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Characterization of Salt Quality from Artemia (*Artemia franciscana*) Cultured Water with *Dunaliella salina* as Natural Feed

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Abstract

The high demand for domestic salt cannot be achieved by local salt production from traditional salt and PT. Garam fluctuates every year. In addition, the quality of traditional salt is still low. This causes the need for domestic salt consumption to be met by imported salt. Therefore, it is necessary to have an alternative to meet the domestic salt demand, namely the revitalization of salt ponds for the development of mass *Artemia* cultivation that produces both *Artemia* production and biomass and produces salt in one location. In its natural habitat, *Artemia* uses microalgae as its main food source. The quality of microalgae feeds greatly determines the production of *Artemia franciscana*. The availability of nutrients is a factor that determines the rate of growth, so the amount and quality of feed are the main factors to meet the nutritional content for optimal development. Therefore, the salt production in this study used saltwater cultured *Artemia* with *Dunaliella salina* as a natural feed with a water age of 3^{rd},7^{th}, and 14^{th} days. This study aimed to determine the best age of *Artemia* water cultured with *D. salina* as a natural feed in salt production. The results showed that the different ages in *Artemia* water cultured with *D. salina* had a significant effect ($p < 0.05$) on salt characteristics. The best result was D7 which is salt from *Artemia* cultured water with *D. salina* as a natural feed with water age 7th day. Their respective values are 0.66 ± 0.33 water content, 91.55 ± 0.32 NaCl, 0.87 water-insoluble part, $0.39\% \pm 0.01$ Ca, and 1.25 ± 0.05 Mg.

INTRODUCTION

Salt is an inferior product that is only produced in tropical countries like Indonesia. However, salt production in Indonesia has not been developed optimally. The high demand for salt cannot be domesticated by local salt production from traditional salt and PT. Garam fluctuates every year. The need for salt every year always increases along with the increase in population and industrial growth. In 2019, the national salt demand was estimated to increase by 5.98% to 4.2 million tons. Most of Indonesia's salt consumption needs are from imports of salt rather than domestic production (Setiawati, 2020). The main problem faced by Indonesia in salt production is that the quality (NaCl) produced does not meet the Indonesian National Standard (SNI).

This is caused by the production technology used and the location or production area that is not right (Kurniawan *et al*., 2019). The main cause of impurities from NaCl crystals or low salt quality, is the occurrence of co-precipitation of salt impurities such as magnesium and crystallized calcium simultaneously with NaCl (Marihati *et al*., 2014). Coprecipitation caused by bacteria Halophilic nourishes Luria Bertani on NaCl purification. Salt ponds themselves have the potential to increase the income of salt farmers through the development of mass *Artemia* cultivation. One of the efforts to increase the productivity of salt ponds is the revitalization of salt ponds for the development of mass *Artemia* cultivation which produces *Artemia* cysts and biomass and produces traditional salt in one salt pond location. Salt field operations using biological systems can increase the evaporation, quality, and quantity of salt produced. The biology system involved is the life of plankton, *Artemia* sp., and halophilic bacteria. *Artemia* can be used as a substitute for e Luria Bertani for very expensive halophilic bacteria nutrition (2.8 million rupiahs per kilogram). Therefore, we need an alternative as a substitute for LB, namely *Artemia* sp. (200 thousand rupiahs per kilogram) containing 52% protein and 15.49% carbohydrates.

Salt cultivation itself can be used to increase the income of salt farmers through the development of mass *Artemia* cultivation. However, *Artemia* cultivation in salt ponds is not yet growing greatly. In addition, the salt pond land has not been managed properly. One of the efforts to increase the productivity of salt ponds is to revitalize the salt pond for the development of mass-producing *Artemia* cultivation *Artemia* cysts and biomass to produce cross salts in one salt pond location. *Artemia* uses microalgae as the main food source (Mohebbi *et al*., 2016). Microalgae feed quality determines the production of *Artemia*. Availability of nutrition is a factor that determines the rate of growth, so the amount of and the quality of feed is the main factor to meet the nutritional content for *Artemia* to develop optimally. Apart from being viewed from a nutritional point of view, microalgae can play a role in the bioremediation process in waters.

Bioremediation is an innovative technology that uses living organisms or biomass to remove heavy metals or other pollutants from the marine environment. In the biological structure, microalgae have the potential to bind metal ions because of their cell wall structure which contains polysaccharides, proteins, and lipids (Pratiwi *et al*., 2020). Diatoms are one of the types of microalgae that are often used as environmental bioindicators because of their high diversity, good sensitivity to changes in water, and their fundamental role in the food chain. The microalga *Chaetoceros calcitrans* is one of the diatom microalgae from the class Bacillariophyceae and is the basis of the dominant marine food chain. Besides *C. calcitrans*, the microalgae *Dunaliella salina* and *Tetraselmis chuii* can also be used as an indicator of the fertility of a water or act as a biosorbent because it easily accumulates with pollutants such as heavy metal lead (Pb) that enter the waters (Tamalonggehe *et al*., 2020).

Artemia cultured water that utilizes *D. salina* as natural feed can be used as raw material in better quality salt production when compared to traditional salt that uses seawater. This is due to the presence of *Artemia* which will eat plankton in the salt field hatchery ponds and will be released as products that are not easily destroyed because they are wrapped in products such as pellets so that they will settle to the bottom of the hatchery (Marihati *et al*., 2014). Therefore, the raw material for making salt water will be clearer and evaporation will occur faster. In addition, *Artemia* carcasses and feces can be a nutrient for the life of halophilic bacteria on the crystallization table. *Artemia franciscana* can withstand various environmental conditions such as high temperature, hypersalinity, low oxygen, and ionic variations. Based on these problems, it is necessary to conduct research on salt quality characterization from the water culture of *Artemia* (*A. franciscana*) with different microalgae as natural feed.

METHODOLOGY

Place and Time

This research was carried out from October 2021 until February 2022. Tests in this study which include yield test, water content test, sodium chloride (NaCl) test, and the water-insoluble part were carried out in the Chemical Laboratory Analysis of the Faculty of Fisheries and Marine Affairs, Airlangga University. While the tests of calcium (Ca), and magnesium (Mg) were carried out at PT. Sucofindo Analytical Laboratories, Surabaya.

Research Materials

The materials used in this study were seawater (negative control), *Artemia* cultured water with *D. salina* 3^{rd},7^{th}, and 14^{th} days. Other materials used for testing consisted of $AgNO_3$ SAP, K_2CrO_4 SAP, NaOH SAP, KI SAP, $K_2Cr_2O_7$ SAP, and aquadest.

Research Design

The experimental design in this study used a Completely Randomized Design (CRD). The total sample is four samples of water as raw material for the production of salt which will then be characterized by three replications.

Work Procedure

Salinity measurement is carried out during culture water sampling of *Artemia*, which is about 40-50 ppt. Then the *Artemia* cultured water is put into a 25 L jerry can. The process of making salt begins with taking *Artemia* cultured water from jerry cans as much as 3 L. Then an assessment process will be carried out using a vacuum pump and nylon paper. The process aims to separate or remove *Artemia* impurities or other physical contaminants like sand. After that, heating will be carried out by induction using the stove temperature of $\pm$300 °C. Heating is carried out until crystals form a salt which is dry and takes $\pm$10-12 hours. The dry parameter of salt crystals can be seen from the sticking or not of salt crystals on the stem stirrer. Heating is stopped when the salt crystals are not stopped at the stirring rod.

In this study, the yield test was carried out by calculating the percentage of salt produced per 3 L of weight. The water content test was carried out using a moisture analyzer. Samples as much as 0.75 grams are weighed in a special container in a moisture balance analyzer. Analysis of Sodium Chloride (NaCl) in this study using titration Mohr's method of geometry is used to determine chloride levels with standard silver nitrate ($AgNO_3$) and potassium chromate (K_2CrO_4) as an indicator. The water-insoluble part test is carried out by calculating the principle of the weight of the material remaining on the filter paper at a temperature of 105 ± 2 °C. The water-insoluble part is tested based on SNI 3556: 2010. Calcium and magnesium tests were carried out at PT. Sucofindo Analytical Laboratories, Surabaya.

Data Analysis

The resulting data are tested for normality first to find out whether the data are normally distributed or not. Furthermore, the distributed normal data will be tested using Completely Randomized Design (CRD) with ANOVA (Analysis of Variance) one factor. If there is a difference, it will continue with Duncan's Multiple Range Test (DMRT) to find out which treatment is the best among the different treatments and not significantly different.

RESULTS AND DISCUSSION

The presence of *Artemia* sp. in the hatchery can have a positive effect on salt production, the first is that it can prey on plankton that causes turbidity so that it can accelerate evaporation and increase water brightness; the second is that *Artemia* carcasses (organic detritus) can be a natural food for halophilic bacteria on the salting table so that it can increase salt quality and purity of NaCl (Marihati *et al.*, 2013). *Artemia* salt crystallization results can be seen in Table 1.

Table 1. Results of *Artemia* salt crystallization.

Code	NC	D3	D7	D14
Appearance of salt				
Final weight (g)	115.11 ± 5.87	127.05 ± 8.16	117.06 ± 5.64	136.54 ± 13.46

Description: (NC) negative control, (D3) *Artemia* cultured water with *D. salina* (3^{th} day), (D7) *Artemia* cultured water with *D. salina* (7^{th} day), (D14) *Artemia* cultured water with *D. salina* (14^{th} day).

Salt from *Artemia* cultured water with *D. salina* as natural feed produces salt that is more refined and purer white when compared to traditional salt (NC). Sales of salt from pond farmers are classified into several classes based on its quality such as appearance and NaCl content. The first quality (K1, very good) is salt with >95% NaCl, the second quality (K2, good) is salt with NaCl between 90-95%, and the third quality (K3, moderate) is salt with <90% NaCl (Susilowati and Perkasa, 2021).

The majority of traditional salt is categorized as K3, this is because the color of the salt is slightly cloudy and large in size. Salt crystal size will affect the quality of salt washing result, the smaller the size of the salt crystal, the quality of salt produced after washing is generally better because it is easier to separate materials, the impurities are mainly impurities contained in the salt crystal, and the salt becomes whiter. Cloudy color is caused by physical impurities such as dust or chemical impurities such as Ca and Mg. Overall, the salt texture of the cultivation of *Artemia* has a smooth texture, different from traditional, roughly textured salt in general. This is due to the process of evaporation of seawater using temperature.

High evaporation is done by boiling seawater until salt crystals are formed (Firmansyah, 2018). The basic principle is that, when water is heated, then the water temperature will rise and if it has reached a boiling point it will form gas bubbles. In principle, the heating process of water with high temperature will increase energy and kinetic water-building particles. This high kinetic energy makes the salt crystals form smooth because there is pressure from the kinetic energy of water particles which breaks the salt crystal into small particles. The influence of temperature is what makes commonly produced traditional salt have a rough texture due to the temperature in the process Crystallization is not optimal. The quantity of salt production can be affected by the salinity of the brine raw material. The higher the salinity, the higher the number of salt crystals produced (Purwati *et al.*, 2020). Salt has a salty taste, but sometimes it tastes bitter. The bitter taste in salt can be caused by the $MgCl_2$ content that is too high (Abdullah and Susandini, 2018). The visual classification of salt quality can be seen in Table 2.

Table 2. Visual classification of salt quality.

Classification of salt quality	Color	Crystal Size
K1	White tends to be clear	Mas 0.5 mm
K2	Pure white but a bit dull	Less than 3 mm
K3	Dull white, mixed with dirt	Less than 4 mm

Based on the visual quality of the salt, the salt produced from *Artemia* cultured water can be categorized into K2 (good). In addition, the quality of the salt is also influenced by the cleanliness of other compounds that are not like $CaSO_4$, $MgSO_4$, and $MgCl_2$ in the crystal. Chemically, the quality of salt is determined by the level of NaCl contained in the salt (Maulana *et al.*, 2017). Consumption salt has a minimum of 94% NaCl and must meet the quality requirements of consumption salt, industrial salt has a minimum of 97% NaCl. Specifically for the food industry, the levels of Ca and Mg are <0.06% (Wibowo, 2020). The chemical characteristics of salt from *Artemia* cultured water can be seen in Table 3.

Table 3. Chemical characteristics of salt from *Artemia* cultured water.

Treatments	Parameters				
	Water content (%)	NaCl (%)	Water-insoluble part (%)	Ca (%)	Mg (%)
NC	$1.01^{ab} \pm 0.3$	$81.06^{a} \pm 0.43$	$0.86^{ab} \pm 0.01$	$0.84^{c} \pm 0.02$	$3.34^{c} \pm 0.11$
D3	$1.11^{ab} \pm 0.23$	$89.10^{b} \pm 0.32$	0.86^{a}	$0.38^{a} \pm 0.01$	1.28^{a}
D7	$0.66^{a} \pm 0.33$	$91.55^{c} \pm 0.32$	0.87^{b}	$0.39^{ab} \pm 0.01$	$1.25^{a} \pm 0.05$
D14	$0.96^{b} \pm 0.22$	$89.40^{b} \pm 0.42$	$0.88^{c} \pm 0.01$	$0.40^{b} \pm 0.01$	$1.6^{b} \pm 0.28$

Description: Different superscript letter notations in the same column show a very significant difference in the differences ($P < 0.05$). However, the same superscript letter indicates that the treatments were not significantly different ($P > 0.05$).

Based on the one-way ANOVA test in Table 3, shows that the difference in the age of *Artemia* cultured water affects the characteristics of the salt ($P < 0.05$). The best concentration of NaCl was D7 which is the salt from *Artemia* cultured water with *D. salina* as natural feed. In addition, the concentrations of Ca and Mg in D3 and D7 were not significantly different and were the lowest concentrations. It was stated that the lower the impurities such as Ca and Mg, the higher the purity of the salt with the higher the concentration of NaCl. This study shows that the use of *Artemia* cultured water can produce better salt compared to traditional salt that uses only seawater (NC). It is based on visual and chemical characteristics. Live *Artemia* biomass, apart from being a by-product of salt fields, can also purify salt water with a transparency value of up to 90% so that it can accelerate evaporation in the hatchery, while detritus and carcass of *Artemia* biomass can be a halophilic growth medium which is a catalyst for purification of NaCl on the crystallization table (Marihati *et al.*, 2013).

Artemia can absorb magnesium and calcium for growth, form an exoskeleton and carry out metabolism. The ability of *Artemia* can be utilized in salt ponds to reduce the magnesium and calcium content in seawater. Magnesium plays a very important role in the growth and life of *Artemia*. Magnesium also has an important role in the metabolism of lipids, proteins, and carbohydrates as a cofactor in enzymatic reactions and metabolism. Low concentrations of magnesium ions can cause high respiration rates (Gozan *et al.*, 2017). High respiration rates at low magnesium concentrations cause stress and decreased life expectancy. In addition, magnesium plays a role in activating energy, by absorption into the hemolymph and releasing it to activate energy in *Artemia*.

The concentration of NaCl at D7 is highest and salt from *Artemia* cultured water with *D. salina* is higher when compared to traditional salt that uses seawater (NC) as raw material. However, the NaCl in the salt produced in this study did not meet the standard (less than 94%). Therefore, further research is needed to improve the quality of salt to meet the standards for consumption of salt and industrial salt. These efforts can be done chemically and physically. Improving the quality of salt chemically can be done by adding chemicals such as sodium carbonate (Na_2CO_3), disodium phosphate (Na_2HPO_4), sodium hydroxide (NaOH), barium chloride ($BaCl_2$), and calcium hydroxide ($Ca(OH)_2$).

Meanwhile, physical improvement of salt quality can be done by washing and recrystallization processes (Purwati *et al*., 2020). Rahman *et al*. (2010) said that impurities in salt can be removed by adding NaOH which can convert $MgCl_2$ and $MgBr_2$ to $Mg(OH)_2$ which precipitates. The addition of sodium carbonate converts $CaCl_2$ to $CaCO_3$ precipitate. Ca^{2+} can be removed from the brine through a stepwise crystallization process where the main fractions of $CaCO_3$ and $CaSO_4$ are precipitated from the salt solution. The following is the reaction equation produced in the purification process.

CONCLUSION

The results showed that the different ages of *Artemia* water cultured with *D. salina* as natural feed in *Artemia* culture had a significant effect ($p < 0.05$) on the salt characteristics. The best result was D7 which is salt from *Artemia* cultured water with *D. salina* as a natural feed with water age 7th day. Their respective values are $0.66^a \pm 0.33$ water content, 91.55 ± 0.32 NaCl, 0.87 water-insoluble part, $0.39\% \pm 0.01$ Ca, and $1.25^a \pm 0.05$ Mg.

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