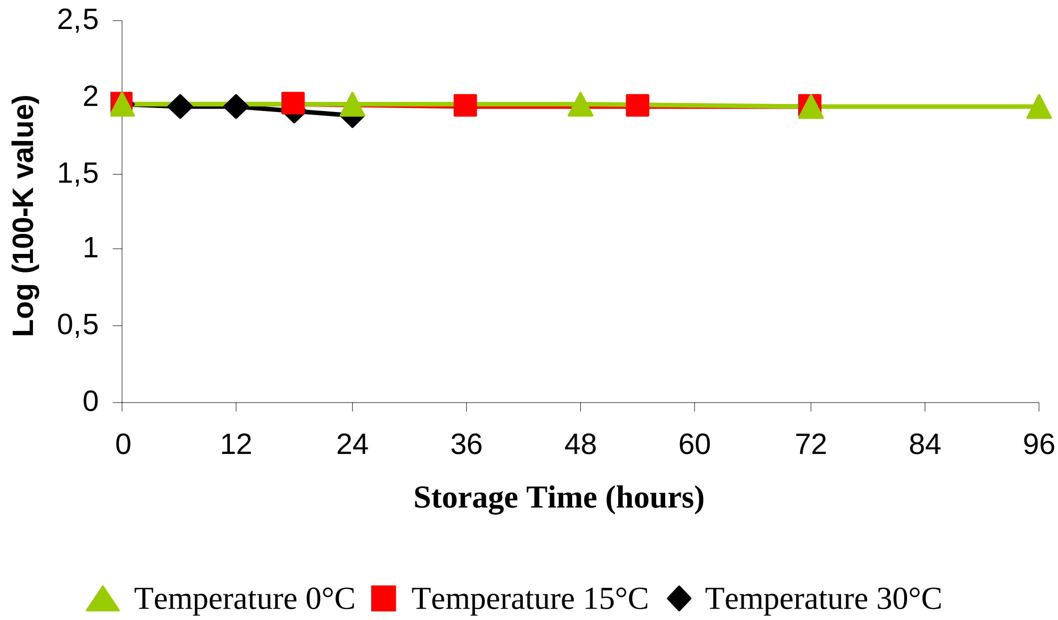

Fig 3. Reaction rate curves at different storage temperatures

Equation K '(= 100 - K value) = a. Exp (-kf.t) (Miki and Nishimoto in Agustini, *et al*., 2001) and then converted into $Y = A - k_f.X$.
With description:

Y is the value of K '
A is a constant
X is the storage time (hours)
kf is the constant rate of reaction

The negative sign in this equation only shows the direction of a decreasing linear line, there was no effect on the constant reaction rate.

Statistical calculations of reaction rate at a temperature of 0°C resulted in the following equation: Y = 1,960-0,0002 X. Value of constants of 1.960 explained that if the variable time constant, the average reaction rate was 1.960%. Value of 0.0002 explained that the reaction rate constant on the 0°C storage temperature was 0.0002.

Statistical calculations of reaction rate at a temperature of 15°C produces the following equation: Y = 1,959-0,0004 X. Value of constants was 1.959 explained that if the variable time constant, the average reaction rate was 1.959%. Value of 0.0004 explained that the reaction rate constants on the 15°C storage temperature was 0.0004.

Statistical calculations of reaction rate at temperature 30°C produces the following equation: Y = 1,963-0,003 X. Value of constants was 1.963 explained that if the variable time constant, the average reaction rate was 1.963%. Value of 0.003 explained that the reaction rate constants on the 30°C storage temperature was 0.003.

From those three equation of reaction rate, constant reaction rate at a temperature of 0°C was 0.0002, while the temperature of 15°C and 30°C, the value were 0.0004 and 0.003, respectively. Based on the reaction rate value, the largest value was found in the storage temperature of 30°C. It could be argued that the treatment of storage temperature of 30°C has the largest reaction rate compared to other temperature or in other words, at temperature of 30°C there was a faster quality deterioration rate occurring than the other storage temperatures (15 ° C and 0 ° C).

Study on the rate of reaction using yellow fin tuna samples with storage temperature treatments applied between 20°C to -40°C was also been done by Miki and Nishimoto (1984). The results showed that K values continue to increase as the time of storage prolonged on the storage temperature of 20°C to -40°C and the higher storage temperature showed the faster the reaction rate, which means that the higher storage temperature caused the fish to be faster deteriorated. For example, the study of Miki and Nishimoto (1984) to achieve 30% of K value of the samples stored at 5°C, 0 ° C and -3°C required different storage time, for 3.85 days, 7.2 days and 15.5 days, respectively.

The results of this study showed that to reached K values of ± 13%, the milk fish that stored at 0°C, 15°C and 30°C required different time of storage, namely 48 hours or 2 days, 36 hours or 1.5 days and 6 hours or 0.25 days.

CONCLUSION

The differences of storage temperature have an impact on the rate of freshness quality change of K value in both types of fishes. K values of mackerel increased more rapidly than milk fish.

Storage of fish samples at higher temperatures performed faster rate of fish freshness deterioration that could be seen in the higher reaction rate.

Fresness Testing Paper (FTP III) proved to be a fast method of determining the index of fish freshness, easy and not too expensive.

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