Open Access

Int. J. Trop. Vet. Biomed. Res.
Vol. 7 (2): 15-19; November 2022

www.jurnal.unsyiah.ac.id/IJTVBR
E-ISSN : 2503-4715

Detection of Reductase and Catalasse Enzymes in Goats Milk (*Capra aegaggrus hircus*) Sold in Banda Aceh

Andi Novita[1*], Aulia Nuddi Yanti Putri[2], Herialfian[3], M. Isa[3], T.Armansyah TR[4], M.Hasan[5]

[1]*Laboratory of Veterinary Public Health, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia*
[2]*Bachelor of Veterinary Medicine, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia*
[3]*Laboratory of Biochemistry, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia*
[4]*Laboratory of Pharmacology, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia*
[5]*Laboratory of Clinic, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia*
**Corresponding author: andinov@unsyiah.ac.id*

Abstract

This study aims to determine of reductase and catalase enzymes in pasteurized goat's milk sold in Banda Aceh. The sample used was pasteurized goat's milk which was sold in Banda Aceh as many as 30 samples were taken by census. The research method used is a survey study method with a cross-sectional approach. Sample testing using reductase test and catalase test. The results showed that the reductase time of 30 samples of pasteurized goat's milk averaged 3-5 hours and the catalase number of 30 samples of pasteurized goat's milk was below 3 ml. Based on the results, it shows that there are reductase enzymes and catalase enzymes in goat's milk and have good quality in Banda Aceh.

Keywords: Goat's milk, reductase enzyme, catalase enzyme

Background

Milk is a food ingredient that has high nutritional value because it contains complete and balanced food substances such as proteins, carbohydrates, fats, vitamins, and minerals needed by humans (Susilawati *et al*., 2013). One of the producers of animal protein other than cow's milk is goat's milk. Goat milk is known and trusted by the public to have benefits for good body health due to the high content of nutrients that maintain a healthy body (Anggreani *et al*., 2019). The advantage of goat's milk is that it does not contain -lactoglobulin or compounds that can trigger allergic reactions such as respiratory tract disorders, red effects on the skin, and digestive disorders (Olsen *et al*., 2021).

The content of high nutritional value causes milk to be the preferred medium for microorganisms (Sholikah *et al*., 2021). According to Harjanti *et al*. (2020) Milk is very susceptible to contamination, besides being caused by bacterial contamination from the environment, milk is also easily contaminated by the number of somatic cells originating from intra-mammary inflammation, known as mastitis. The quality of milk will decrease if there are spoilage bacteria in it (Arini and Ifalahma, 2019). The quality of milk can be seen by the composition of the milk and the physical state of the milk. The reductase time and catalase number is an examination of the state of milk which is useful for determining the presence of microorganisms in milk (Rahmawati, 2018).

The reductase enzyme in milk is formed by microorganisms, which can reduce the methylene blue dye to become colorless, the faster the methylene blue disappears, indicating that the milk contains a lot of microorganisms. Bacterial growth is strongly influenced by environmental factors, including temperature, pH, oxygen and the availability of food substances (Sari *et al*., 2013). The principle of this reductase test is the color change of methylene blue by

microbial activity. The color of methylene blue will be changed to white by microbes in milk (Puspitarini and Inggit, 2015).

Catalase is an enzyme found in various foods, one of which is found in milk. The catalase enzyme can cause milk to spoil quickly (Nurlita and Anwarudin, 2019). The higher the number of catalases found in milk, the higher the bacterial population found in milk. The presence of the catalase enzyme is indicated by the emergence of oxygen bubbles due to the breakdown of hydrogen peroxide into water and oxygen (Pupitasari *et al*., 2018). The catalase enzyme that breaks down H_2O_2 occurs during respiration, where bacteria form a defense system from the toxic H_2O_2 they produce themselves (Bulu *et al*., 2019).

To reduce the risk of damage to goat's milk, in addition to milking milk from clean and healthy udders and clean cage sanitation, post-milking handling must also be considered, especially in storage. One way to overcome this problem is heating milk by pasteurization (Oslen *et al*., 2021). Milk pasteurization is the act of pasteurizing milk at a predetermined temperature and time (Prasetyo, 2020). There are two processes of pasteurization or heating, namely at a temperature of 62ºC for 30 minutes, or heating at a temperature of 72ºC for 15 seconds (Hayuningtyas *et al*., 2019).

Pasteurization cannot kill non-pathogenic bacteria, especially spoilage bacteria. To extend the shelf life of pasteurized milk, the cooling method is carried out at a maximum temperature of 10°C. Spoilage microbes cannot grow and develop at a temperature of 3 – 10°C (Sholikah *et al*., 2021). Pasteurization in a short time serves to prevent damage or maintain the nutritional value of milk, organoleptic properties, and obtain commercially sterile food products.

Pasteurization with temperatures that are not properly controlled still exists so that the applied temperature does not match the required temperature. Milk that is too ripe or still contains live microbes in the product will eventually occur if the temperature is too high or too low (Rahmawati, 2018). Therefore, it is necessary to study the quality of pasteurized goat's milk sold in Banda Aceh and its surroundings in terms of the reductase and catalase enzymes from the milk.

Materials and Method

Research on the detection of catalase and reductase enzymes in goat milk sold in Banda Aceh and its surroundings was carried out at the Veterinary Public Health Laboratory, Faculty of Veterinary Medicine, Universitas Syiah Kuala. This research will be conducted in April 2021. The sample used in this study was goat milk sold in Banda Aceh and its surroundings as many as 30 samples were taken by census. The tools used in this research are reductase tube, catalase tube, dropper pipette, incubator. The materials used in this study were 10 bottles of goat's milk sold in Banda Aceh, Methylene blue solution and 0.5% H_2O_2 solution. The research method used is a cross sectional study. Sampling was in the form of purchasing goat's milk which was sold in Banda Aceh and its surroundings, then taken to the laboratory using an ice box and tested at the Veterinary Health Laboratory, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh.

The catalase test was carried out in the Veterinary Public Health Laboratory, Faculty of Veterinary Medicine, Universitas Syiah Kuala as much as 60 mL was inserted into a catalase tube. Added 30 mL of 0.5% Hydrogen Peroxide into it, homogenized by inverting the tube. Milk is placed in the vertical part of the tube that has a scale at the top, so that there are no air bubbles at the top. The tube was plugged with cotton, put in an incubator at 37ºC for 3 hours. Milk is said to be good if the volume of O_2 gas collected is a maximum of 3 mL.

The reductase test was carried out in the Veterinary Public Health Laboratory of the Faculty of Veterinary Medicine, Universitas Syiah Kuala. The goat milk was taken and put into a reductase tube that had been sterilized and each 0.5 ml of methylene blue solution was filled into the tube using a 0.5 ml pipette. The reductase tube was plugged with aluminum foil and tied with a rubber band. Then back and forth until the blue color is evenly distributed. Then it was incubated in an incubator at 37°C. Every

half hour the treatment was checked for color changes. The reductase number is determined based on the time (hours) when the methylene blue color changes to colorless. The data obtained were analyzed descriptively.

Results and Discussion

The results of the reductase time and catalase numbers in Table 1 show the presence of reductase and catalase enzymes in normal pasteurized goat's milk. The microbes in milk principally produce reductase enzymes that can reduce the blue dye from methyline blue to colorless, when the milk is dripped with methyline blue, the milk will turn blue and change color when there are microbes in the milk (Arini and Ifalahma, 2019).

Table 1. Average reductase time and catalase number of sellers I-VI of goat's milk in Banda Aceh and surrounding areas.

Seller	Average reductase time	Average catalase number
I	5 hours	0,16 ml
II	4,6 hours	0,36 ml
III	3,3 hours	0,56 ml
IV	3,6 hours	0,85 ml
V	3,7 hours	0,59 ml
VI	3,2 hours	0,65 ml
Overall average	3,9 hours	0,52 ml

According to Umar *et al*. (2014) milk quality is acceptable if the blue color disappears for more than 2 hours and less than 6 hours with the number of bacteria per ml is 4,000,000 - 20,000,000. The color change of methylene blue in milk is influenced by the number of bacteria in milk (Sari *et al*., 2013).

The principle of the reductase test is based on the ability of the bacteria present in milk to grow using dissolved oxygen, thereby reducing the oxidation-reduction strength of the mixture. So that the added methylene blue will be reduced to white (Arini and Ifalahma, 2019). From many studies, it turns out that there is a relationship with the number of bacteria with the amount of reducing power in milk. Therefore, the reduction power test is used as one of the procedures to determine the quality of fresh milk and pasteurized milk. Storage time treatment has a very significant effect on milk quality. There is an interaction between pasteurization time and storage time on milk quality.

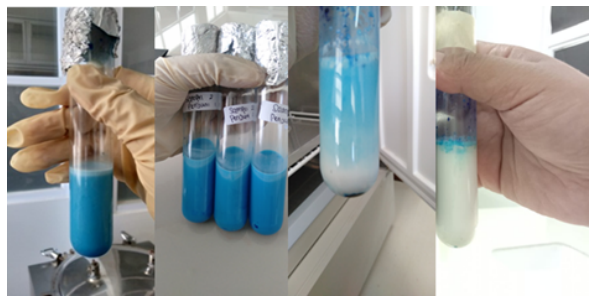

Figure 1: The results of the reductase test indicated by a color change from blue to colorless

The longer it is stored, the faster the process of changing from blue to white. This is presumably because the longer the milk is stored, the greater the number of bacteria in the milk, so the ability to reduce methylene blue is faster (Asmaq and Marisa, 2020). Organisms that grow in milk produce available oxygen and when oxygen is depleted, an oxidation-reduction reaction occurs for microbial survival (Puspasari *et al*., 2018).

To determine the ability of bacteria to produce the enzyme catalase. The presence of the catalase enzyme is indicated by the emergence of oxygen bubbles due to the breakdown of hydrogen peroxide into water and oxygen (Puspasari *et al*., 2018). The catalase enzyme that breaks down H_2O_2 occurs during respiration, where bacteria form a defense system from the toxic H_2O_2 they produce themselves (Anastiawan, 2014). Catalase enzyme is thought to be important for aerobic growth because H_2O_2 formed with the help of various respiratory enzymes is toxic to microbial cells (Putri *et al*., 2018).

According to Anastiwan (2014), the process of the catalase enzyme breaks down H_2O_2, namely during respiration, bacteria produce various components, one of which is H_2O_2. Bacteria that have the ability to break down H_2O_2 with the catalase enzyme immediately form a defense system from the toxic H_2O_2 they produce themselves. Positive catalse bacteria will break down

H_2O_2 into H_2O and O_2 where the parameter indicating the presence of catalase activity is bubbles in the form of oxygen bubbles. Catalase negative bacteria do not produce these bubbles. This means that the given H_2O_2 is not broken down by catalase negative bacteria so that it does not produce oxygen. Catalase negative bacteria do not have the enzyme catalase that can break down H_2O_2.

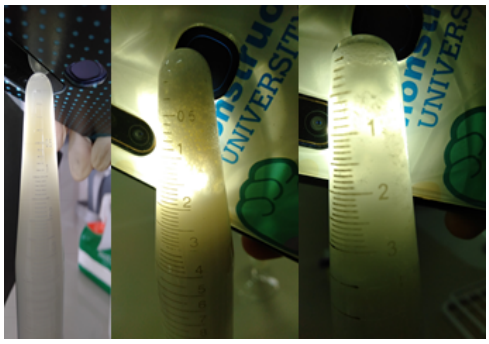

Figure 2: Catalase test results indicated by the presence of air bubbles at the end of the catalase tube.

Bacteria in milk can come from the milk itself or due to bacterial contamination from outside, as well as due to milking techniques and the use of unclean tools (Sari *et al*., 2013). Catalase enzyme to break down hydrogen peroxide into water and oxygen which is characterized by the formation of oxygen gas bubbles. (Goyal, 2012).

Conclusion

Based on the results of the study, it can be concluded that the presence of reductase and catalase enzymes in normal conditions in goat's milk sold in Banda Aceh and its surroundings from six sellers with an average reductase time of 3-5 hours and catalase numbers below 3 ml.

References

Anastiawan. 2014. Isolation and characterization of probiotic bacteria derived from intestines of broiler ducks *Anas domesticus*. Thesis. Department of Biology, Faculty of Mathematics and Natural Sciences, Hasanuddin University.

Anggreani, Y. D. A., N. P. Mariani and I. A. Okarini. 2019. Physicochemistry and microbiology of Etawah crossbreed goat milk during storage at room temperature. *Journal of Tropical Animal Science*. 7(1):9-20.

Arini, L.D.D and Ifalahma, D. 2019. Examination of the total number of bacteria in fresh cow's milk from a cattle farm in the Kalijambe area of Sragen. *Journal of Health*. 8(2): 128 - 139.

Asmaq, N and Marisa, J. 2020. Physical and organoleptic characteristics of fresh milk in single field. *Indonesian Journal of Animal Husbandry*. 22(2): 168-175.

Bulu, S., M.E.S. Ledo, and A.D.N. Rupidara. 2019. Identification of lactic acid bacteria in fresh palm sap (Borassus flabellifer linn). *Jambura Edu Biosphere Journal*. 1(2):47-52.

Goyal, R. 2012. Characterization of the lactobacillus isolated from different curd samples. *African Journal of Biotechnology*. 11(79): 14448–14452.

Harjanti, D. W., D. Wulandari, R. Hartanto, and A. Muktiani. 2020. Mammary inflammation level and milk stability of subclinical mastitis cows receiving herbal supplements and Zn-Se proteinate. *Livestock and Animal Research*. 18(1): 132 - 141.

Hayuningtyas, A.B., N. Ibrahim, and E. Wulandari. 2019. Quality detection of cow's milk purity using digital image processing method binary large object and learning vector quantization classification. *e-Proceeding of Engineering*. (2): 1-7.

Nurlita, Y and Anwarudin, W. 2019. The formulation of antioxidant candy preparations from mangosteen rind extract (*Garcinia mangostana* l.) and cow's milk. *Journal of Herbal and Pharmacological*, 1(1): 1-6.

Olsen, E., A. Qisthon, V. Wanniatie, and A. Husni. 2021. The degree of acidity and reductase rate of pasteurized ettawa crossbreed goat milk with different shelf life at 4°C refrigerator temperature. *Journal of Animal Husbandry Research and Innovation*. 5 (2): 114-118.

Prasetyo, M. S. 2020. Heat transfer analysis of milk pasteurizer. *Nusantara Machinery Journal*. 3(1): 1-8.

Puspasari, E.R., Yanuartono, S. Hartati, S. Rahardjo, A. Nururrozi, and S.

Indarjulianto. 2018. Isolation and identification of *Staphylococcus epidermidis* in the milk of PFH cows with subclinical mastitis in Wukirsari, Cangkringan, Sleman, DIY. *Journal of Animal Sciences*. 28 (2): 121 – 128.

Puspitarini, O.R and Inggit, I. 2015. Effect of storage time in the refrigerator on the quality of pasteurized goat's milk. *Dynamics of Animals*. 1(1):41-44.

Putri, A.A., Erina and Fakhrurrazi. 2018. Isolation of lactic acid bacteria of the genus *Lactobacillus* from faeces of sambar deer (*Cervus unicolor*). *JIMVET*. 2(1): 170-176.

Rahmawati, Y.N. 2018. Commercial sterile food analysis using f0 factor to achieve food safety in UHT milk processing at PT. Ultrajaya. Thesis. Pasundan University Bandung.

Sari, M., B.N.S. Ida, and K.A. Kadek. 2013. The quality of milk of post-thawing etawah crossbreed goats in terms of reductase time and catalase number. Indonesia Medicus *Veterinus*. 2(2): 202-207.

Sholikah, N., A.A. Mufid, A.S. Bachrul, T.R. Hidayat, and Y. Yoga. 2021. Processing cow's milk into pasteurized milk to increase milk value and marketability. *Journal of Community Empowerment Learning*. 2(1): 75-79.

Susilawati, T., S. B. M. Abduh and S. Mulyani. 2013. Reduction of bacteria and methylene blue as well as changes in browning intensity and PH of milk due to heating at 80°C in various periods. *Animal Agriculture Journal*. 2(3): 123-131.