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The Effect of Immersion Time on the Longevity of Puffy Fish (Rastrelliger Sp.) Fresh Using Beluntas (*Pluchea Indica L.*) Leaf Extract

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Abstract: This study aims to determine the effect of beluntas leaf extract (Pluchea indica L.) on the quality of fresh mackerel (Rastrelliger sp.). Treatment factors were beluntas leaves with a concentration of 100% for 3 hours, 6 hours, 9 hours, 12 hours and 15 hours. This study was designed using RAL for chemical and microbiological test results, while organoleptic data were analyzed by Kruskall-wallis. All data that had a significant effect were tested by Duncan's further test. The results showed that fresh mackerel (Rastrelliger sp.) soaked in 100% solution of beluntas (Pluchea indica L.) indicated that the immersion time had a significant effect on the hedonic quality of the eyes, gills, mucus, odor, flesh and texture of mackerel. . The organoleptic value of hedonic quality at immersion time of up to 15 hours meets the standard of SNI 2729-2013 concerning fresh fish for all hedonic quality parameters with a value of 7. The treatment duration of immersion up to 15 hours had a significant effect on the amount of TPC with values between 4.62 CFU/gr – 5.57 CFU/gr and pH ranging from 6.13 – 6.42. These results still meet the requirements of SNI 2729-2013 regarding fresh fish.

Keywords: *Beluntas leaves, mackerel, hedonic quality, Total Plate Count (TPC), pH*

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PRELIMINARY

Mackerel (Rastrelliger sp.) is one type of pelagic fish that has economic value and potential in Indonesia. Mackerel production experienced an average volume increase of 3.63% from 2002 to 2007 (DKP, 2007). In Gorontalo Province, the potential for mackerel in 2012 – 2017 reached 699.50 tons – 874,375 tons (DKP Gorontalo, 2017).

Fish is one of the food products from fisheries that is needed by humans because in fish meat there are compounds that are needed by the body consisting of protein, fat, carbohydrates, vitaminsand mineral salts. Fish also contains quite high water, which is 76%, which is a suitable medium for the life of spoilage bacteria or other microorganisms, so that fish undergo the process of decay very quickly (Irawan, 1997).

Freshness of fish is a very important factor and is closely related to durability. Fresh fish has good quality so that the selling value is high, otherwise if the fish is not fresh it has low quality so the price is low (Murniyati and Sunarman, 2000).

One of the efforts to maintain the freshness of fish is by adding natural additives that are safe for consumption, considering that today many fish sellers use harmful additives such as formalin to maintain the freshness of fish. Additives that are safe for consumption and natural that can be used as preservatives to keep fish fresh are using beluntas (Pluchea indica L.) leaves.

According to Dalimartha (2008), beluntas leaves have compounds such as flavonoids, essential oils, phenolics, tannins and alkaloids that have potential as antibacterials as evidenced by the research of Widyawati (2009) on the antibacterial activity test of beluntas leaves against Pseudomonas aeruginosa and Staphylococcus aureus bacteria. These compounds can inhibit the growth of bacteria. The results of the research by Septika (2014) in Adriyana (2017) stated that the beluntas leaf extract used in the preservation of snakehead fish for 6 hours of immersion, the bacterial population was only 15.05 x 105 CFU/mL. However, the research was only conducted on a few types of fish, not on all types of fish, especially on the types of fish that are often found in traditional markets. so this study was conducted to determine the effect of beluntas leaves on seawater fish, namely mackerel, because these fish are fish that are often found in traditional markets in Gorontalo Province. Based on this background, research on "The Effect of Beluntas Leaves (Pluchea indica L.) on the Durability of Fresh Mackerel (Rastrelliger sp.)" needs to be done.

RESEARCH METHODOLOGY

This research was conducted from January to August 2018 in Gorontalo City, Gorontalo Province. Organoleptic testing (hedonic quality) will be carried out at the Laboratory of Biotechnology and Characterization of Fishery Product Quality. Microbiological and chemical testing of samples will be carried out at the Center for Development and Quality Testing of Fishery Products (BPPMHP).

Tools and materials

The tools used in the study included a coolbox as an immersion container, organoleptic score sheets, grinding machines or blenders, scales, digital scales, knives, cutting boards, plates, magnetic stirrer, hot plates, autoclaves, cups, incubators, ovens, micropipettes, labels, baking sheet, erlenmeyer tube, beaker, test tube, Thermolyne, freezer, water bath, stamped paper, aluminum foil, Laminary air flow, cotton. The materials used were fresh mackerel, ice cubes, beluntas solution, Butter Field Phosphate (BFP), Plate Count Agar (PCA) and aquades.

Research Procedure

The initial stage, namely the provision of mackerel used in this study, was obtained from the TPI Tenda Kota Gorontalo, then the fish preparation stage was carried out, after preparation of fresh fish and packaged in a coolbox that had been cleaned and given ice in a ratio of 1:1, then it was ready to be taken to the kitchen. BPPMHP testing site. This study refers to research conducted by Adriyana (2017) where the results of his research showed the best quality of red snapper in the treatment (beluntas leaves 60g and soaking time 60 minutes) with the number of bacterial colonies 2.17 x 106 cfu/g.

Based on the preliminary study, the soaking time was 6 hours as the basis of reference in the main study to determine the soaking time of mackerel with beluntas leaves. Mackerel that has been soaked with 100% beluntas leaf solution was soaked for A (3 hours), B (6 hours), C (9 hours), D (12 hours) and E (15 hours) as treatment, then analyzed for organoleptic hedonic quality. which includes eyes, gills, mucus, flesh, smell and texture; and microbiological testing (TPC) and pH.

RESULTS AND DISCUSSION

Results of Hedonic Quality Analysis of pufferfish (Rastrelliger sp.)

The organoleptic test of mackerel that was carried out was a hedonic quality test. Assessments for this test on fish include appearance (eyes, gills, mucus), flesh, smell, and texture. This organoleptic test was carried out by 25 semi-trained panelists. The data from the organoleptic test results of the three parameters are described as follows.

Fish eye

Histogram of the average hedonic quality of mackerel eyes during 15 hours of immersion can be seen in Figure 1.

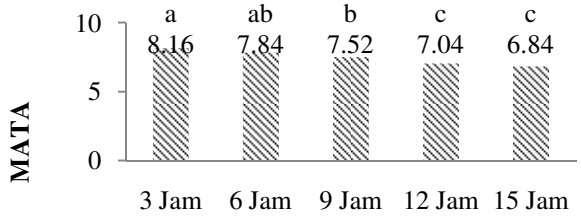

Figure 1. Histogram of the average hedonic quality of mackerel eyes with different immersion times. The same letters indicate that the results of the treatment are not significantly different and the same letters indicate that the results are not significantly different.

Figure 1 shows the value of the hedonic quality of mackerel eye treatment results which decreases along with the longer immersion with 100% beluntas concentration. The lowest hedonic quality value in the 15 hour immersion treatment was 6.84 (rounded up to 7) with criteriaflat eyeball, slightly cloudy cornea, slightly grayish pupil, slightly shiny specific type of fish. The highest quality value was found in 3 hours of immersion, namely 8.16 (rounded up to 8) with the criteriaflat eyeball, clear cornea and pupil, slightly shiny fish species. If it is seen from the quality standard of fresh fish set by SNI (2729-2013) that mackerel eyes treated with 3 hours to 15 hours of immersion statistically meet the organoleptic value requirements of 7.

Based on the Anova test, different immersion time treatments had a significant effect on the resulting eye appearance (Appendix 5). Duncan's test results showed that 3 hours of immersion was not significantly different from 6 hours, 6 hours was not significantly different from 9 hours but significantly different from 12 hours and 15 hours of immersion, but 12 hours and 15 hours of immersion were not significantly different.

During the soaking time of 3 hours to 15 hours, the eyelets still met the requirements, this was due to the presence of beluntas solution which was thought to inhibit the process of deterioration of the quality of the eyelets due to microbial activity. The decrease in organoleptic value can be inhibited by anti-bacterial substances such as phenol from the beluntas solution used. Phenol can inhibit the activity of bacterial enzymes, which in turn will interfere with the metabolism and the survival process of these bacteria (Basjir, et al. 2012).

Along with the longer immersion, which is 15 hours, the fish eyes begin to experience a decrease in quality, this is closely related to the antibacterial compounds in fish. According to Agati, et al (2007) in Herawati (2011) the permeability of cell membranes in fish eyes is disturbed due to the presence of phenolic compounds. The phenolic compounds contained in beluntas affect the organoleptic value of eye appearance, so changes occur very quickly in fish eyes.Fisheye

appearance is related to the number of TPC colonies. At 15 hours of storage, TPC colonies showed a higher number than other treatments.

In accordance with Widyasari's (2006) statement that the longer the shelf life of fish, the value of eye appearance will continue to decrease, this is caused by organoleptic, chemicaland microbiological changes. Physical changes are caused by the presence of a number of bacteria and enzymes that actively carry out activities in all parts of the fish so that the fish are quickly damaged.

Gill

Histogram of the average hedonic quality of puffed gills for 15 hours of immersion can be seen in Figure 2.

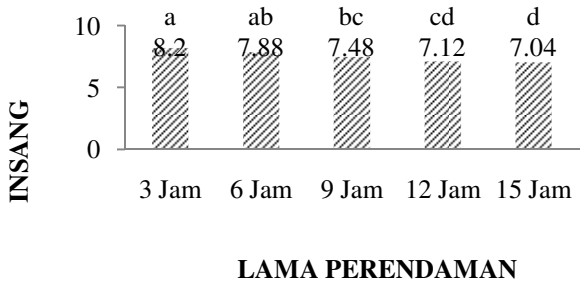

Figure 2. Histogram of the average hedonic quality of mackerel gills with different immersion times. The same letters indicate that the results of the treatment are not significantly different and the same letters indicate that the results are not significantly different.

Figure 2 shows the value of the hedonic quality of the mackerel gills as a result of treatment which decreases along with the longer immersion with a concentration of 100% beluntas. The lowest hedonic quality value in the 15 hour immersion treatment was 7.04 rounded 7 with the criteria of pink or light brown gill color with a little cloudy mucus. The highest quality value was found in 3 hours of immersion, namely 8.2 which was rounded up to 8 with the criteria wthe color of the gills is dark red or reddish brown, less bright with a little transparent mucus. When viewed from the quality standard of fresh fish set by SNI (2729-2013) that the gills of mackerel fish treated with a soaking time of 3 hours to 15 hours statistically met the organoleptic value requirements of 7.

Based on the Anova test, different immersion time treatments had a significant effect on the appearance of the gills produced (Appendix 5). Duncan's test results showed that 3 hours of immersion was significantly different from all treatments, but not significantly different from 6 hours, 6 hours was not significantly different from 9 hours, 9 hours was not significantly different from 12 hours, 12 hours was not significantly different from 15 hours.

At a duration of 3 hours to 15 hours of immersion, fish gills can be maintained by using antimicrobials in the beluntas. These antimicrobials are used to prevent the activity of bacteria that cause a decrease in the quality of mackerel. The use of antimicrobials can play a role in maintaining the freshness of fish, including the gills. The use of beluntas solution containing anti-microbial compounds is able to maintain the freshness of mackerel gills.Beluntas contains phenolic compounds, mAccording to Hakim, et al (2015) flavonoids are phenolic compounds that function as antibacterials by forming complex compounds against extracellular proteins.

The results showed that the longer the immersion the organoleptic value of the gill appearance of mackerel decreased. The decline in the quality of the gills is inseparable from the performance of the gills in filtering oxygen in the water during respiration so that the gills become a place for microbial accumulation (Fujaya, 2004).

The active compounds contained in beluntas leaves are alkaloids, flavonoids, triterpenoids, tannins and phenols as well as other essential oil derivatives (Nahak, 2012 and Dalimartha, 1999). Flavonoids and phenolic compounds contained in beluntas leaves have antibacterial activity, namely inhibiting the growth of Escherichia coli bacterial cells (Susanti, 2006). The results of research by Nahak et al., (2007) showed that pure extract of beluntas leaves can reduce 70% of the number of salivary bacteria.

Mucus

Histogram of the average hedonic quality of bloated mucus for 15 hours of immersion can be seen in Figure 3.

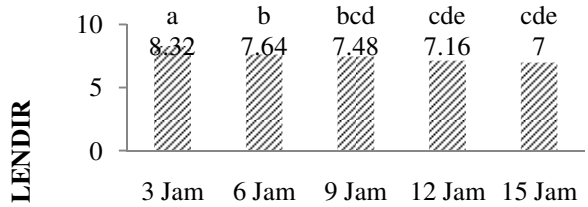

Figure 3. Histogram of the average hedonic quality of mackerel slime with different immersion times. The same letters indicate that the results of the treatment are not significantly different and the same letters indicate that the results are not significantly different.

Figure 3 shows the value of hedonic quality of mackerel slime as a result of treatment which decreases with increasing duration of immersion with a concentration of 100% beluntas. The lowest hedonic quality value in the 15 hour immersion treatment was 7 with the criteria of the mucus layer starting to become slightly cloudy. The highest quality value was found in immersion 3, namely 8.32 which was rounded up to 8 with the criteria of a clear mucus layer, transparent and quite bright. When viewed from the quality standard of fresh fish set by SNI (2729-2013) that the mucus of mackerel fish treated with a soaking time of 3 hours to 15 hours statistically met the organoleptic value requirements, namely 7.

Based on the Anova test, different soaking time treatments had a significant effect on the mucus produced (Appendix 5). The results of Duncan's test showed that 3 hours of immersion was significantly different from all treatments, 6 hours of immersion was not significantly different and 9 hours was significantly different from other treatments. The immersion time of 9 hours was not significant with all treatments except 3 hours.

At 3 hours to 15 hours of immersion, the mucus in mackerel still meets the requirements, this is because the beluntas solution is used to inhibit the growth of bacteria due to the presence of antibacterial compounds such as flavonoids. Flavonoids are the largest group of phenolic compounds (Sjahid, 2008). The mechanism of action of flavonoids is to function as an antibacterial by forming complex compounds against extracellular proteins that disrupt the integrity of the bacterial cell membrane. Its mechanism of action is by denaturing bacterial cell proteins and damaging cell membranes beyond repair (Juliantina, et al. 2008).

The results showed that the longer the immersion the hedonic quality of mackerel mucus decreased. The decrease in the value of hedonic mucus is related to the activity of bacteria that utilize fish mucus. The mucus consists of glucose protein and becomes a good substrate for bacterial growth. The presence of the beluntas leaf extract solution as an antibacterial was only able to maintain the quality of the mucus for up to 12 hours, because the longer immersion caused the antibacterial in the beluntas leaf extract solution to be no longer effective.

This is related to the results of the TPC bacterial colony test with the organoleptic results of the hedonic quality of the appearance of mucus in mackerel, it can be seen that the longer the immersion the bacterial growth increases, thus the activity of bacteria on the surface of the skin begins to carry out the process of decay which is marked by a layer of mucus that begins to develop. looks a bit cloudy on the surface of the skin.

Meat

The histogram of the average hedonic quality of puffed meat for 15 hours of immersion can be seen in Figure 4.

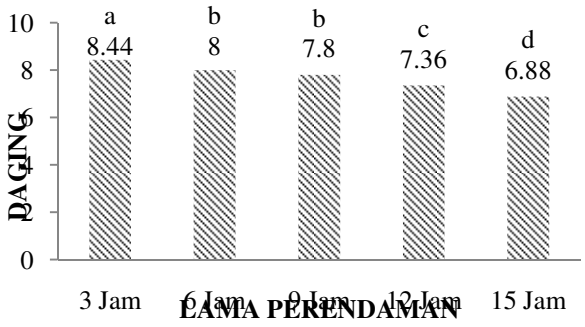

Figure 4. Histogram of the average hedonic quality of mackerel meat with different soaking times. The same letters indicate that the results of the treatment are not significantly different and the same letters indicate that the results are not significantly different.

Figure 4 shows the hedonic quality value of mackerel meat as a result of treatment which decreases along with the longer immersion with 100% beluntas concentration. The lowest hedonic quality value in the 15 hour immersion treatment was 6.88 which was rounded 7 with the criteria of a slightly less brilliant cut, strong meat tissue. The highest quality value was found in 3 hours of immersion, namely 8.44 which was rounded up to 8 with the criteriaSpecific brilliant cut meat, strong flesh tissue. . When viewed from the quality standard of fresh fish set by SNI (2729-2013) that the mackerel meat treated with a soaking time of 3 hours to 15 hours statistically met the organoleptic value requirements of 7.

Based on the Anova test, different soaking time treatments had a significant effect on the meat produced. The results of Duncan's test showed that the 3 hour immersion time was different from all treatments. The immersion time of 6 hours was not significantly different from that of 9 hours. the immersion time of 12 hours was significantly different from the immersion time of 15 hours.

The results showed that at 3 hours to 15 hours of immersion the quality of the fish still met the requirements, because the content of beluntas as an antibacterial such as one of the essential oils. According to (Benkeblia, 2004) essential oils have antibacterial activity that works by inhibiting the formation of bacterial cell membranes so that the membrane or cell wall is not formed or is formed imperfectly. The cell membrane has a function including controlling the entry and exit of various substances and is the location of the active substance transport system, for that the occurrence of bacterial inhibition can be caused by damage to the structural components of the bacterial cell membrane.

Along with the length of immersion the organoleptic value of the meat began to decrease, this was influenced by the presence of several bacteria. According to Afrianto and Liviawaty (2011), the appearance of meat, especially the color of the incision is influenced by the oxidation reaction between oxygen and fat components in dull fish. Fish meat consists almost entirely of transverse

striped flesh formed by meat fibers. The sensory attributes of meat were also related to the abdominal wall of the fish, at 15 hours of storage, the cuts of meat were not bright enough.

Smell

The histogram of the average hedonic quality of bloated odor during immersion for 15 hours can be seen in Figure 5.

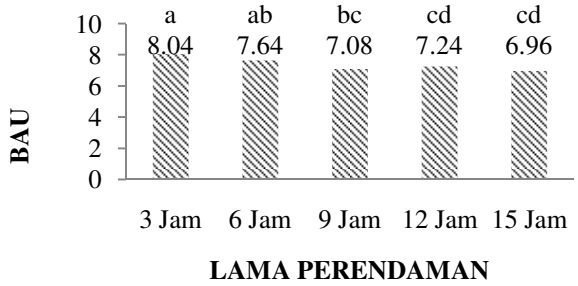

Figure 5. Histogram of the average hedonic quality of the smell of mackerel with different immersion times. The same letters indicate that the results of the treatment are not significantly different and the same letters indicate that the results are not significantly different.

Figure 5 shows the hedonic quality of mackerel odor as a result of treatment which decreases as the duration of immersion increases with 100% beluntas concentration. The lowest hedonic quality value in the 15-hour immersion treatment was 6.96 which was rounded 7 with criteriafresh, less specific type. The highest quality value was found in 15 hours of immersion, namely 8.04 which was rounded up to 8 with the criteria of fresh, specific type. If it is seen from the quality standard of fresh fish set by SNI (2729-2013) that the mackerel meat treated with a soaking time of 3 hours to 15 hours statistically meets the organoleptic value requirements of 7.

Based on the Anova test, different immersion time treatments had a significant effect on the resulting odor. Duncan's test results showed that the immersion time was 3 hours, not significantly different from 6 hours, but significantly different from 9 hours, 12 hours and 15 hours.

The results showed that the soaking time of 3 hours to 15 hours of fish eyes still met the requirements, this was due to the presence of beluntas solution which was thought to inhibit the process of deteriorating fish odor quality due to microbial activity. The decrease in organoleptic value can be inhibited by anti-bacterial substances such as phenol from the beluntas solution used. Phenol can inhibit the activity of bacterial enzymes, which in turn will interfere with metabolism and the process of survival of these bacteria (Basjir, et al. 2012).

The decrease in organoleptic quality of mackerel odor was in line with the decrease in microbiological quality based on the number of TPC bacteria. The results of the organoleptic analysis of fish odor and the results of the fish TPC test showed interrelated results. The higher the number of bacteria in mackerel, the lower the organoleptic odor value, and vice versa. This is because the smell that arises from fish, is an odor that arises due to damage to fish components. The damage is caused by the activity and reforms carried out by bacteria.

texture

The histogram of the average hedonic quality of mackerel texture during 15 hours of immersion can be seen in Figure 6.

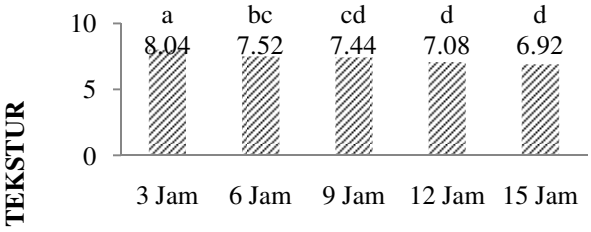

Figure 6. Histogram of the average hedonic quality of mackerel texture with different immersion times. The same letters indicate that the results of the treatment are not significantly different and the same letters indicate that the results are not significantly different.

Figure 6 shows the hedonic quality value of mackerel texture as a result of treatment which decreases along with the longer immersion with 100% beluntas concentration. The lowest hedonic quality value in the 15-hour immersion treatment was 6.92 (rounded up to 7) with the criteria being somewhat soft, somewhat elastic. The highest quality value was found in 3 hours of immersion, namely 8.04 rounded up to 8 with the criteriasolid, compact, elastic. If you look at the quality standard of fresh fish set by SNI (2729-2013) that the texture of mackerel fish treated with 3 hours and 15 hours of immersion meets the organoleptic value requirements of 7.

Based on the Anova test, different immersion time treatments had a significant effect on the resulting texture. The results of Duncan's test showed that 3 hours of immersion was significantly different from that of 6, 9, 12 and 15 hours, but the immersion time of 12 hours and 15 hours was not significantly different.

The results showed a decrease in the organoleptic value of the texture along with the immersion time. However, statistically the value of the decline is still categorized as fresh fish according to SNI. This is due to the presence of beluntas solution which can inhibit the process of quality deterioration due to microbial activity by antibacterial compounds. Because microbial activity is inhibited, the autolysis and enzyme processes are inhibited, thus inhibiting the protein denaturation process as a medium for bacterial growth.

The longer the immersion, which is 15 hours, the texture quality decreases, the decrease in texture quality is triggered by the activity of microbial enzymes that change the fish meat to become softer, the texture is also related to the water holding capacity which is also related to the denaturation of fish protein.

Fish spoilage is caused by destructive microbial activity. Fish spoilage occurs due to the breakdown of proteins into simple molecules (such as amino acids). This breakdown causes the cells in the fish's body to rot. The allicin compound from the beluntas extract effectively inhibits the degradation process of fish texture by the bacteria destroying the beluntas slowing down microbial metabolism (Widaningrum and Winarti, 2010).

Microbiological Test Results (Total Plate Count) Fresh Mackerel (Rastrelliger sp.)

Based on the results of the study there was an increase in the number of microbes at different immersion times. The test results showed that the highest TPC log value was in the sample with 15 hours of immersion, which was 5.57 CFU/gr, while the lowest was 4.62 CFU/gr.

The maximum limit of TPC value in fresh fish by the national standardization agency is a maximum of 5 x 105 colonies/gram (maximum log value of 5.7 CFU/g). Overall, the results of the TPC test for all immersion time treatments still meet the SNI requirements because each TPC value is still below the maximum TPC limit (BSN, 2013). However, in the 15-hour treatment, the growth of

bacteria increased or was not inhibited by active compounds such as phenolic compounds so that it was still possible to experience a faster deterioration of microbiological quality.

Based on the ANOVA test, different immersion time treatments had a significant effect on the resulting TPC value (Appendix 8). The results of Duncan's test showed that the immersion time of 3 hours was significantly different from all treatments. The immersion time of 6 hours was significantly different with all treatments. The immersion time of 6 hours was significantly different with all treatments. However, the immersion time of 12 hours and 15 hours was not significantly different.

The low number of bacteria indicates that the beluntas solution can suppress bacterial growth because beluntas has antibacterial compounds including flavonoids and tannins. While the increase in the number of bacteria is due to the presence of substances that support the growth of bacteria in fish. Bacteria grow thanks to compounds such as proteins that can be found in fish muscles as a food source. At first, bacteria grow on the surface and then multiply.

pH value

The results showed that the data from the pH test showed that the pH of mackerel fish with different immersion time was in the interval from 6.13 to 6.42. The lowest value was found in the 15 hour soaking time treatment and the highest value was found in the 3 hour treatment. Referring to the quality standard of fresh fish set by BSN (2729-2013) that the pH of mackerel fish treated with soaking time of 3 hours, 6 hours, 9 hours, 12 hoursand 15 hours is not required. However, according to Junianto (2003) the pH value of fresh fish ranges from 5.2 to 6.8. So that the soaking time of 3 hours to 15 hours qualify as fresh fish.

Based on Anova test different immersion time treatments have a significant effect on the resulting pH value(Appendix 8). Duncan's test results showed that the 3 hour immersion time was significantly different with all treatments. The immersion time of 6 hours and 9 hours was not significantly different. The immersion time of 12 hours and 15 hours was significantly different (Appendix 9).

The results showed that the longer the immersion the fish's pH value was lower. The decrease in pH value indicates the activity of proteolytic enzymes found in fish meat tissue that produces ammonia. While the decrease in pH value occurs due to the activity of microorganisms that degrade carbohydrates in the form of glycogen into lactic acid. The pH value increased due to the formation of ammonia due to the activity of proteolytic enzymes. This is in accordance with Santoso et al. (1999) that the decrease in pH in soaking is due to the formation of lactic acid resulting from the breakdown of glycogen by enzymes contained in meat. In addition, the pH of mackerel can also be influenced by the treatment of soaking time with the beluntas solution. The longer the immersion, the lower the pH value of the fish.

Fish quality can be seen in terms of fish freshness parameters. The fish freshness parameters were measured subjectively (organoleptically) and objectively (pH, TVB, TPC). These parameters have a relationship during the process of deterioration of milkfish quality. Post-mortem changes occur after the fish dies and blood flow stops. The result of the cessation of blood circulation is a very complex series of reactions in the muscles. The immediate effect of cessation of blood circulation and removal of blood from muscle tissue is the lack of oxygen delivery to the tissues. As a result, the tissue is unable to re-form adenosine triphosphate (ATP) as a cellular energy source, because the electron transport mechanism and oxidative phosphorylation are immediately stopped. This causes anaerobic respiration which causes the production of lactic acid so that the pH drops. After the pH drops, proteolytic enzymes, especially cathepsin, are free and active and degrade proteins. The

breakdown of protein will stimulate bacterial growth so that fish will increasingly show signs of decay (Eskin, 1990).

Conclusion

Based on the results of the study, it can be concluded that fresh mackerel (Rastrelliger sp.) using 100% beluntas (Pluchea indica L.) solution with immersion time of 3 hours, 6 hours, 9 hours, 12 hours and 15 hours, shows that the immersion time has an effect on significantly on the value of hedonic quality of eyes, gills, mucus, odor, meat and texture of mackerel. This treatment had a significant effect on the amount of TPC and pH so that it still met the requirements of SNI 2729-2013 regarding fresh fish.

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