Potential of Seawater Electrolyte as a Preservative of Fresh Fish

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ABSTRACT

Background: *The use of cooling and freezing techniques in maintaining the quality of fresh fish requires more expensive production costs in the fresh fish business. Therefore, we need an alternative method with low cost, one of which is by utilizing seawater electrolytes. Seawater electrolytes are effective in killing microbes, thus prolonging the shelf life of fishery products.*

Objective: *This study aimed to determine the effect of seawater electrolysis on the total plate count of selar (Selaroides sp.) fresh fish.*

Methods: *The research design used is an experimental design with the following treatments: Treatment A: Selar fish soaked in 100% electrolytic seawater for 1 hour; Treatment B: Selar fish soaked in 50% electrolysis seawater for 1 hour; Treatment C: Selar fish soaked in ice (1:1 ratio) for 1 hour; and Treatment D: Selar fish left at room temperature without treatment for 1 hour. The parameter measured was the total plate count of each treatment, ORP (Oxidation Reduction Potential) and pH of electrolyzed seawater.*

Results: *The results showed that soaking in 50% and 100% electrolyzed seawater significantly affected the total plate count of fresh fish during 1 hour of storage (P>0.05). The best treatment is treatment A, with a yield of 4.65 ± 0.26 Colony/gram. The ORP (Oxidation Reduction Potential) value obtained from the electrolysis is 770.5 mv. The pH value obtained from the electrolysis of seawater is 6.3.*

Conclusion: *This study findings that the concentration of 100% and 50% electrolysis seawater had the same effect as ice treatment on the total plate count of selar fish (Selaroides sp.) during one hour of storage.*

Keywords: ORP, pH, Selar, Total Plate Count

Introduction

Food safety is a fundamental provision for consumers in determining the decision to buy a product. Fresh fish are susceptible to contamination and deterioration in a relatively short period (Bath *et al.*, 2012; Palawe*et al.*, 2016). The quality of fresh fish as raw material for processed fish products affects the final product. Therefore, the fish processing process should avoid cross-contamination. In addition, geographical conditions, climate, and handling methods affect the quality of fishery products, especially fresh fish (Piramoon and Hosseinimehr, 2016).

One way to maintain the quality of fresh fish is by preservation techniques (Sampels, 2014), and the techniques that are widely applied in handling fresh fish are freezing and the use of ice (Pandit, 2017). A major disadvantage to the use of ice or freezing is the high operational costs. In addition, this method cannot be void of the occurrence of lipid oxidation (Gómez-Guillén and Montero, 2007). Another preservation technique used is a low-temperature long time (LTLT) which is commonly called pasteurization. In addition, the high-temperature short time (HTST) method known as sterilization is widely applied to fishery products (Palawe *et al.*, 2014; Olatunde and Benjakul, 2018).

In preventing the deterioration of fresh fish, techniques such as cooling and freezing are often used in Indonesia (Wibowo *et al.*, 2017). Cooling and freezing techniques add to the cost of production in the fresh fish business (Kykkidou*et al.*, 2009). Therefore, alternative methods with low cost are needed to maintain the quality of fresh fish. One alternative method that has not been widely used in Indonesia is the use of electrolyte water.

Electrolytic water is made from water without the addition of harmful ingredients. Electrolyte water is also widely used in sanitizer products with added HOCl and NaOH (Rahman*et al.*, 2016). The production of electrolyte water can be done and does not require high costs. Electrolyte water is generally used to sterilize medical materials and instruments, food sanitation, and other applications (JETRO, 2009). Electrolyte water is reported to have very strong antibacterial properties against pathogenic bacteria, so it is important in food safety (Huang*et al.*, 2008). The additional material used to manufacture electrolyte water, namely sodium chloride (NaCl) (Kim*et al.*, 2000). Water containing a lot of sodium chloride, such as seawater, has excellent potential to be used as raw material for making electrolyte water (Kasai *et al.*, 2000).

Seawater is the largest natural resource globally that can be used as raw material for electrolyte water. The use of electrolyte water from seawater is still very little; this is unfortunate considering that seawater can be obtained for free, and its availability is abundant. The potential use of seawater as raw material for electrolysis water is extensive because the sodium chloride content in seawater reaches 86% (Gawande *et al.*, 2017). Seawater as an electrolytic water material with a high chlorine ion effectively kills microbes and extends shelf life (Kraft, 2008). Electrolyzed water is adequate for extending shelf life and killing microbes in fishery products due to its high oxidation-reduction potential (ORP), chlorine content (CL_2, HOCl, and –OCl) and pH (Len *et al.*, 2000).

Previous studies showed electrolyzed water could decrease microbes in fisheries has been widely carried out and provides effective results. Liu *et al.* (2006) reported that electrolyzed water could reduce *Listeria monocytogenes* contamination on seafood processing. Furthermore, electrolyzed water with available chlorine at an adequate level for disinfection can be used safely and effectively in various aspects of aquaculture (Katayose *et al.*, 2007). Huang *et al.* (2008) reported electrolyzed water can reduce the colony total of *Escherichia coli* and *Listeria monocytogenes* in fresh salmon. Nowsad *et al.* (2020) reported electrolyzed water could decrease growth of bacteria on fresh fish. Therefore, it is necessary to use seawater as an electrolysis water material to maintain the quality of fresh fish at a lower cost. This study aims to determine the effect of the electrolyzed seawater on the total plate count of fresh fish. In addition, the oxidation-reduction potential (ORP) and pH values of the electrolyzed seawater were also measured.

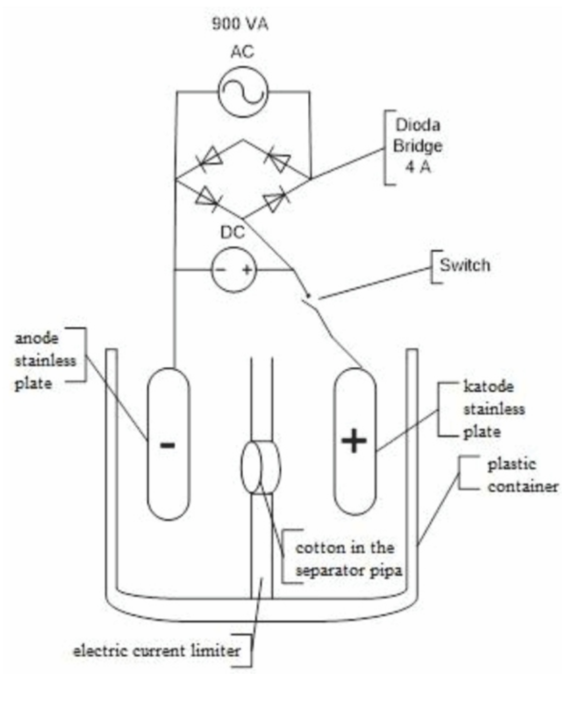

Figure1. Seawater Electrolysis Instrument

MATERIALS AND METHODS

Materials

The materials used in this study were seawater with 3-5% salinity, fresh selar fish, plate count agar, NaCl 0.9%, sterile ice cubes, and aquades. Seawater samples took from Tahuna waters, Sangihe Islands, Indonesia, at a depth of 2 meters.The tools used are scales, polypropylene plastic containers, digital pH meters digital (trans instrument BP 3001), ORP meters (eutech instrument PCD 50), refractometers, laminar air flow, incubators, autoclaves, analytical balances, magnetic stirrers, micropipettes, hot plates, test tubes, erlenmeyer flasks, petri dishes. The tools used in the manufacture of electrolysis instruments, namely plastic containers, plastic paralon pipes, sterile cotton, plastic glue, electrical wires, stainless steel plates, diode bridges 4 A, switches and electrical plugs.

Research Procedure

The fresh fish used to observe the total plate count, namely selar (*Selaroides* sp.). The research design used in this study was a completely randomized design with four treatments and three replications, namely:

(A) Selar fish soaked in 100% electrolytic seawater for 1 hour;

(B) Selar fish soaked in 50% electrolysis seawater for 1 hour;

(C) Selar fish soaked in ice (1:1 ratio) for 1 hour; and

(D) Selar fish left at room temperature without treatment for 1 hour

Electrolysis Instrument Manufacturing

Instruments are made with simple electrolysis at a low cost. The series of electrolysis instruments showed in Figure 1.

The production of electrolyzed seawater was carried out using seawater raw materials with a salinity level of 3-5%. The seawater filtered through a six mesh sieve to filter out impurities carried from the sea, then inserted as much as 4 liters of seawater into the electrolysis instrument. The electrolysis process was carried out for ± 3 minutes until the pH became 6.3. Electrolyzed water used as a preservative is at the anode (-) due to the formation of hypochloric acid, which functions as an antibacterial. After that, the electrolyzed seawater was filtered using a filter cloth. The electric power used is DC 900 VA.

Total Plate Count Assay (Arifan et al., 2019; Salanggon et al., 2020)

Aseptically, 25 g of fresh fish samples were weighed, then put into a sterile container. Added 225 ml of 0.9% NaCl solution, stirred for 1–2 minutes (this solution is considered a 10^{-1} dilution). Then, added 1 ml of the 10^{-1} dilution to 9 ml of NaCl solution to obtain a 10^{-2} dilution. This step was carried out until obtained a solution was with a dilution of 10^{-4}. Then take 1 ml from 3 series of dilutions 10^{-2}, 10^{-3}, 10^{-4} and put into a sterile petri dish and performed in duplicate for each series of dilutions. After that, add 12–15 ml of plate count agar (PCA) with a temperature of 44–46 °C to each petri dish that already contained the sample solution. Then, it rotated back and forth so that the sample solution and PCA media were completely mixed. After that, it was left until the PCA media hardens. PCA media was incubated for 48 hours at 35°C with the Petri upside down. Then, the bacterial colonies that grew with the colony counter were counted. The total microbial count was carried out using the Harrigan method with the equation

$$\text{Total colony (colony gram}^{-1}\text{)} = \frac{\sum N}{[(n1 \times 1) + (n2 \times 0.1) \times D]}$$

Description:

N : The number of bacterial colonies in the range of 25-250

n : The number of plates whose colonies can be counted

D : Lowest dilution level

Data analysis

Data analysis used regression analysis, correlation coefficient analysis, normality analysis using the Kolmogorov Smirnov method, homogeneity analysis of the Bartlett method, one-way parametric analysis of variance followed by Duncan's test with a 95% confidence level.

RESULTS

ORP (Oxidation Reduction Potencial)

Changes in the ORP value in the electrolysis process for 3 minutes (Figure 2) showed an increase. ORP value increased from 33.2 mv to 770.5 mv. The ORP linear regression test results on electrolysis time obtained the equation y = 3.989x + 20,557, with a correlation coefficient of 99%. The results of this test indicate that the longer the electrolysis time affects the increase in the ORP value. :

pH

Changes in pH in the electrolysis process for 3 minutes (Figure 3) showed a decrease. The decrease in pH occurred from the initial pH of 7.2 to 6.3. The results of the linear regression test of decreasing pH for electrolysis time obtained the equation $y = 7.268 - 0.005x$, with a correlation coefficient of 95%. The results of this test indicate that the longer the electrolysis time affects the decrease in the pH value.

Total Plate Count (TPC)

The results of TPC were by using the square root transformation method. Then tested the normality of the Kolmogorov Smirnov method and the homogeneity of the variance of the Bartlet

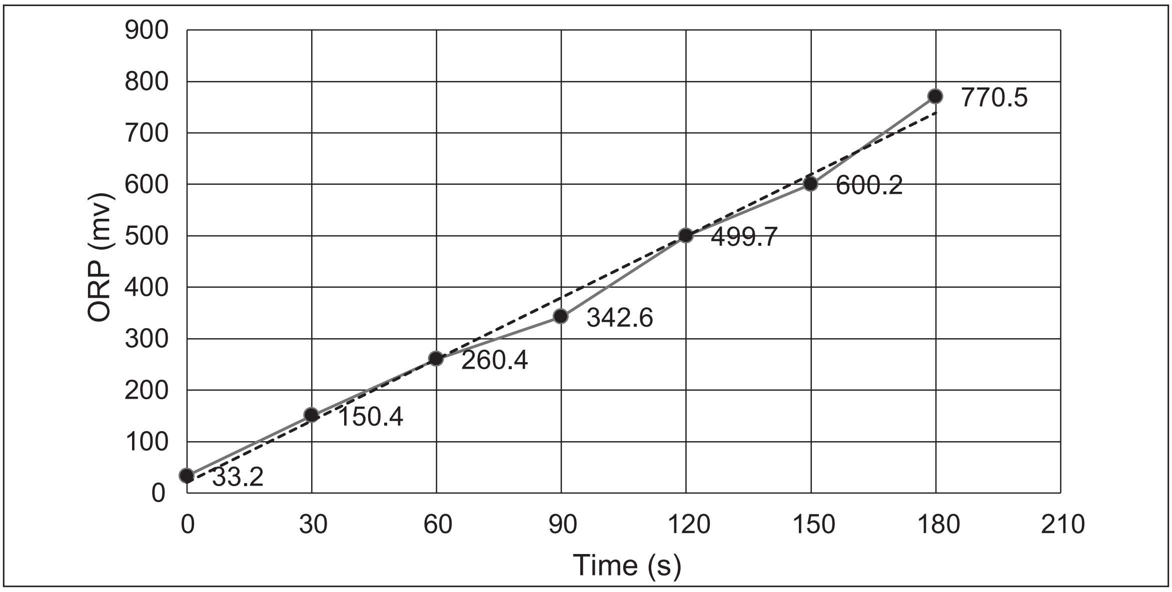

Figure 2. Relationship between ORP and time during electrolysis

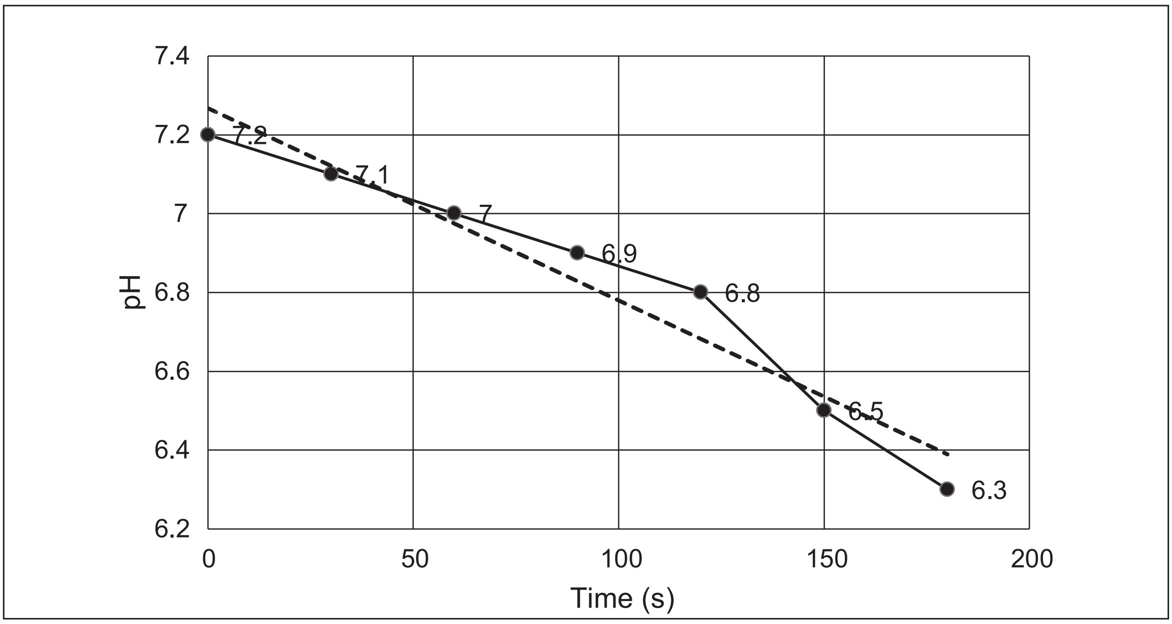

Figure 3. Relationship between pH and time during electrolysis

Table 1. Fresh fish total plate count with 1-hour storage

Treatment	TPC (Colonygram^{-1})
A (electrolyzed seawater 100%)	$4.65 \pm 0,26^{a}$
B (electrolyzed seawater 50%)	$4.73 \pm 0,14^{a}$
C (Ice 1:1)	$4.87 \pm 0,24^{a}$
D (Control)	$5.26 \pm 0,13^{b}$

Note: The difference in letters indicates the level of significance ($p<0.05$)

method so that data that meets the assumptions of parametric testing was obtained. Parametric analysis was carried out using a one-way analysis of variance (ANOVA). The results obtained from the ANOVA test showed a significant level of difference. It continued with the Duncan method of differentiation test, and the results were obtained as shown in Table 1.

The results showed that electrolyzed water with ORP 77 0.5 mv and pH 6.3 could significantly inhibit bacterial growth. The results of parametric statistical tests showed that treatments A, B, and C gave better results than treatment D. The results showed that the effect of electrolysis water with a concentration of 50% and 100% gave the same effect as the use of ice.

DISCUSSION

The ORP value of this study were supported by previous studies, namely the application of electrolyzed water with ORP >700 mv for 5 minutes can inhibit the growth of suspensions of *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Listeria monocytogenes* (Kim *et al*., 2000). Electrolyzed water with ORP 724 mv could inhibit the growth of aerobic bacteria, coliform bacteria, *Bacillus cereus*, mold ad yeast (Koseki *et al*., 2002). Al-Haq *et al*. (2005) reported electrolyzed water with ORP 800 mv or less show antimicrobial activity. Application of electrolyzed water in the food industry with ORP between 800-900 mv (Colangelo*et al*., 2015).

The pH value obtained from the electrolysis process in this study, which is 6.3, is intended for application as a preservative, pH between 6.2 – 6.5 (Chow *et al*., 2009). Electrolyzed water is safe for humans, so it is suitable as a food preservative (Dewi *et al*., 2017; Zhao *et al*., 2021). Previous studies showed that the use of electrolyzed water with a pH range of 6.5 can inhibit the growth of *Escherichia coli*, *Enterococcus faecalis*, *Staphylococcus aureus* (Guentzel *et al*., 2008), *Listeria monocytogenes*, and *Salmonella typhimurium* (Yang et al., 2003). The use of electrolyzed water was also applied to potatoes, radishes, and spinach to reduce aerobic bacteria growth (Len et al., 2000).

The application of electrolysis water affected inhibiting total microbial in fishery products. Previous studies, the use of electrolyzed water in carp reduces the growth of aerobic bacteria (Mahmoud *et al*., 2004), in tilapia reduces the growth of *Escherichia coli* and *Vibrio parahaemolyticus* (Huang *et al*., 2006), in fish skin reduces histamine-producing bacteria (Phuvasate and Su, 2010), and in salmon, electrolyzed water could reduce the growth of *E. coli* and *Listeria monocytogenes* (Ocer and Demirici, 2006). The use of electrolyzed water in livestock products, namely chicken meat reduces the growth of *E. coli* and *Salmonella typhimurium* (Fabrizio *et al*., 2002; Fabrizio and Cutter, 2005); beef sausage decreased the growth of *L. monocytogenes* (Huang *et al*., 2008). While in agricultural products, the use of electrolytic water in carrots, potatoes, and radishes reduces the growth of aerobic bacteria (Len *et al*., 2000), in cucumbers, electrolyzed water could reduce the growth of aerobic bacteria, coliforms, and fungi (Koseki *et al*., 2004).This study proves that seawater can be used as electrolyte water and can inhibit the growth of bacteria in fresh fish. Electrolyzed seawater can potentially be used to inhibit bacterial growth during storage as a substitute for ice. The use of electrolyzed seawater can be used in the fisheries business in maintaining the quality of fresh fish.

CONCLUSION

This study findings that the concentration of 100% and 50% electrolysis seawater had the same effect as ice treatment on the total plate count of selar fish (*Selaroides* sp.) during one hour of storage. The ORP and pH values of electrolyzed seawater were 770.5 mv and 6.3, respectively.This study shows that electrolyzed seawater can inhibit the growth of bacteria, thus indicating that it can be applied as an alternative method of preserving fresh fish.

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ONFLICTS OF INTEREST

All authors declare all sources of support for the research and any association with a product or subject that may constitute no conflict of interest.

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