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THE USE OF FERMENTATION OF RICE WASHING WATER AND SALT ON THE SHELF LIFE OF RED TILAPIA FILETS BASED ON THE NUMBER OF MICROBES AT LOW TEMPERATURE STORAGE

Penggunaan Fermentasi Air Cucian Beras dan Garam Terhadap Masa Simpan Filet Nila Merah Berdasarkan Jumlah Mikroba pada Penyimpanan Suhu Rendah

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ABSTRACT

Red tilapia is very popular with Indonesian people, because its meat tastes delicious and thick, similar to red snapper. One of the tilapia fish products is tilapia fillet with skin, which has the advantage of containing high nutritional value, especially protein and fat content, but has the disadvantage of quickly decreasing in quality. A strategy to reduce the number of bacteria can be through adding natural preservative compounds. Fermentation of rice washing water with added salt concentration produces lactic acid compounds which are anti-bacterial so they can slow down the growth of bacteria. This research aims to determine the best salt concentration in fermented rice washing water for the shelf life of red tilapia fillets based on the number of microbes at low temperature storage (5°-10°C). The research method used was experimental with four treatments, namely soaking tilapia fillets for 30 minutes with the addition of fermented rice washing water at each concentration (0%, 2%, 3%, 4%). The research results showed that the average pH value of untreated red tilapia fillets during storage tended to be higher when compared to treated red tilapia fillets. This is because there are no compounds that control the fermentation of rice washing water. Untreated red tilapia filets only have a shelf life of 8 days, while treated red tilapia filets have a longer shelf life of around 9 -10 days. Based on the research results and discussion description, it can be concluded that adding a salt concentration of 3% to fermented rice washing water is the best concentration for shelf life during low temperature storage (5°-10°C) with an acceptance limit of 10 days, with a total number of bacteria of $6{,}5x10^7$cfu/g, and a pH value of 6.70.

Keywords: Fermented Rice Washing Water, Microbes, pH, Red Tilapia Filet

ABSTRAK

Ikan nila merah banyak digemari oleh masyarakat Indonesia, dikarenakan rasa dagingnya gurih dan tebal mirip daging ikan kakap merah. Salah satu produk ikan nila adalah filet nila dengan kulit, memiliki keunggulan yaitu mengandung nilai gizi yang tinggi terutama

kandungan protein dan lemak, namun memiliki kekurangan yaitu cepat mengalami penurunan mutu. Strategi untuk mengurangi jumlah bakteri bisa melalui penambahan senyawa pengaw*et al.,*ami. Fermentasi air cucian beras yang ditambah dengan konsentrasi garam menghasilkan senyawa asam laktat yang anti bakteri sehingga dapat memperlambat pertumbuhan bakteri. Penelitian ini bertujuan untuk menentukan konsentrasi garam pada fermentasi air cucian beras yang paling baik terhadap masa simpan filet nila merah berdasarkan jumlah mikroba pada penyimpanan suhu rendah (5°-10℃). Metode penelitian yang digunakan adalah eksperimental dengan empat perlakuan, yaitu melakukan perendaman selama 30 menit terhadap filet nila dengan penambahan fermentasi air cucian beras pada setiap konsentrasi (0%, 2%, 3%, 4%). Hasil penelitian menunjukan bahwa rata-rata nilai pH filet nila merah tanpa perlakuan selama penyimpanan cenderung lebih tinggi jika dibandingkan dengan filet nila merah yang diberi perlakuan. Hal ini dikarenakan tidak adanya senyawa yang mengontrol fermentasi air cucian beras. Filet nila merah tanpa perlakuan hanya memiliki masa simpan selama 8 hari, sedangkan filet nila merah dengan perlakuan memberikan masa simpan lebih lama sekitar 9 -10 hari. Berdasarkan hasil riset dan uraian pembahasan, dapat disimpulkan bahwa penambahan konsentrasi garam 3% pada fermentasi air cucian beras merupakan konsentrasi yang paling baik terhadap masa simpan selama penyimpanan suhu rendah (5°-10℃) dengan batas penerimaan 10 hari, dengan jumlah total bakteri sebanyak 6,5x10^7cfu/g, dan nilai pH 6,7.

Kata Kunci: Fermentasi Air Cucian Beras, Filet Nila Merah, Mikroba, pH

INTRODUCTION

Aquaculture in Indonesia is an important component, one of which is red tilapia cultivation. According to records from the Ministry of Maritime Affairs and Fisheries, tilapia fish production in Indonesia in 2021 will reach 1.35 million tons with a value of IDR 33.62 trillion (KKP, 2022). Red tilapia is widely popular and consumed by Indonesian people, due to high market demand, white flesh, delicious and thick taste similar to red snapper, also quite high nutritional content, especially low protein and fat, besides that the price is relatively stable (Amir *et al.,* 2014). Tilapia has few spines so it can be made into various processed products (Riyadi *et al.,* 2019).

One of the tilapia fish products is tilapia fillets with skin. Filet products can also be further processed into various other processed products, and marketed with an attractive presentation. Tilapia filets with skin have the disadvantage of quickly decreasing in quality, because in the fish filleting process, the skin becomes a place for bacteria to grow, which can cause the fish meat to easily experience fat oxidation and become contaminated with microbes (Rostini, 2013).

Bacterial contamination of tilapia fillets can cause a decrease in the quality of the fish fillets. The problem of high bacterial contamination in tilapia fillets needs to be addressed, strategies to control contamination during handling of fish fillets are very necessary. Many filet companies use chlorine type disinfectants for washing filets, and to inhibit microorganisms. The cheap price and effectiveness in killing microorganisms are the advantages of chlorine (Al-sa'ady *et al.,* 2020). Based on issues regarding the addition of food additives, such as the use of formaldehyde and chlorine, it is necessary to have alternative fish fillet preservatives that are not harmful to health and have affordable prices. Using natural preservatives can overcome this problem. Natural ingredients have the potential for preserving fish, because they contain natural ingredients that can inhibit microbial activity (Syamsir, 2007).

Organic acids are natural preservative compounds produced by lactic acid bacteria from fruit fermentation, and essential oil components from plant extracts (Mapiliandari, 2008). The fermentation process from the use of vegetable and fruit waste can produce lactic acid bacteria which can inhibit the putrefaction process in fish, because it kills putrefactive bacteria (Siagian,

2013). Carbohydrates are used as a substrate for the growth of lactic acid bacteria. Rice contains 85% carbohydrates, 8% protein and 80% vitamin B1 (Haryadi, 2006).

Waste rice washing water is very easy to obtain compared to several types of substrates which are capable of growing other lactic acid bacteria, because rice is the main food ingredient for Indonesian people, and around 10% of rice washing water is used by the community, even in certain cases, such as being used to water plants. Fermented rice washing water contains Lactobacillus and Streptococcus, which are types of lactic acid bacteria that can inhibit putrefactive bacteria (Susilawati, 2018).

During the lactic acid bacteria fermentation process, it is necessary to add salt as an environmental control. According to Ali *et al.,* (2014) adding salt in the fermentation process can help reduce the solubility of oxygen in water and can inhibit the activity of proteolytic bacteria. Salt functions as a selective inhibitor for contaminant microbes, as an inhibitor for the growth of other microbes, especially pathogenic microbes, and the addition of salt can lower the pH (Handayani, 2023).

Based on this description, lactic acid bacteria produced from fermentation of rice washing water have the potential as an alternative natural preservative for tilapia filets. To find out whether rice washing water can extend the shelf life of tilapia filets, information is needed about the product's shelf life. Therefore, it is necessary to conduct research on the shelf life of red tilapia fillets based on the number of microbes at low temperature storage by immersion in fermented rice washing water and salt. This research aims to determine the best salt concentration in fermented rice washing water for the shelf life of red tilapia fillets based on the number of microbes at low temperature storage (5°-10°C).

METHODS

Place and Time

The research process for fermenting rice washing water, making fillets, counting microbes, storing tilapia fillets at low temperatures was carried out at the Fisheries Product Processing Technology Laboratory, Faculty of Fisheries and Marine Sciences, Padjadjaran University. The fish acclimatization process was carried out at the Aquaculture Laboratory, Faculty of Fisheries and Marine Sciences, Padjadjaran University. The research process for sterilizing tools and materials, making media is carried out at the Biotechnology Laboratory, Faculty of Fisheries and Marine Sciences, Padjadjaran University. Research was conducted in February - March 2024.

Tools and Materials

The tools used in this research are digital scales, glass jars, colony counters, pH meters, disposable petri dishes, basins, incubators, hot plate stirrers, magnetic stirrers, Bunsen lamps, autoclaves, filters, latex gloves, measuring pipettes, Erlenmeyer tubes. , test tube lids, test tubes, test tube racks, hand counters, label paper, brown paper, beaker glass, tissue, knives, cutting boards, stationery, cellphones, mica plastic mats, refrigerators, plastic wrapping, mortars, pestles, food tongs , drainer, fiberglass tub, styrofoam box, cool box, aerator hose, scissors, vortex mixer, Scott bottle, micro pipette, blue tips, laminar air flow, tweezers and spatula. The ingredients used are distilled water, salt, 95% alcohol, nutrient agar (NA), rice, rice washing water fermentation solution, red tilapia fish, red tilapia fillet, ice curia, spirit solution, pH 4 and pH 7 buffer solutions.

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Procedure for Making Fermented Rice Washing Water

The procedure for making fermented rice washing water in this research refers to research by Nisah *et al.,* (2021) which is modified as follows:

1) Rice is washed with clean water once

2) The ratio of rice to water is 1:2
3) Wash the 500 ml jar first until clean, then drain until dry
4) Jars are sterilized before use
5) Put 150 ml of rice washing water into a sterilized jar, then double the level of rice washing water in the jar
6) The salt is put into the jar according to the weight of the rice washing water, after which the solution is homogenized
7) The jar is closed tightly and covered with plastic wrapping and fermented fpr 6 days at room temperature

Filet Making Procedure

The process of making fish fillets begins by killing the fish first. The first step to reduce fish activity is to put the fish in a plastic container filled with cold water for 10 minutes so that the fish is not stressed, then put the fish in a styrofoam box containing twice the weight of the fish, then leave it for 30 minutes. minutes for the fish to die slowly (Liviawaty, 1998). After the fish is dead, proceed to making skinned fillets. The procedure for making fillets according to (Liviawaty, 1999) is as follows:

1) Tilapia is weeded by cleaning the fish scales, then washing the fish
2) Cutting the back of the head and root of the tail with a fillet knife is carried out in an air-condition room with a temperature range of 19° – 23°C
3) The cut is made from head to tail along the dorsal fin and parallel to the spine, then at an angle towards the ribs
4) The fish is then turned over, slashed at the back of the head and base of the tail, then cut the flesh from the base of the tail towards the head.
5) The sheet of meat is opened and the cutting continues
6) The resulting fillets are washed with cold water (± 10°C), then drained for 1 minute.

Application of Fermented Rice Washing Water to Red Tilapia Filet

The application of fermented rice washing water to fish fillets refers to research (Insani, 2016) which is modified as follows:

1) Tilapia fish fillets are soaked in fermented rice washing water (according to treatment) for 30 minutes
2) The soaked filet is then removed and drained for 2 minutes
3) Each filet is placed in a mica tray measuring 16 cm x 10 cm lined with tissue and perforated plastic then covered with cling wrap.
4) After the fish fillets are packaged, they are stored in a refrigerator at a low temperature with a temperature range of 5 - 10°C
5) Each treatment was tested for pH parameters, and TPC at a salt concentration of 0% was carried out on storage days 1, 3, 6, 7, 8, and 9, salt concentrations of 2%, 3%, 4% were carried out on storage day 1, 4, 7, 8, 9, 10, 11, and 12.

pH Testing Procedure

Determination of pH is carried out using a pH meter. According to Bawinto (2015), the work order for using a pH meter is as follows:

1) Standard solution of pH 4, and 7 are prepared to calibrate the pH meter
2) The test sample that has been ground using a mortar is weighed at 5 grams
3) The finely ground sample is put into a beakerglass which has been filled with 45 ml of distilled water and homogenized.
4) Then the pH value is measured, at a salt concentration of 0% the pH value is measured on days 1, 3, 6, 7, 8, and 9 of storage, at a salt concentration of 2%, 3%, 4% the pH value is measured on days of storage 1st, 4th, 7th, 8th, 9th, 10th, 1th, and 12th.

Total Plate Count (TPC)

The TPC determination method is used to determine the total number of bacteria in fish fillets that have been soaked with fermented rice washing water to determine the presence of bacterial growth in fish fillet samples using solid media with the final result being colonies that can be observed visually and can be counted. The maximum limit for bacteria in fish fillets is $5x10^6$ cfu/g (Connell, 1991). The working principle of TPC analysis is to count the number of bacteria in a sample (fish fillet) by dilution. The plate count method is an effective way to determine the number of bacteria. from. TPC calculations were carried out at a salt concentration of 0% on storage days 1, 3, 6, 7, 8, and 9, salt concentrations of 2%, 3%, 4% on storage days 1, 4, 7, 8, 9, 10, 11, and 12. The procedure for testing total bacteria using the Total Plate Count (TPC) method based on modified SNI 2332.3:2015 is as follows:

1) A sample weighing 5 g is weighed then placed I a sterile container or plastic and added with 45 mL of physuilogical NaCl solution
2) The sample was homogenized for 2 minutes. Homogenant is a 10^{-1} or P1 dilution solution
3) 1 ml of P1 is taken using a pipette and put into 9 mL of physiological NaCI solution to obtain a dilution of 10^{-2} or P2
4) Then 1 mL of P2 is taken and put into 9 mL of physiological NaCI solution to get a dilution of 10-3 or P3
5) Each dilution is shaken at least 25 times
6) Next do the same thing for dilutions 10^{-4} (P4), 10^{-5} (P5) and so on according to the sample conditions
7) Take 1 ml of the results of each dilution above and put them in a sterile petri dish. This step was carried out in duplicate for each dilution
8) Add 12-15 mI NA to each cup containing the sample. So that the sample and NA media are completely mixed, rotate the cup forward - backward and left – right
9) After that, the plates were incubated in an inverted position. Then put it in an incubator at 38°C

According to SNI 2332.3:2015, the following formula is used to calculate the number of microbial colonies:

$$N = \frac{\Sigma C}{[(1\ x\ n_1) + (0{,}1\ x\ n_2)]\ x\ (d)}$$

Information:

N = number of product colonies, expressed in colonies per ml / colonies per g
ΣC = number of colonies on all plates counted
n_1 = number of plates in the first dilution calculated
n_2 = number of plates in the second dilution calculated
d = the first dilution is calculated

Data analysis

The results of pH observations and bacterial counts were analyzed comparatively descriptively. The comparative descriptive analysis method compares the similarities and differences of two or more facts from the object being investigated based on a certain framework of thought, and describes the facts of an object or subject being studied systematically and then analyzed (Nazir, 2005). The pH data is plotted in graphical form to see the decrease and increase in pH of fish fillets during storage. Data on total bacteria are plotted in graphical form, then compared with the maximum acceptance limit for bacteria in fish fillets that are still suitable for consumption ($5x10^6$ cfu/g) (Connell, 1991). These two data are also interpreted with each other.

RESULT

The pH results of red tilapia fish fillets by immersion in fermented rice washing water and salt during storage at low temperatures (5°-10°C) can be seen in Table 1.

Table 1. Average pH Value of Red Tilapia Fillets Soaked in Fermented Rice Washing Water and Salt During Storage at Low Temperature (5°-10°C)

Day of storage-	Salt Concentration in Fermented Rice Washing Water (%)			
	0	2	3	4
1	6,30	6,10	6,00	6,20
3	6,00	-	-	-
4	-	5,75	5,70	5,80
6	6,45	-	-	-
7	6,55	6,30	6,00	6,40
8	**6,60**	6,50	6,40	6,60
9	7,20	**6,60**	6,60	**6,70**
10	-	6,80	**6,70**	6,90
11	-	6,90	6,90	7,00
12	-	7,00	7,00	7,10

Information:
- : pH measurement was not carried out

The research results based on table 1 explain that the pH value of red tilapia fillets soaked in fermented rice washing water and salt concentrations of 2%, 3%, and 4%, as well as the control treatment stored at low temperature (5°-10°C) has a pH value diverse. The pH value of red tilapia fillet on the 1st day of observation was around 6.00– 6.30, on the following day the pH value of red tilapia fillet decreased and increased. The results of observations of the total number of bacterial colonies can be seen in Table 2.

Table 2. Total Number of Bacterial Colonies (cfu/gram) of red tilapia fillets soaked in fermented rice washing water and salt during storage at low temperature (5°-10°C)

Day of storage	Number of Bacteria (cfu/g) Red Tilapia Filet by Soaking in Fermented Rice Washing Water			
	0	2	3	4
1	$1,9x10^5$	$1,8x10^5$	$1,5x10^5$	$2,0x10^5$
3	$2,5x10^5$	-	-	-
4	-	$3,8x10^5$	$2,5x10^5$	$3,3x10^5$
6	**$1,6x10^6$**	-	-	-
7	$1,2x10^7$	**$1,1x10^6$**	$1,5x10^6$	**$1,6x10^6$**
8	$5,1x10^7$	$2,3x10^7$	**$2,1x10^6$**	$1,7x10^7$
9	$8,7x10^7$	$9,4x10^7$	$5,8x10^7$	$7,9x10^7$
10	-	$1,8x10^8$	$6,5x10^7$	$9,4x10^7$
11	-	$2,5x10^8$	$1,6x10^8$	$2,3x10^8$
12	-	$3,8x10^8$	$2,3x10^8$	$3,0x10^8$

Based on observations, the number of spoilage bacteria at the beginning of the shelf life was around 10^5cfu/gram for all treatments. The total number of spoilage bacteria in the treatment of soaking red tilapia filets in fermented rice washing water and salt concentrations of 2%, 3% and 4% increased longer than the control treatment. This can be seen from the total number of spoilage bacteria in the control treatment of 10^6cfu/gram on day 6, the total number of spoilage bacteria in the treatment of soaking red tilapia fillets in fermented rice washing water and salt concentration of 2%, and 4% was 10^6cfu/gram on the 7th day, and the total number of spoilage bacteria in the treatment of soaking red tilapia filets in fermented rice washing water and 3% salt concentration was 10^6cfu/gram on the 8th day.

The research results showed that untreated red tilapia filets only had a shelf life of 6 days, while treated red tilapia filets had a longer shelf life of around 7-8 days. The difference in the number of days at the acceptable limit of 3% salt concentration is due to more optimal effectiveness and function in inhibiting microbial growth. Meanwhile, at concentrations of 2% and 4% it becomes ineffective, because at a concentration of 2% the salt which controls the fermentation of rice washing water is too little, while at a concentration of 4% it becomes ineffective because increasing the concentration does not always have a stronger inhibitory effect on bacterial growth. Increasing the concentration does not provide a longer shelf life because the nitrogen and protein compounds contained in certain amounts can be utilized by bacteria and stimulate their growth (Krisanti, 2005). The number of bacteria was 10^7 cfu/g, even though it was no longer accepted based on Connell (1991), it cannot be concluded that the red tilapia filet was no longer suitable for consumption. It is suspected that the high number of bacteria was dominated by lactic acid bacteria because the pH value was still classified as acidic, namely 6.60.-6.70 and the aroma is still neutral without any foul smell.

DISCUSSION

Lactic acid bacteria will break down lactose into lactic acid, a decrease in the pH value will occur due to the formation of lactic acid (Hidayat *et al.,* 2013). In accordance with research by Oktavian (2017), the pH value of catfish that was treated with soaking in breadfruit leaf extract showed a low pH value when compared to catfish fillets that were not treated. The pH value of tilapia filets treated with basil leaf extract during low temperature storage fluctuates as the shelf life increases. The pH value of tilapia filets treated with basil leaf extract soaking is lower compared to untreated tilapia filets (Azzahra, 2023). Basil extract affects the pH value of fish because basil contains flavonoids, saponins and tannins, antimicrobial activity which can inhibit bacterial growth (Nipa, 2022). The decrease in the pH value of tilapia fillets is because after the fish dies, blood circulation stops and oxygen supply decreases (Junianto, 2003).

The glycolysis process converts glycogen in the fish's body into lactic acid which will reduce the pH value of red tilapia fillets (Saputra & Tati, 2014). Chemical changes in fish flesh caused by the activity of the glucosinase enzyme in the fish's body cause a decrease in the pH value (Afrianto *et al.,* 2014). The use of low temperatures can affect the fluctuation of pH values in red tilapia fillets (Munandar *et al.,* 2009). Storage at low temperatures (5°-10°C) can inhibit the activity of enzymes in fish fillet meat, resulting in a slowdown in quality deterioration. The addition of salt can lower the pH, this decrease is due to the breakdown of the NaCl compound into its components, namely: Na^+ and Cl^- ions. Lactic acid bacteria need Na+ as a growth supporting factor, while Cl^- ions bind with free water, causing an acidic atmosphere due to the formation of HCl compounds (Handayani, 2023).

The increase in pH is caused by the formation of alkaline compounds, for example ammonia, the result of the breakdown of protein in fish flesh by enzymes and bacteria (Liviawaty & Afrianto, 2010). In fish fillets, protein is broken down into simpler compounds in the form of peptides, amino acids and ammonia, thereby increasing the pH (Yunizal, 1998).

The increase in pH is caused by the formation of volatile base compounds. The activity of microorganisms can also produce these compounds during the decomposition of complex compounds contained in fish flesh (Pangestika *et al.,* 2022).

The average pH value of untreated red tilapia fillets during storage tends to be higher when compared to treated red tilapia fillets. This is because there are no compounds that control the fermentation of rice washing water. According to Anggraeni, (2021), salt concentration affects water content, total lactic acid content, total bacterial count, sensory value and consumer acceptance. The activity of lactic acid bacteria is more effective at low salt concentrations compared to high salt concentrations, causing higher lactate concentrations. This is also the reason why red tilapia fillets, if not treated, can only survive until the 6th day. Red tilapia fillets soaked in fermented rice water with a salt concentration of 3% have a low pH compared to other processing methods. In line with research by Roni & Herawati (2012) on fermenting cabbage waste, the optimal salt solution concentration for fermentation is 3%, because it produces more lactic acid.

The increase in the number of bacteria during storage is due to autolysis which occurs in the fish's body through the action of enzymes. The autolysis process is always followed by an increase in the number of bacteria, because all the enzyme breakdown products produced during the autolysis process are a very suitable medium for the growth of bacteria and other microorganisms (Afrianto & Liviawaty, 1989). The number of bacteria increases with the length of storage because there is an optimal environment for bacterial growth, so that bacteria can grow optimally (Afrianto & Liviawaty, 2010).

Bacterial growth in the soaking treatment of red tilapia fillets in fermented rice washing water and salt concentrations of 2%, 3% and 4% continued to grow, even though the packaging and storage process in the refrigerator at low temperatures (5°-10°C) of red tilapia fillets had done. This shows that red tilapia filets contain types of bacteria that are psychrophilic and psychrotrophic. The groups of bacteria that can damage food stored in the refrigerator are the psychrophilic and psychrotrophic bacteria (Supardi & Sukamto, 1999). Psychophilic bacteria are microbes that are able to grow at temperatures below 5°C, but their growth occurs rapidly at temperatures of 10°-25°C (Sopandi, 2014). Psychotropic bacteria are able to grow at a minimum temperature of -4°C - 5°C, able to grow at an optimum temperature of 25°-30°C, able to grow at a maximum temperature of 30°-35°C.

Treated red tilapia fish fillets have a longer shelf life than untreated red tilapia fish fillets. In addition, the number of bacteria in red tilapia fillets that were not treated increased more quickly compared to red tilapia fillets that were treated. This is because there are no compounds that control the fermentation of rice washing water. According to Anggraeni (2021), salt content affects water content, total lactic acid content, total bacterial count, sensory value and consumer acceptance. In the plant fermentation process, the use of less than 2.5% salt can cause the growth of putrefactive bacteria and proteolytic bacteria which inhibit the fermentation process, while salt concentrations above 10% can cause the growth of halophilic bacteria which can inhibit fermentation (Azka *et al.,* 2018).

CONCLUSION

Based on the research results and discussion description, it can be concluded that adding a salt concentration of 3% to fermented rice washing water is the best concentration for shelf life during low temperature storage (5°-10°C) with an acceptance limit of 10 days, with a total number of bacteria of $6{,}5x10^7$ cfu/g cfu/g, and a pH value of 6.70.

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