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Antibacterial activity of honey in preserving high-pressure cooked milkfish stored at room temperature

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Abstract. High-pressure cooked milkfish is susceptible to decay during the storage process at room temperature because it has a relatively high water and protein content. The use of honey as a natural preservative containing antibacterial compounds is expected to improve the quality and shelf life of high-pressure cooked milkfish. This study aims to determine the effect of honey as a natural preservative against the total amount bacteria and organoleptic value during storage at room temperature. The treatment in this study was the use of a honey solution concentration as the preservative using samples consisting of 0% (without honey or control), 20%, 25%, 30% and 0.1% sodium benzoate solution. The results showed that the concentration of 30% for the honey solution was able to maintain the quality of the high-pressure cooked milkfish during storage at room temperature for up to 72 hours with the total bacteria being $2,3 \times 10^5$ colonies/gram ($< 5,0 \times 10^5$ colonies/gram) with an average organoleptic value of 7,45.

1. Introduction

High-pressure cooked milkfish are one type of the fish product that has the advantage of its bones being soft which means that all parts of the body can be consumed. The content of the dissolved protein, total protein and water content in fried soft-bone milkfish with a cooking time of 90 minutes was 15.96%, 32.36%, and 37.42% respectively [1]. High-pressure cooked milkfish is susceptible to decay during storage at room temperature because it has a relatively high water and protein content that is suitable for the growth of microorganisms, especially bacteria and fungi [2]. Food products consisting of water, fat, carbohydrates, proteins, and a small proportion of organic and mineral compounds will trigger microbial growth as they are a source of energy [3]. The maximum limit for microorganisms present in food products is 5.0×10^5 colonies/grams. [4]. The various damages and food poisoning caused by these microorganisms can be prevented through the use of preservation techniques to prevent or slow the growth of microbes. Heating (pasteurization and sterilization) is a preservation technique that can disable microorganisms [5].

High-pressure cooked milkfish is a processed product cooked using a heating technique at a high temperature of 115 to 121°C and with pressure of one atm [6]. To extend their shelf life, high-pressure cooked milkfish need to be preserved. Honey is a natural ingredient that can be used as a preservative because it has been recorded to demonstrate antibacterial activity against various spoiling bacteria [7]. Honey is produced from the nectar of flowers (honey flowers),



the secretions of the plant and/or the excretions of insects, called honeydew. Bees collect these sweet substances, enrich them with their own substances and store them in beehives. Honey promotes antibacterial activity against Gram-positive and negative bacteria. Although originating from different sources of flowers and countries, honey has been shown to promote antibacterial activity against *S. aureus*, *E. coli* and *Salmonella sp* [8]. Mundo [9] has proven the ability of honey in inhibiting the growth of spoilage microorganisms and bacteria causing food-borne diseases so then it can be considered as appropriate for use as a food preservative under suitable conditions. This study aims to determine the effect of high-pressure cooked milkfish immersion with honey on the total amount of bacteria and the organoleptic value at room temperature storage.

2. Materials and methods

2.1. Materials

The research material consisted of a 200 gram fresh milkfish purchased from the market and the original bee honey obtained from Sukapura, Probolinggo. Other materials used in the research were Plate Count Agar (PCA), physiological NaCl, 70% alcohol, distilled water, and sodium benzoate. The PCA was a medium for assessing the total bacteria from the sample. The PCA composition was 0.5% peptone, 0.25% yeast extract, 0.1% glucose and 1.5% agar with a neutral pH kept at 32°C for 48 hours.[10]. The additional ingredients used to cook the high-pressure cooked milkfish consisted of garlic, salt and turmeric.

2.2. Methods

High-pressure cooked milkfish, as the test milkfish product, was soaked in a honey solution. The use of a concentration of honey solution to soak milkfish product was 20%, 25% and 30% respectively. The negative control was a milkfish product that had not been soaked in the honey solution, and the positive control was a milkfish product that had been soaked in a chemical preservative of 0.1% sodium benzoate solution. Soaking was done for 30 minutes and then it was cooked in the oven for 30 minutes at 50°C. The dried product was removed in a clean container and stored at room temperature (20-25°C).

An observation of the total bacteria, organoleptic, pH value and moisture content was conducted before storage (0 hours) and after 24 hours, 48 hours, 72 hours, and 96 hours in storage. The observation of the milkfish product stored for 96 hours was done in order to determine the ability of honey to act as a preservative compared with other commonly used natural preservatives.

The calculation of the total number of milkfish product bacteria was done using the Total Plate Count (TPC) method. The TPC determination was done by counting the total number of bacterial colonies in the petri dish and then comparing it with the quality standard. [11]. The TPC method used a pour plate [12] which was then incubated at 37°C for 24 hours with the petri dish position reversed.[13]. The observation of the organoleptic milkfish product was done using an assessment sheet based on SNI 4106.1-2009. The panelists consisted of 30 students from the Faculty of Fisheries and Marine of Universitas Airlangga. Organoleptic testing is subjective because it only relies on the senses and sensitivity of the panelists [11]. The measurements of the pH of the milkfish product meat solution were homogenized using the pH meter. The water content in the milkfish product was determined by drying the meat, as much as five grams, in the oven at 105-110°C for three hours or until a constant weight was obtained. The difference in the weight of the material before and after drying was due to the water content in the milkfish product [14].

3. Result

The total bacterial count of milkfish product has been shown in Table 1. The total bacterial count in the milkfish product in all treatments continued to increase during storage for up to 96 hours at room temperature. The results showed that the total bacterial count in treatment D (honey concentration 30%) and treatment E (0.1% sodium benzoate) in up to 72 hours storage was 2.3×10^5 colonies/gram and 9.3×10^4 colonies/gram respectively. According to the National Standardization Agency of Indonesia [4], the maximum limit of microbial contamination in fishery products is 5.0×10^5 colonies/gram.

Table 1. The average of the total bacterial count (colonies/gram) of the milkfish product

Duration of storage	Treatment				
	A	B	C	D	E
0 jam	$1,3 \times 10^2$	$8,5 \times 10^1$	$7,5 \times 10^1$	$4,5 \times 10^1$	$2,0 \times 10^1$
24 jam	$3,9 \times 10^3$	$8,1 \times 10^2$	$5,8 \times 10^2$	$3,5 \times 10^2$	$5,7 \times 10^2$
48 jam	$2,6 \times 10^6$	$8,5 \times 10^4$	$6,6 \times 10^4$	$3,0 \times 10^4$	$2,6 \times 10^4$
72 jam	$6,9 \times 10^7$	$3,9 \times 10^6$	$8,0 \times 10^5$	$2,3 \times 10^5$	$9,3 \times 10^4$
96 jam	$1,9 \times 10^9$	$2,5 \times 10^7$	$7,3 \times 10^6$	$3,5 \times 10^6$	$2,6 \times 10^6$

Description: A: without soaking honey (control), B: 20% concentration of the honey solution, C: 25% concentration of the honey solution, D: 30% concentration of the honey solution, E: 0,1% sodium benzoate solution

The organoleptic value of the milkfish product as determined by 30 untrained panelists has been shown in Table 2.

Table 2. The average result of the organoleptic value of the milkfish product

Duration of storage	Treatment				
	A	B	C	D	E
0 jam	8,08	8,08	8,14	8,24	8,09
24 jam	7,90	7,85	7,95	8,03	7,96
48 jam	6,10	7,35	7,51	7,81	7,50
72 jam	4,70	6,91	7,17	7,45	7,03
96 jam	3,67	5,49	6,45	7,01	6,58

Description: A: without soaking honey (control), B: 20% concentration of the honey solution, C: 25% concentration of the honey solution, D: 30% concentration of the honey solution, E: 0,1% sodium benzoate solution

The results of the organoleptic test of the milkfish product showed that treatment D (honey concentration 30%) was the best treatment because it was able to maintain the average organoleptic value of the milkfish product at room temperature during for up to 96 hours with a value 7.01. Nevertheless, the safe limit of the milkfish product consumption with a 30% honey concentration was at 72 hours storage at room temperature with the average organoleptic value being 7.45. The pH values and water content of the milkfish products have been shown in Tables 3 and 4.

Table 3. The average pH value of the milkfish product.

Duration of storage	Treatment				
	A	B	C	D	E
0 jam	6,05	5,65	5,65	5,60	6,00
24 jam	6,06	5,73	5,70	5,65	6,03
36 jam	6,33	5,80	5,80	5,66	6,08
72 jam	6,35	5,85	5,85	5,83	5,88
96 jam	7,33	5,90	5,98	5,88	5,88

Description: A: without soaking honey (control), B: 20% concentration of the honey solution, C: 25% concentration of the honey solution, D: 30% concentration of the honey solution, E: 0,1% sodium benzoate solution

Table 4. The average water content (%) of the milkfish product.

Duration of storage	Treatment				
	A	B	C	D	E
0 jam	62,03	41,93	41,48	40,42	62,23
24 jam	62,58	43,05	43,22	42,30	62,43
36 jam	63,09	44,98	45,46	43,95	63,69
72 jam	64,06	47,86	47,76	45,23	64,86
96 jam	64,88	53,97	51,95	49,09	65,19

Description: A: without soaking honey (control), B: 20% concentration of the honey solution, C: 25% concentration of the honey solution, D: 30% concentration of the honey solution, E: 0,1% sodium benzoate solution

4. Discussion

The results showed that honey with 30% concentration had the ability to inhibit the growth of the microorganisms during the storage of the milkfish product for 72 hours at room temperature as well as when using the chemical preservative of 0.1% sodium benzoate (Table 1). However, the organoleptic tests showed that the use of 30% honey concentrations had a higher organoleptic mean value compared with 0.1% sodium benzoate (Table 2).

Some of the researchers reported that the antimicrobial quality of honey is due to its physicochemical properties such as the high sugar reducing content, high viscosity, high osmotic pressure, low pH, low water activity, low protein content, and the presence of hydrogen peroxide [15]. The main antibacterial factor in honey is hydrogen peroxide [16, 17, 18]. According to Libonatti [8], hydrogen peroxide is the result of the action of glucosidase secreted by the hypopharyngeal gland. Glucosidase breaks down glucose into gluconic acid and hydrogen peroxide. Glucosidase activity stops when the condition of acid pH and little water is available, but glucosidase activity is continued when the honey is diluted with water. The extraordinary antimicrobial properties of honey are caused by hydrogen peroxide and gluconic acid [19]. According to Allen [20], the antibacterial activity is because honey contains non-peroxide components such as acidity, osmolarity, flavonoids, phenolic compounds and lysozyme. Non-peroxide antibacterial activity is not sensitive to heat and light and remains intact, even though it can be stored for long periods of time [21].

The use of a 30% honey solution proved to be more effective than the 0.1% sodium benzoate solution for up to 72 hours at room temperature. The research of Ariyani and Yennie [22] proved that chitosan is able to preserve the pindang of layang fish (the fish product is a processed fish through a combination of boiling and salting) in an overpass for 72 hours, while Rasdyta [23]

used coconut shell liquid smoke to preserve fish for 72 hours. Cahyadi [24] argued that the use of natural preservatives is considered to be safer for consumption than chemical preservatives. Excessive use of chemical preservatives and unregulated doses may cause poisoning and preservatives accumulated in the organs can be carcinogenic.

The greatest quality decline was seen in the parameter of pH value in the negative control treatment equal to 7,33. The pH value was the optimum condition for microorganisms to grow. The pH value of the milkfish product soaked in honey solution still has an acidic condition because honey is acidic. Amenu [25] stated that honey has a fairly low pH (3.2-4.5) that allows it to inhibit and prevent bacterial growth at a pH range of 7.2-7.4. The mechanism of action for honey is done by changing the pH of the material that it is added to. Changes in the pH value will inhibit the work of the microorganism enzymes contained in the material and prevent the growth of said microorganisms [24].

The data in Table 4. shows that the moisture content in the milkfish product of each treatment increased during storage at room temperature up to 96 hours. Changes in the moisture content during storage at room temperature are thought to be due to the free water formed as a result of protein degradation by the microorganisms. The water content in the honey solution treatment of 30% was the lowest compared to the other treatments, even with a positive control of 0.1% sodium benzoate solution. The lower content of the water caused the shelf life of the preserved milkfish product using the 30% honey solution to be up to 72 hours (three days) at room temperature with the microbial contamination in the product being less than 5.0×10^5 colonies/gram. According to Winarno [14], the water content in the food ingredients also determines the freshness and durability of the food. The high water content in the food ingredients is a good medium for the growth and activity of the microorganisms [26].

5. Conclusion

The concentration of 30% honey solution was able to maintain the quality of the milkfish product during storage at room temperature for up to 72 hours. The total bacteria was $2,3 \times 10^5$ colonies/gram ($< 5,0 \times 10^5$ colonies/gram) and the average organoleptic value is 7,45, according to the National Standard of Indonesia.

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