Exhibition and Seminar on Science and Creative Technology, University of Al-Azhar Indonesia (EXSACT-A 2024) Proceeding

DOI: https://doi.org/10.36722/exc.v2i1.2244

The Effect of Crude Nipa Leaf Extract (*Nypa fruticans*) on the Quality and Shelf Life of Fresh Milkfish (*Chanos chanos*)

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Abstract - **In the fishermen's environment, in general, fresh fish is preserved so that it does not spoil quickly by using ice, but the durability of ice is limited, so fishermen usually add formalin; formalin is generally used as a preservative for corpses and is prohibited from being added to food. Nipah leaf extract is very good to be used as an anti-bacterial compared to the fruit, so that it can be used as a natural preservative. This study aimed to determine the optimum concentration of the addition of crude nipa leaf extract on the quality of fresh milkfish. The sample in this study used milkfish preserved using nipa leaf extract as a natural preservative. This research was divided into two stages, namely, in the first stage, it was used to find the best concentration in making nipah leaf extract solution, and the second stage was carried out to determine the effectiveness of nipa leaf solution as a natural preservative. This study found that Nipah leaf extract with a concentration of 22.5% could increase the shelf life for 24 hours with a bacterial growth rate of 5.63±0.03 log cfu/g. This study concludes that Nipah leaf extract can inhibit decay in milkfish.**

Keywords— *milkfish, nipah, anti-bacterial.*

INTRODUCTION

Fish is one of the aquatic commodities that has the potential to be utilized. The market need for fish continues to increase along with the increase in population. Milkfish is a fishery product that is often consumed by the public. Milkfish is a fishery commodity that tastes good and tasty, so the public loves it. Apart from being quite tasty and tasty, the price of milkfish is affordable for all levels of society [1]. Fish that are handled properly can be consumed safely by humans.

Fish that is not cured is only fit for consumption for a short time. Various ways of preserving fish are done, but some of them need help with the natural properties of fish. [2]. A *preservative* is a substance that can prevent food spoilage in terms of taste, colour and smell because preservatives aim to inhibit the growth of spoilage bacteria [3]. In the fishermen's environment, fresh fish are generally preserved so they do not spoil quickly by using ice, but the durability of ice is limited, so fishermen usually add formalin. Adding formalin aims to keep the fish fresh as long as possible until it reaches the consumer, even though formalin is generally used as a preservative for corpses, not as a food preservative [4]. According to [5], appropriate antimicrobials can extend shelf life and ensure the safety of food products. Therefore, alternative antimicrobial ingredients are needed from natural ingredients that are harmless when consumed and can inhibit microbial growth in products so that they function to inhibit food spoilage due to microbial activity.

According to [6], mangroves have many benefits that directly intersect with human life, ranging from ecological benefits to food and medicinal sources. The Nipa palm plant has antibacterial properties. Nipah leaf extract is very good to use as an antibacterial compared to the fruit. According to [7], previous research showed that the Nipah plant contains active antioxidant and antibacterial compounds. Based on [8]said that flavonoids, saponins, terpenoids, phenolics and tannins are also active compounds that function as antimicrobial compounds. This research aims to determine how adding crude palm leaf extract to fresh milkfish affects spoilage bacteria during storage.

METHOD

Materials

The tools used in the extraction process include glass bottles, glass funnels, filter paper, and rotary evaporators. The equipment used in the analysis process were digital scales, spatulas, weighing bottles, oven, crushable pliers, desiccators, 100 ml beaker glass, test tubes, test tube racks, serological pipettes, suction balls, coolboxes, analytical scales, knives, vials, Erlenmeyer, evaporator cups, dropping pipettes, 100 ml measuring cups, spatulas, pans, trays, stirring rods, glass funnels, Conway dishes, 1 ml pipettes, incubators, Petri dishes, colony counters, glass bottles, vertical condensers, and pH meters.

The materials used in the extraction process are filter paper (Whatman no.42), plastic wrap, aluminium foil, label paper, nitrogen gas, distilled water solvent, and young Nipah leaves (Nypa fruticans). The materials used in the analysis process were distilled water, 2 N sulfuric acid, concentrated sulfuric acid, concentrated HCl, chloroform, ethanol, Meyer's reagent, 10% FeCl3, magnesium powder, concentrated ammonia, anhydrous acetic acid, Artemia salina Leach larvae, seawater, label paper, tissue, sample fish meat, boric acid, HCl, NaCl and nutrient agar (NA).

Method

This research is divided into two stages: preliminary and main. Preliminary research was conducted to determine the best concentration using distilled water with a concentration of 5%, 10%, 15%, and 20%, which will be used in the main research. Determination of the best concentration is determined using the Zeleny method, which seeks the highest yield value, the lowest water content, and the lowest LC_{50} value.

The main research was carried out using the best nipah leaf extract, which was then given treatment with a narrower range (X-2.5%, X%, and X+2.5%). The fresh banging was soaked in the three nipa leaf extract solutions for 24 hours at room temperature (27ºC), and samples were checked at 0 hours, 12 hours, and 24 hours with the TVB test, TMA test, TPC test, and pH.

RESULT AND DISCUSSIONS

Preliminary Research

Yipa fruticans Leaf Extract Yield

The results of the ANOVA yield value of the crude extract of Nypa fruticans leaf flour at each concentration were not significantly different (P>0.05). The yield of the crude extract of Nypa fruticans leaf flour can be seen in Figure 1 below.

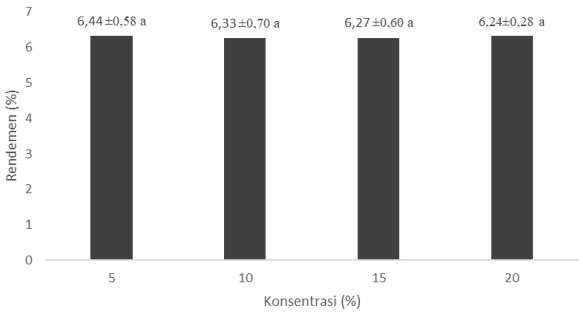

Figure 1. Results of Nypa fruticans Leaf Extract Yield Test

Based on the graph in Figure 1, the highest yield was obtained by an extract with a concentration of 5% with a yield value of 6.44% ± 0.58, while the lowest yield with a yield value of 6.24% ± 0.70 was obtained by an extract with a concentration of 20%. The resulting yield is the amount of compound extracted by various concentrations of distilled water.

The high yield of Nypa fruticans leaf extract using distilled water cannot be separated from the nature of distilled water as a polar solvent [9], making it easy to extract the compounds contained in Nypa fruticans leaf powder. [10]stated that the higher the yield percentage, the more organic compounds contained in the extract.

Water Content of Nypa fruticans Leaf Extract

The ANOVA results showed that the water content of the crude extract of Nypa fruticans leaf flour at each concentration was not significantly different (P>0.05). The results of the average water content of the crude extract of Nypa fruticans leaf flour can be seen in Figure 2 below.

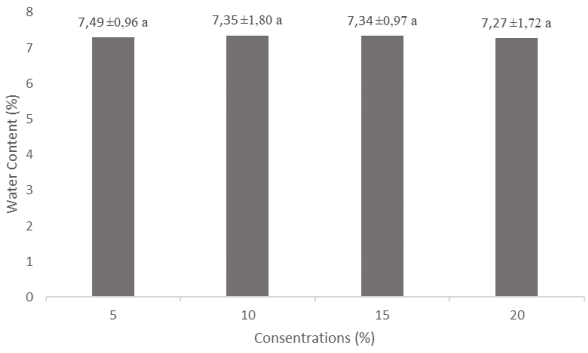

Figure 2. Test Results for Moisture Content of Nypa fruticans Leaf Extract

Based on the water content test results in Figure 4, Nypa fruticans leaf extract with a concentration of 5%

has the highest average water content of 7.49% ± 1.80. At the same time, the lowest water content was owned by Nypa fruticans leaf extract with a concentration of 20% of 7.27% ± 1.72. The difference is because the concentration of Nypa fruticans leaf extract affects the water content value. The difference aligns with [11], which states that lower extract concentrations result in higher water content.

A low amount of water can make a material last longer when stored for a relatively longer period, so the possibility of damage due to mould during storage is very low. According to [12], a plant extract is declared viscous if its water content ranges from 5-30%, so Nypa fruticans leaf extract with distilled water is included in the thick category.

Toxicity of Nypa fruticans Leaf Extract (LC_{50})

Toxicity testing was conducted to identify whether Nypa fruticans leaf extract is toxic. One of the methods that can be used to test for toxicity is the Brine Shrimp Lethality Test method with Artemia Salina Leach as a bioindicator. The LC_{50} value of each treatment was obtained from calculating the concentration log on the probit value. The LC_{50} value indicates the concentration of a test substance that can cause death by 50% of the amount of Artemia salina Leach after 24 hours of treatment.

The average results of calculating the toxicity of Nypa fruticans leaf extract can be seen in Figure 3 below.

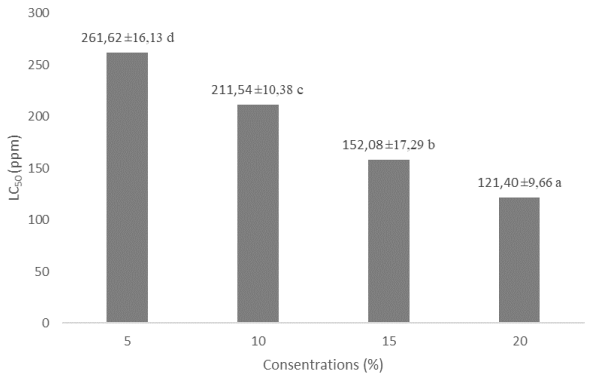

Figure 3. Nypa frutican Leaf Extract Toxicity test results

Based on the graph in Figure 3, it can be seen that Nypa fruticans leaf extract treated with different concentrations produced different LC_{50} values. The results of ANOVA calculations on the toxicity test of the crude nipa leaf flour extract showed significantly different results ($P<0.05$). Extract with a concentration of 20% showed the lowest average yield with an LC_{50} value of 121.40 ± 9.66 ppm. At the same time, the highest average LC50 yield was obtained by a 5% concentration of 261.62 ± 16.13 ppm.

The lower the LC_{50} value obtained, the stronger the material's toxicity. Toxic properties can be identified according to the number of dead larvae at a certain concentration. [13] states that an extract can be toxic if it has an LC_{50} value (a concentration that can kill 50% of shrimp larvae) of less than 1000 µg/mL after 24 hours of contact. These results indicate that Nypa fruticans leaf extract using aqua dest is toxic because it has an LC_{50} below 1000 ppm. Phytochemical compounds (flavonoids, tannins, triterpenoids and saponins) contained in the crude extract of nipa leaf flour can potentially cause death in *A. Salina Leach* larvae. These compounds act as stomach poisons. Therefore, the digestive system will be disrupted if this compound enters the larvae's body. In addition, this compound can also inhibit taste receptors in the mouth of the larvae. This results in the larvae being unable to stimulate taste, so they cannot recognize their food. As a result, the larvae starve to death [14].

Best Solution Determination

The best solution in this preliminary study was an extract with a 20% solution with a yield value of 6.24%, a water content of 7.27%, and an LC_{50} of 121.40 ppm.

Main Research

Total Volatile Base (TVB) Test

The TVB value at room temperature storage was evaluated by ANOVA, showing a significant difference ($P<0.05$) concerning storage time. The TVB value of milkfish is shown in Figure 4 below.

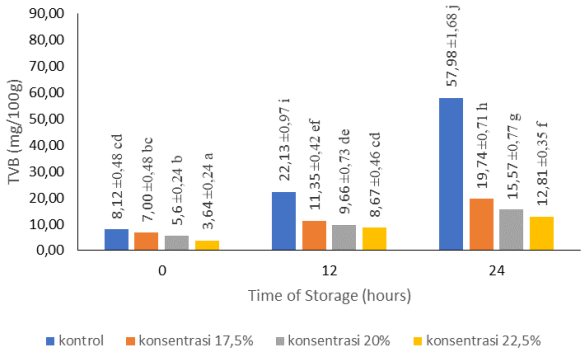

Figure 4. Milkfish TVB Value

Based on Figure 4, the TVB value at the 0th hour of storage in control was 8.12 ± 0.48 mg/100g; 17.5% concentration of 7.00 ±0.48 mg/100g; 20% concentration of 5.6 ±0.24 mg/100g; and at a concentration of 22.5% of 3.64 ±0.24 mg/100g. The

TVB value continued to increase until the 24th hour of storage, namely in the control of 57.98 ± 1.68 mg/100g; 17.5% concentration of 19.74 ±0.71 mg/100g; 20% concentration of 15.57 ±0.77 mg/100g; and 22.5% concentration of 12.81 ±0.35 mg/100g. The difference shows that milkfish produce volatile compounds that accumulate.

According to [15], the work of bacteria in breaking down proteins and amino acids into simpler compounds to be able to grow and reproduce produces residual compounds such as NH3, trimethylamine and their derivative compounds where these compounds belong to the group evaporated words. The TVB value increases as the number of bacteria increases because one of the results of the bacteria decomposition is a compound that belongs to volatile bases [7]. Based on [16], for aquatic products, a TVB-N level of less than 12 mg/100 g is considered fresh, and values ranging from 20 – 25 mg/100 g or more than 25 mg/100 g are believed to be early spoilage. Judging from the TVB value, it can be concluded that the shelf life of milkfish is no more than 12 hours without treatment; after being given treatment, the shelf life becomes longer.

Trimetilamine (TMA) Test

The TMA value of milkfish evaluated by ANOVA showed a significant difference (P<0.05) concerning storage time. The TMA value of milkfish is shown in Figure 5 below.

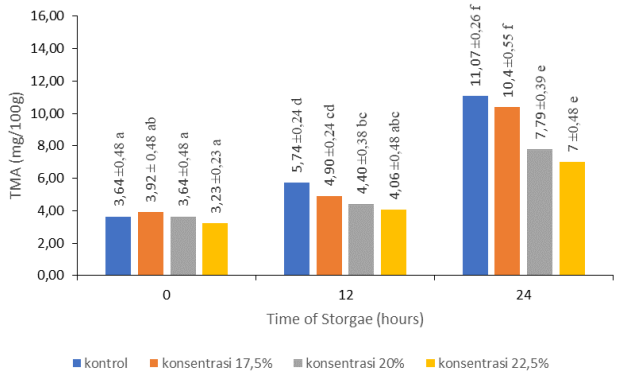

Figure 5. TMA Value of Milkfish

Based on Figure 5, it can be seen that the TMA value of milkfish at 0 hours was 3.64 ± 0.48 mg/100g in control, 3.92 ± 0.48 mg/100g at a concentration of 17.5%, 3.64 ±0.48 mg/100g at a concentration of 20%; and 3.23 ±0.23 mg/100g at a concentration of 22.5%. The TMA value of milkfish increased until the 24th hour of storage to 11.07 ± 0.26 mg/100g in control, 10.4 ±0.55 mg/100g at a concentration of 17.5%, 7.79 ±0.39 mg/100g at a concentration of 20%; and 7 ±0.48 mg/100g at a concentration of 22.5%.

The TMA value in this study was considered good because it was still below the maximum limit of SNI, namely 15 mg/100g (National Standardization Agency, 1994). Trimethylamine is due to the addition of formalin in the TMA testing process; it is suspected that the results obtained are not derived from formalin produced from milkfish but rather from the added formalin. The study by [17] stated that when detecting formaldehyde content in fish, the formaldehyde detected was total formaldehyde derived from added formaldehyde and natural formaldehyde.

Total Plate Count (TPC) Test

The TPC value at room temperature storage evaluated by ANOVA showed a significant difference (P <0.05) concerning storage time. The TPC value of milkfish is shown in Figure 6 below.

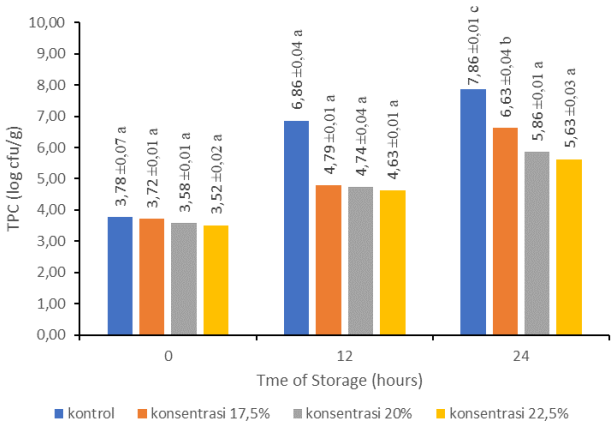

Figure 6. TPC in Milkfish

From the data in Figure 6, it can be seen that the microbial content in milkfish at 0 hours, the control was 3.78 ± 0.07 log cfu/g; concentration of 17.5% obtained 3.72 ±0.01 log cfu/g; 20% concentration obtained 3.58 ±0.01 log cfu/g; and a concentration of 22.5% obtained 3.52 ± 0.02 log cfu/g. The TPC value of milkfish increased until the 24th hour of storage, namely in the control of 7.86 ±0.01 log cfu/g; 17.5% concentration of 6.63 ±0.04 log cfu/g; 20% concentration of 5.86 ±0.01 log cfu/g; and at a concentration of 22.5% of 5.63 ±0.03 log cfu/g. The microbial content in fresh milkfish without treatment is below the SNI standard for the maximum number of milkfish microorganisms, namely 5.70 log cfu/g (Indonesian Standardization Agency, 2009), so milkfish should be stored no more than 12 hours. However, after being given treatment, the TPC value

in milkfish decreased, so the shelf life of milkfish was longer.

The growth of microorganisms indicates that the crude extract of Nypa fruticans leaf flour can only inhibit the growth of microorganisms. [7] stated that crude extracts could only inhibit the growth of microorganisms, not kill them. The higher the concentration of an antibacterial substance, the more it contains anti-bacterial compounds, so bacteria absorb more antibacterial compounds and cause slower bacterial growth [17].

pH Test

The pH value of milkfish evaluated by ANOVA showed a significant difference (P<0.05). The graph of the pH value of milkfish is shown in Figure 7 below.

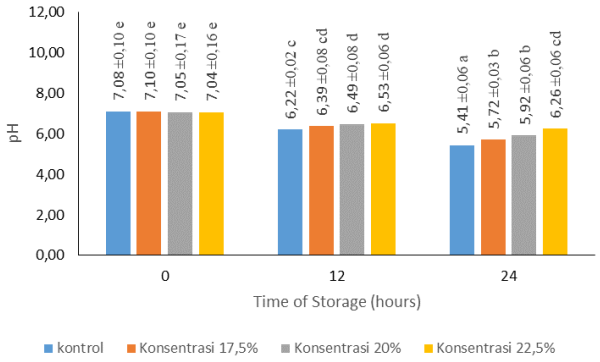

Figure 7. Milkfish pH value

The pH value of milkfish at 0 hours in the control was 7.08 ± 0.10; a concentration of 17.5% of 7.10 ± 0.10, 20% concentration of 7.05 ± 0.17; and a concentration of 22.5% of 7.04 ± 0.16 which indicates the condition of the fish is quite good and still fresh. In Zummah and Wikandari's (2013) study, the pH in the fresh milkfish sample was 6.27, which showed that the pH was close to neutral and still suitable for consumption. Then it decreased gradually at 12 hours to 6.22 ± 0.02 in control; 6.39 ±0.08 at a concentration of 17.5%; 6.49 ±0.08 at a concentration of 20%; and 6.53 ± 0.06 at a concentration of 22.5% then at 24 hours, the pH in control was 5.41 ± 0.06; concentration of 17.5% to 5.72 ±0.03; concentration of 20% to 5.92 ±0.06; and at a concentration of 22.5% it becomes 6.26 ± 0.06.

From the above results, it can be seen that there was a decrease in pH levels. The decomposition of fish meat occurs due to the activity of enzymes in the fish's body. Fish that expend more energy before dying will cause the pH to decrease rapidly and result in the kalepsin enzyme being able to decompose proteins; this decomposition will increase the volatile bases so that the pH value will increase [18]. Bacteria found in food produce compounds that are acidic and basic. Thus, the pH value is influenced by the dominant compound. In the early storage stages, the decomposition produces lactic acid compounds, which can lower the pH [19]. [20] explained that after rigour mortis occurred, the lowest pH achieved by meat was 5.1, and the highest was 6.2. In this process, there is a change in glycogen into lactic acid, which lasts until the glycogen in the meat runs out.

CONCLUSION

Based on the results and discussion, it can be concluded that the addition of *Nypa fruticans* leaf extract can affect the quality and shelf life of milkfish (*Chanos chanos*), where soaking milkfish (*Chanos chanos*) in an extract with a concentration of 22.5% can increase the shelf life for 24 hours.

The addition of the crude extract of *Nypa fruticans* leaf flour cannot kill bacteria but can only inhibit bacterial growth; this can be seen in the growth of bacteria in fish without treatment at 24 hours of 7.86 ± 0.01 log cfu/g, then fish that had been added with the extract at a concentration of 22.5% only had a bacterial growth rate of 5.63 ± 0.03 log cfu/g.

REFERENCES

[1] E. Susanto, “Pengolahan Bandeng (Chanos chanos) Duri Lunak,” in *Seri Materi Penyuluhan Bagi Masyarakat Pesisir*, 2010, pp. 1–19.

[2] D. T. Mareta and S. N. Awami, “Pengawetan Ikan Bawal dengan Pengasapan dan Pemanggangan,” *MEDIAGRO*, vol. 7, no. 2, pp. 33–47, 2011.

[3] Y. Zuraidah, “Faktor-faktor yang Berhubungan dengan Penggunaan Formalin pada Pedagang Tahu di Pasar Flamboyan Kota Pontianak,” *Pannmed*, vol. 2, no. 1, pp. 9–12, 2007.

[4] Nurmasari, “Pengaruh Formalin Terhadap Mukosa Yeyunum Tikus Putih,” Skripsi, Universitas Muhammadiyah Malang, Malang, 2008.

[5] F. W. Mahatmanti, W. Sugiono, and W. Sunarto, “Sintesis Kitosan dan Pemanfaatannya Sebagai Anti Mikroba Ikan Segar,” *Jurnal Sains dan Teknologo (Sainteknol)*, vol. 8, no. 2, pp. 101–111, 2010.

[6] H. Purnobasuki, "Potensi Mangrove sebagai Tanaman Obat," *J Biota*, vol. 9, no. 2, pp. 125–126, 2004.

[7] M. Yunita, Y. Hemdrawan, and R. Yulianingsih, "Analisisi Kuantitatif Mikrobiologi pada Makanan Penerbangan (Aerofood ACS) Garuda Indonesia Berdasarkan TPC (Total Plate Count) dengan Metode Pour Plate," *Jurnal Keteknikan Pertanian Tropis dan Biosistem*, vol. 3, no. 3, pp. 237–248, 2015.

[8] A. Ajizah, "Sensitivitas Salmonella typhirium terhadap ekstrak daun Psidium Guajava," *BIOSCIENTIVE*, vol. 1, no. 1, pp. 31–38, 2004.

[9] H. Sa'adah and H. Nurhasnawati, "Perbandingan Pelarut Etanol Dan Air Pada Pembuatan Ekstrak Umbi Bawang Tiwai (Eleutherine americana Merr) Menggunakan Metode Maserasi," *Jurnal Ilmiah Manuntung*, vol. 1, no. 2, pp. 149–153, Jan. 2017, doi: 10.51352/jim.v1i2.27.

[10] A. J. N. Parhusip, "Kajian Mekanisme Antibakteri Ekstrak Andaliman (Zanthoxylum acanthopodium DC) Terhadap Bakteri Patogen Pangan," Disertasi, Sekolah Pascasarjana Institut Pertanian, Bogor, 2006.

[11] Ismarani, "Potensi Senyawa Tanin Dalam Menunjang Produksi Ramah Lingkungan," *CEFARS : Jurnal Agribisnis dan Pengembangan Wilayah*, vol. 3, no. 2, pp. 50–54, 2012.

[12] N. Khoirani, "Karakterisasi Simplisia dan Standarisasi Ekstrak Etanol Herba Kemangi (Ocimum americanum L.)," Skripsi, UIN Syarif Hidayatullah, Jakarta, 2013.

[13] L. H. Meyer, *Food Chemistry*. Westport: The AVI Publishing Company Inc. University of California, 1982.

[14] I. Budaraga, A. Y. Marlinda, and U. Bulanin, "Antibacterial Properties of Liquid Smoke from the Production of Cinnamonhow Purification and Concentration of Different," *International Journal of Thesis Projects and Dissertations (IJTPD)*, vol. 4, no. 2, pp. 265–274, 2016.

[15] A. Zummah and P. R. Wikandari, "Pengaruh Waktu Fermentasi dan Penambahan Kultur Starter Bakteri Asam Laktat Lactobacillus plantarum B1765 Terhadap Mutu Bekasam Ikan Bandeng (Chanos chanos)," *UNESA Journal of Chemistry*, vol. 2, no. 3, pp. 14–24, 2013.

[16] S. Liu *et al.*, "Quality Evaluation of Traypacked Tilapia Fillets Stored at 0°C Based on Sensory, Microbiological, Biochemical and Physical Attributes," *Afr J Biotechnol*, vol. 9, no. 5, pp. 692–701, 2010.

[17] Yulneriwarni, H. Silfia, and S. Handayani, "Aktivitas Antibakteri Ekstrak Makroalga Padina australis dan Laurencia nidifica di Kepulauan Seribu terhadap Bakteri Staphylococcus aureus dan Escherichia coli," *Jurnal Pro-Life*, vol. 3, no. 3, pp. 153–166, 2016.

[18] M. Nurilmala, M. Wahyuni, and H. Wiratmaja, "Perbaikan Nilai Tambah Limbah Tulang Ikan Tuna (Thunnus sp.) Menjadi Gelatin Serta Analisis Fisika-Kimia," *Jurnal Buletin Teknologi Hasil Perikanan*, vol. 9, no. 2, pp. 22–33, 2006.

[19] E. Afrianto, E. Liviawaty, O. Suhara, and H. Hamdani, "Pengaruh Suhu dan Lama Blansing terhadap Penurunan Kesegaran Filet Tagih Selama Penyimpanan pada Suhu Rendah," *Jurnal Akuatika*, vol. 5, no. 1, pp. 45–54, 214AD.

[20] K. Suradi, "Pengaruh Lama Penyimpanan pada Suhu Ruang Terhadap Perubahan Nilai pH, TVB dan Total Bakteri Daging Kerbau," *Jurnal Ilmu Ternak*, vol. 12, no. 2, pp. 9–12, 2012.