



## Analysis of Shelf Life and Antibacterial Actvity of Sumbawa Wild Horse Milk against *Escherecia Coli* and *Staphylococcus Aureus* Bacteria

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**Abstract** — Sumbawa wild horse mik is pure milk produced by a mare which is bred in sumbawa and is a healthy drink that has antibacterial activity and longer shelf life duration without using preservatives. This study aims to analyze the shelf life and antibacterial activity of Sumbawa wild horse milk (SWHM) against *Escherecia coli* and *Staphylococcus aureus* bacteria, with test parameters including water content, fat content, protein content, pH, and testing was carried out on the 15th day and 30th day after milking and storage at room temperature. The results showed that SWHM did not decay or changes in levels that were far different during the storage period of 15-30 days at room temperature, with the results of fat content of 86.182% and 1.6650%, protein content of 2.1624% and 2.2961%, 86.1821% water content and pH 5.21 and 4.00. Antibacterial activity plays more role in inhibiting *Escherichia coli* bacteria compared to *Staphylococcus aureus* with a concentration of 50%, 75%, and 100% with a maximum inhibition of 12.77 mm.

**Keywords** — Antibacterial, Sumbawa wild horse milk, Shelf life

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### I. INTRODUCTION

Sumbawa Wild Horse Milk is one of the pure milk that is milking from a horse farmed by farmer in Sumbawa Island. Milk is nutritious liquid produced by the mammary glands of both humans and animals. Milk contains many compounds needed by the body such as vitamins, minerals, fat, calcium, and protein [1].

Health problems in humans most commonly occur due to infection, one of which is caused by a bacterial infection. The bacteria that cause the most infections are *Escherichia coli* and *Staphylococcus aureus* [2].

One of the most beneficial milks for our bodies comes from the Susu Kuda Liar Sumbawa Wild Horse Milk (SWHM). SWHM is a livestock animal that is widely cultivated in Sumbawa which has many health benefits. Sumbawa Wild Horse Milk can be drunk directly, without going through the pasteurization process and can last up to a month without using preservatives. Based on the research that has been done, it was found that a compound called galactoferrin in milk has excellent antimicrobial activity [3].

Therefore it is important to research on whether SWHM can last a long time without using preservatives and how its nutritional content is stored for one month.

### II. MATERIAL AND METHODS

#### A. Material

The tools used in this study are glass cup (pyrex), pH meter electrode (PHB-4), measuring cup (pyrex), waterbath (B-One), oven (kirin), wattman filter paper (Whatman), flask sokhlet (scott duran), erlenmeyer flask (pyrex), dropper pipette, petri dish, volume pipette (pyrex), autoclave (all american), test tube (pyrex), micropipette (microlit), spatel, calipers (vernier), rod stirrer, analytical balance (fujitsu), incubator (thermo scientific), UV-VIS spectrophotometry (tecan), butyometer bottle (Funke Gerber), kjeldahl flask (Duran flask), selotif (Morris), cotton (Medisoft), and paper disk (Macherey Nagel).

The materials used in this study, as follows wild horse sumbawa's milk, pH 4 and 7 buffer solutions (Merck), aquades, hexane (Merck), NaOH (Merck), H<sub>2</sub>C<sub>2</sub>O<sub>2</sub>H<sub>2</sub>O (Merck), Na thio sulfate (Emsure), HCl (Merck), K<sub>2</sub>SO<sub>4</sub>

(Pundak Scientific), Selenium, blank, mixed indicator bromocresol-red methyl pink (Merck), Agar medium (Merck), Amoxicillyn trihydrate ( unbranded generics), sterile milli-Q, and the *Escherichia coli* (Novapharin atcc 11229) and *Staphylococcus aureus* bacteria (Novapharin atcc 6538).

### B. Methods

The research was carried out in several stages including collecting Sumbawa wild horse milk samples, taken from horse breeders in Dompu Bima Sumbawa, NTB. The manual milking process is using clean hands and the milk has gone through the filtering process and without pasteurization. Physical analysis such as color, aroma, taste and texture were tested. Then carried out measurements of pH, moisture content, fat content using the Soxhlet method, and measuring protein levels using the Kjeldhal method. Sumbawa wild horse milk was tested on the 15th and 30th day after milking to see the comparison of the storage capacity of Sumbawa Wild Horse Milk (SWHM) and nutritional content.

Testing the antibacterial activity SWHM using the disk diffusion method or disc, and after obtaining the results then look at the MIC (Minimum Inhibitory Concentration) of SWHM on bacterial growth. The data obtained were then analyzed, whether SWHM has an inhibition zone or kills bacterial growth.

### III. RESULT AND DISCUSSION

The Sumbawa Wild Horse Milk (SWHM) is milk originating from Sumbawa which is different from horse milk in other areas, where the milk obtained from the mother horse which is released is free to feed itself on land that has grass on a land area of about 5 hectares. SWHM has many benefits such as antibacterial, curing heart disease, hypertension, lungs, bronchitis, typhus, and can be a substitute for breast milk [3].

The collection of samples of milk from the Sumbawa wild horse was carried out in Dompu, Bima, Sumbawa, NTB province. All milk used for this study have the same treatment, which aims to obtain more constant research data results.

In this study, SWHM test was carried out on the shelf life at room temperature, without going through the pasteurization process and without adding preservatives. From the physical analysis test, the results showed that the milk did not appear to be lumpy, cracked, and not damaged, it was just that the taste became a little sour due to the natural fermentation process. This is some the characteristics of damaged milk are such as dirty milk containers, pungent aroma, lumpy or separate texture, and discoloration.

The storage capacity test of Sumbawa horse milk can be seen from the physical analysis testing such as taste, aroma, texture, and color which is then compared with the quality requirements of SNI 01-6054-1999 horse milk. After testing

on the 15th and 30th day of the milked SWHM, the results obtained were in accordance with the SNI horse milk quality requirements where the texture of the liquid SWHM was white, had a strong sour aroma, with a sour taste.

This can occur because horse milk generally contains lactoferrin. Lactoferrin can act as an antibacterial because there are macrophage cells where the phagocyte cells destroy bacteria by binding Fe<sup>3+</sup> so that it can inhibit bacterial growth. Lactoferrin can be obtained from human cholestrum, human tears, cow's milk, horse milk, and saliva, but in different amounts. Based on research, the levels of lactoferrin in horse milk are 9.89%, cow's milk is 8.38%, colostrum is 5-7 mg/mL, breast milk is 1-2 mg/mL, tears 2.2 mg/mL, and saliva 7-10 µL [4].

TABLE 1.

Sumbawa Wild Horse Milk Analysis

No	Analysis	Level	Level	SNI 01-6054-1999
		(%) Day 15 <sup>th</sup>	(%) Day 30 <sup>th</sup>	
1	Water Content	86,1821	86,0093	
2	Fat	1,6582	1,6650	Min. 1,3 %
3	Protein	2,1624	2,2961	Min. 2,0 %
4	pH	5,21	4,00	Min. 3,0

Information: Day 15<sup>th</sup> and Day 30<sup>th</sup> after milking

Measurement of the pH value the milk of the Sumbawa wild horse after the milking was decreased where on the 15th day the pH was 5.21 and on the 30th day the pH was 4.0 This is caused by lactic acid bacteria such as *Streptococcus lactis*, where lactose becomes lactic acid, causing a lower pH [5].

Measurement of moisture content was using the Gravimetric method because the procedure is simple and the data obtained is quite good and accurate. In this measurement, a desiccator is used to help the process of drying, absorbing moisture, and cooling the material before weighing the water-free sample to test the water content [6]. After dried proses in 3 hours, the result water content was constant. From the measurement of the water content there was no significant change on the 15th day the result was 86.1821% and on the 30th day the result was 86.0093%.

Fat is a source of energy that is needed for daily activities and than has function as a solvent for vitamins A, E, D, and K. Determinated fat of Sumbawa wild horse milk in this research used the Soxhlet method, this method was chosen because it uses less solvent, adjustable heating, simple, and the data obtained is more accurate, the analysis of fat content using the Soxhlet method uses n-hexane solvent because it

has stable analysis and is more suitable for food, such as extracting vegetable and soybean oil [7]. The results obtained was not much different, the results obtained on the 15th day was 1.6583%, and on the 30th day the result was 1.6650%. The results obtained from the sample of Sumbawa wild horse milk are in accordance with the SNI requirements, where the quality requirements of Sumbawa horse milk is at least 2.0% [8].

Protein is one of the essential nutrients for the body to build muscle and other body tissues. Based on the Indonesian Ministry of Health's Nutrition Adequacy Rate (RDA), the standard protein adequacy rate for Indonesians is around 56-66 grams per day for women and 62-66 grams per day for men. The test of protein content in SWHM used the Kjeldahl method due to the simpler method for determining protein, the results obtained were not much different where on day 15th the result was 2.1624% and on day 30th the result was 2.2961%. Protein levels in SWHM, the longer the storage has increased due to the fermentation process, which makes protein levels increase where lactic acid bacteria such as *Streptococcus lactis* convert lactose into lactic acid, as happened in the yogurt fermentation process [5].

From the test results of pH, fat content, water content, and protein content on SWHM, there are several parameters that have decreased milk quality, but overall still meet the requirements of the SNI for horse milk. If the result is decrease in the quality of the milk, this may also be due to the length of storage of SWHM and the influence of temperature during storage where the packaging can provide protection against the product from sunlight, dirt, pollution, physical damage and withstand gas transfer, and natural fermentation processes from the Sumbawa Wild Horse Milk.

Antibacterials are drugs that can inhibit or kill the growth of microorganisms, by inhibiting bacterial cell wall synthesis, disrupting the permeability of bacterial cell membranes, and destroying bacterial nucleic acids [3]. Where this research uses Amoxicillin positive control because it has a broad spectrum. However, it is not known whether it only inhibits or can kill bacteria, therefore using amoxicillin. This research was conducted on two bacteria, namely *Staphylococcus aureus* and *Escherichia coli*, where these two bacteria represent gram-positive and gram-negative bacteria.

TABLE 2.

Test of the Inhibition of Milk of the Sumbawa Wild Horse against *Escherichia coli* bacteria ATCC 1229, After Day 15<sup>th</sup>

Sample	Concentration	Inhibition	Average	Information
		Diameter (d/mm)	Diameter (mm)	
C1				

C2					
Control +	100 ppm	13,10	13,10	13,10	Activated *
Control -		6,00	6,00	6,00	Inactivated
	12,5%	6,00	6,00	6,00	Inactivated
	25%	6,00	6,00	6,00	Inactivated
SWHM	50%	8,60	8,60	8,60	Activated
Testing Result after Increasing Concentration Level and After Day 30 <sup>th</sup>					
Control +	100 ppm	12,70	12,85	12,77	Activated*
Control -		6,00	6,00	6,00	Inactivated
	50%	7,45	8,90	8,17	Activated
SWHM	75%	10,10	9,30	9,70	Activated
M	100%	12,70	12,85	12,77	Activated
Information:					
* : Activated, can inhibited and killed bacteria					
Control + : Amoxicillin					
Control - : Disc Paper 6 mm using miliQ					
C1 : 1st Cup					
C2 : 2nd Cup					

TABLE 3.

Test of the Inhibition of Milk of the Sumbawa Wild Horse against *Staphylococcus aureus* Bacteria ATCC 6538, After Day 15<sup>th</sup>

Sample	Conc.	Inhibition	Average	Information	
		Diameter (d/mm)	Diameter (mm)		
C1 C2					
Control +	100 ppm	32,30	31,75	32,03	Activated*
Control -		6,00	6,00	6,00	Inactivated
	12,5%	6,00	6,00	6,00	Inactive
SWHM	25%	6,00	6,00	6,00	Inactivated
	50%	8,00	6,45	7,23	Activated

Testing Result after Increasing Concentration Level, After Day 30 <sup>th</sup>					
Contol					
+	100 ppm	33,85	31,75	32,8	Activated*
Contro					
1-		6,00	6,00	6,00	Inactivated
	50%	6,00	6,00	6,00	Inactivated
SWH					
M	75%	6,00	6,00	6,00	Inactivated
	100%	6,00	6,00	6,00	Inactivated

Information:

\* : Activated, can inhibited and killed bacteria

Control + : Amoxicillin

Control - : Paper Discs 6 mm using miliQ

C1 : 1st Cup

C2 : 2nd Cup

In this table, it can be seen that the SWHM sample is more active in *Escherichia coli* bacteria than *Staphylococcus aureus* bacteria. *Staphylococcus aureus* bacteria are more active at a concentration of 50% in the first test compared to 50% in the second test at a concentration of 75% and 100%, this is possible because of the long storage time of SWHM at room temperature because the fermentation process continues to be contaminated by bacteria during storage and content levels Water in SWHM affects the inhibitory power of the *Staphylococcus aureus* bacteria, which cannot survive at a water content of less than 17%, but the milk content is greater, causing *Staphylococcus aureus* bacteria to survive [9].

Base on this research, on the 15th day of initial testing using concentrations of 12.5%, 25%, and 50% but the result obtained is not maximal (optimal) and the concentrations did not actively inhibit bacterial growth. Therefore, on the 30th day an increase was made with concentrations of 50%, 75%, and 100% this aims to see maximum result on the antibacterial activity of Sumbawa wild horse milk.

#### SUMBAWA WILD HORSE MILK ANTIBACTERIAL ACTIVITIES

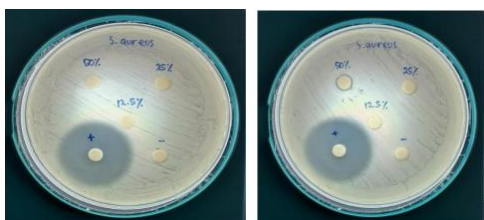


Figure 1. Inhibitory Activity of *Staphylococcus aureus* Bacteria

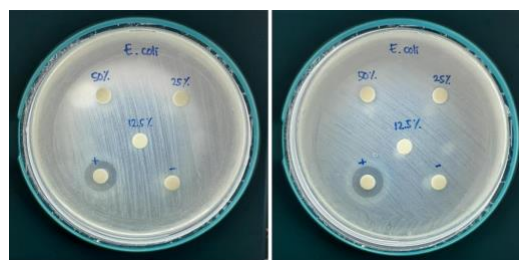


Figure 2. Inhibitory Activity of *Escherichia coli* Bacteria

*Escherichia coli* bacteria can be inhibited at a concentration of 50%, 75%, and 100% where a diameter of 8.60 mm and 8.17 is obtained at a concentration of 50% obtained 9.70 mm at a concentration of 75%, and 12.77 at a concentration of 100%, where the inhibitory power obtained is almost close to the positive control where amoxycillin is 100 ppm with a diameter of 13.10 this causes the best inhibition power is at a concentration of 100%. Sumbawa wild horse milk has antibacterial activity which plays a greater role in inhibiting *Escherichia coli* bacteria compared to *Staphylococcus aureus*.

#### IV. CONCLUSION

Based on the research, it can be concluded that Sumbawa wild horse milk does not experience any damage or changes in levels that are much different in the storage period of 15<sup>th</sup>-30<sup>th</sup> day at room temperature where the results of fat content are 1.6582% and 1.6650% (SNI min. 3%), protein content 2, 1624% and 2.2961% (SNI min. 2%), and pH (SNI min. 3) and 86.1821% moisture content. The antibacterial activity of Sumbawa wild horse milk has a more inhibiting role in *Escherichia coli* bacteria compared to *Staphylococcus aureus* bacteria. The best concentration of inhibitory is 100%, with a maximum inhibition of 12.77 mm.

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