EFFECT OF RENNET ENZYME AND PINEAPPLE EXTRACT (*Ananas comosus*) TOWARDS CLOTTING TIME, pH, TPC, AND CALCIUM CONTENT IN SUBCLINICAL MASTITIS MILK

UNDERGRADUATE THESIS

By:
Asiah Putri Humairo
SIN. 175050101111022

ANIMAL SCIENCE PROGRAM
FACULTY OF ANIMAL SCIENCE
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This undergraduate – thesis is one of the requirements to obtain a bachelor's degree in faculty of Animal Science, Universitas Brawijaya

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UNIVERSITAS BRAWIJAYA

BIOGRAPHY

The author, Asiah Putri Humairo, was born in Balikpapan on September 22, 1999. The first of 2 children of Mr. M. Araya and Mrs. Anna Nurhayati. In 2011, the author graduated from SDIT - ISTIQAMAH Balikpapan, after which I continued to Junior High School at SMPIT - ISTIQAMAH Balikpapan and graduated in 2014 and then continued High School Education at SMA PATRA DHARMA Balikpapan in 2014 and graduated in 2017. In 2017, the author was accepted at the Faculty of Animal Husbandry, Brawijaya University through the SNMPTN route.

During her time as a student, the author joined the English Class Program. The author is also an active member of the Brawijaya Archery Student Activity Unit and the PENA FAPET UB Student Activity Unit. The author carried out Field Work Practices in Thailand at the Chiang Mai Fresh Milk Farm dairy farm in November - December 2018 and wrote a Field Work Practice report entitled "Management of Dairy Cattle Farm in Chiang Mai Fresh Milk Farm, Ban Hong, Lamphun, Thailand" with Prof. Dr. Ir Lilik Eka Radiati, MS., IPU. as internship supervisor.

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EFFECT OF RENNET ENZYME AND PINEAPPLE EXTRACT (*Ananas comosus*) TOWARDS CLOTTING TIME, pH, TPC, AND CALCIUM CONTENT IN SUBCLINICAL MASTITIS MILK

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ABSTRACT

This purpose of this research was to observe the effect of rennet enzyme and pineapple extract towards coagulation time, pH, microbial count, and calcium content in subclinical mastitis milk. This research used an experimental method with Nested Completely Randomized Design (CRD) with 7 treatments and 2 replications. The treatments were namely T0 (subclinical mastitis milk), T1 (subclinical mastitis milk with 1% of rennet enzyme and 1% of pineapple extract), T2 (subclinical mastitis milk with 2% of rennet enzyme and 2% of pineapple extract), T3 (subclinical mastitis milk with 4% of rennet enzyme and 4% of pineapple extract), T4 (subclinical mastitis milk with 1% of pineapple extract), T5 (subclinical mastitis milk with 2% of pineapple extract), and T6 (subclinical mastitis milk with 4% of pineapple extract). The data obtained are analyzed using Analysis of Variance (ANOVA) and followed by Duncan's Multiple Range Test (DMRT). The result showed that the effect of adding rennet enzyme and pineapple

extract towards coagulation time showed significant difference (P<0,01). On pH showed difference (P<0,05) towards the different subclinical mastitis milk used and also showed significant difference (P<0,01) towards treatments. While no difference between milk nor treatments for microbial count. For calcium content between treatments showed significant differences (P<0,01) with increasing amount according to concentrations used for based on each treatment. Suggestions for further research is to know what can effect rennet enzyme and pineapple extract on subclinical mastitis milk towards color changes that can be turned into an indicator.

Keywords: *subclinical mastitis, pH, TPC, clotting, rennet enzyme, pineapple extract*

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SUMMARY

Milk is a white liquid obtained from milking cows or other mammals that can be consumed or used as a healthy food ingredient. Milk is a source of food that is needed for the growth and development of the body as well as in maintaining a healthy body. Cow's milk is a food that has high nutritional value, such as protein, carbohydrates, fats, minerals and vitamins which are very beneficial for human. Milk is counted as perishable food or easily damaged. The perishable nature of milk makes it necessary to pay attention on how to handle milk before and after milking. One of the causes of low production and quality of milk for dairy cows is from the health aspect, namely the presence of mastitis.

Mastitis is a disease caused by various types of bacteria including *Streptococcus agalactiae, Staphylococcus aureus, Pseudomonas aeruginosa*. These bacteria attack the udder causing inflammation and damage to the alveoli cells. Based on clinical symptoms, mastitis is classified into 2 types, namely clinical mastitis and sub clinical mastitis. Clinical mastitis is mastitis that shows symptoms through abnormalities in the physical quality of the milk, swelling of the udder, udder that is

hot when touched, reddish udder color, decreased appetite and cattle that look uncomfortable or painful when milked. Meanwhile, sub clinical mastitis does not show any detectable symptoms such as no visible abnormalities in the physical milk or udder.

The purpose of this research was to determine the percentage of rennet enzyme and pineapple extract added to show which have a significant effect on subclinical mastitis milk on pH, clotting rate, the number of microbes and calcium content.

The method used was experimental research with the basic principles of making cheese is to agglomerate milk. Data obtained from direct observation. Data processing used the experimental method nested completely randomized design (CRD) with 2 types of subclinical mastitis milk, 7 treatments and 2 replications and continued by Duncan's Multiple Range Test if there was any significant difference. The variables tested included pH, coagulation time, Total Plate Count (TPC) and calcium levels. The treatments used were rennet enzyme and pineapple extract given with specific concentration according to the treatments. The result showed that the subclinical mastitis milk given treatments had highly significant effect ($P < 0,01$) on coagulation time, pH value and calcium content. Microbial count using Total Plate Count (TPC) method in treated milk showed a non – significant result. Suggestion for further research, to know the detailed reaction on how the rennet enzyme and pineapple extract affect the subclinical mastitis milk.

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LIST OF ABBREVIATION

CMT	: California Mastitis Test
CRD	: Complete Randomized Design
ANOVA	: Analysis of Variance
DMRT	: Duncan's Multiple Range Test
pH	: Potential of Hydrogen
TPC	: Total Plate Count
AAS	: Atomic Absorption Spectrophotometre
CFU	: Colony Forming Unit
g	: Gram
kg	: Kilogram
l	: Liter
ml	: Millilitre
mg	: Milligram
%	: percent

CHAPTER I
INTRODUCTION

1.1. Background

Milk is a white liquid obtained from milking cows or other mammals that can be consumed or used as a healthy food ingredient. Milk is a source of food that is needed for the growth and development of the body as well as in maintaining a healthy body. Cow's milk is a food that has high nutritional value, such as protein, carbohydrates, fats, minerals and vitamins which are very beneficial for human. The level of fulfillment of milk in terms of quantity is still very low, as evidenced by the level of domestic production in 2009, namely 827.2 tones / year and requires imports of 173,305, 30 tonnes / year (Directorate General of Animal Husbandry and Animal Health, 2011; Riyanto, 2016).

Milk is counted as perishable food or easily damaged. The perishable nature of milk makes it necessary to pay attention on how to handle milk before and after milking. One of the causes of low production and quality of milk for dairy cows is from the health aspect, namely the presence of mastitis. Mastitis is a disease caused by various types of bacteria including *Streptococcus agalactiae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*. These bacteria attack the udder causing inflammation and damage to the alveoli cells. Damage caused by mastitis results in decreased milk production, decreased milk quality and also greatly affects economic value. Decrease in milk quality is a disorder that occurs in milk due to bacterial contamination that causes mastitis which destroys the nutritional composition of the milk. Meanwhile in Indonesia, it was reported that mastitis resulted in a decrease in milk

production by up to 25% of total milk production (Ministry of Agriculture, 2004; Adriani, 2010). Based on clinical symptoms, mastitis is classified into 2 types, namely clinical mastitis and sub clinical mastitis. Clinical mastitis is mastitis that shows symptoms through abnormalities in the physical quality of the milk, swelling of the udder, udder that is hot when touched, reddish udder color, decreased appetite and cattle that look uncomfortable or painful when milked. Meanwhile, sub clinical mastitis does not show any detectable symptoms such as no visible abnormalities in the physical milk or udder. According to Pratomo *et al*., (2013) sub clinical mastitis in Indonesia reaches 97% of all mastitis. Subclinical mastitis is considered more dangerous because it has no known symptoms and also causes very high losses. Another disadvantage caused by sub-clinical mastitis is the increase in production costs for treatment, sometimes cattle affected by sub-clinical mastitis have to be removed earlier from the farm because of the higher maintenance costs of their production.

The California Mastitis Test (CMT) is the only method for detecting sub-clinical mastitis that can be used outside the cow's body (Pratomo *et al*., 2013). This method is currently considered the simplest and fastest method using a device called a paddle and by using a specific reagent that can determine the severity of subclinical mastitis experienced. Reagent mixed with milk which is placed on 4 parts of the paddle as representative of each quarter, after being homogenized for 10 - 15 seconds, it will show changes in the physical milk according to the severity of mastitis.

Rennet enzyme is one of the ingredients used to coagulate casein. This material is obtained in the form of powdered, liquid or flour rennet, which is made from the

abomasum of ruminants. Rennet enzyme which is often called renin is a substance used to coagulate milk in the initial process of making cheese. The addition of enzymes carried out in cheese making aims to reduce the pH to 4.5 - 5.4 where this pH is the isoelectric point of casein milk (Budiman *et al*., 2017). According to Ginting (2015), this enzyme is the most stable; in the pH range 5.3 - 6.3 but has the best relative stability at pH 2.0. Renin has a very high resistance to acids. Renin can maintain its activity at pH 1.6. However, at a high pH, renin undergoes irreversible conformational changes. The enzyme renin is used in the cheese-making process, namely in the milk clotting stage. The enzyme renin that comes from animals is also known as renin extract. Renin extract from calves is effective against cow's milk, so is renin from lambs effective against sheep's milk. An enzyme that is also often used to coagulate milk in addition to the renin enzyme is to add acids. One source of acids that can be easily found is acid derived from fruits, one of which is pineapple (Ananas comosus). In pineapple there is an acid, namely citric acid which contains the enzyme bromelain. Bromelain enzymes can be found in all parts of pineapples such as fruit, stem, crown and skin (Komansilan *et al*., 2019).

1.2. Problems

How is the effect of rennet enzyme and pineapple extract given with certain concentrations on subclinical mastitis milk towards pH, clotting rate, microbial count and calcium levels in mastitis cow's milk.

1.3. Objectives

The purpose of this research was to determine the percentage of rennet enzyme and pineapple extract added to show which have a significant effect on subclinical mastitis milk on pH, clotting rate, the number of microbes and calcium content.

1.4. Advantages

The results of this study are expected to provide information for the public, especially dairy farmers, on the detection method for subclinical mastitis in dairy cattle using rennet enzyme and pineapple extract and can be used as research material for future researchers.

1.5. Conceptual Research Framework

Milk is a white, nutritious liquid produced by mammalian glands. Milk is the main source of nutrition for newborn livestock before it can digest solid food. Fresh cow's milk is a good food material for humans and bacteria. Bacteria that contaminate milk can develop quickly. Navyanti and Adriani (2015) stated that to obtain good fresh milk, all efforts must be aimed at reducing the number of bacteria present in milk by paying attention to several factors that can affect the quality of the milk, for example sanitation and cleanliness of enclosures, animal health and hygiene, health and cleanliness of the milkers and cleanliness of milking equipment. National milk production has not been able to meet domestic milk needs because national milk demand in terms of quantity may be fulfilled but in quality it has not been able to meet the desires of milk producers and consumers, so that domestic milk production can only be accepted as much as 40% while the other

60% is fulfilled by imported milk. The inability to meet the demand for milk is due to the low productivity of Indonesian dairy cows both in terms of quantity and quality (Rosena, 2020; Pratomo *et al*., 2013).

One of the diseases that greatly affects the production, quality and quantity of milk is mastitis. Mastitis is a reaction to inflammation of the udder caused by germs, thermal injury or mechanical injury. inflammation of the udder causes an increase in protein in the blood and white blood cells in the mammary tissue. Mastitis can cause physical, chemical and bacteriological changes in milk and pathology in the mammary gland tissue. The method used to detect mastitis in dairy cows at a low level (sub clinical) is to use the California Mastitis Test (CMT) (Surjowardojo *et al*., 2008). By using the CMT method the results obtained, and the implementation are fast, but not all breeders have CMT test equipment, especially small-scale local dairy farmers. The reagent used in the CMT detection method uses a special reagent containing aryl sulfonate with leukocyte cell DNA which forms a gel mass, so that the agglutination quality or gel consistency that occurs is a picture of the number of leukocyte cells in milk, as a result of the body's response to bacterial infection. The thicker the gel that is formed, the more leukocyte cells in the milk will be (Akers, 2002; Surjowardojo, 2008).

Commercial rennet enzymes are generally obtained from the abomasum or fourth stomach of calves who are still consuming milk. When the calf starts eating other foods besides milk, the renin enzyme will no longer be secreted. This rennet enzyme is a coagulant in casein contained in milk which is used in the initial process of making cheese (Hutagulung, 2017). Rennet enzyme or often referred to as renin or chymosin, is a

proteolytic enzyme that is produced in the stomach of animals. Chymosin has an isoelectric point at a pH of about 4.5. This enzyme is most stable in the pH range 5.3 - 6.3 but is relatively more stable at pH 2.0. Renin is a protease enzyme that can hydrolyze hemoglobin as effectively as pepsin and trypsin. (Ginting, 2015). Because the rennet enzyme is widely used in the food industry, especially cheese processing, the commercial rennet enzyme can be found by consumers easily and affordable. With the same clotting principle as the CMT test method for milk clotting, it is hoped that the renin enzyme can show sensitivity to subclinical mastitis milk so that it can be used as a reagent.

A coagulant that is often used in milk in addition to the rennet enzyme that can be used is the acid contained in fruits, namely citric acid. Pineapple is one type of fruit that can be easily found, in pineapple there is an enzyme that can coagulate milk, which is the bromelain enzyme. Bromelain enzyme can be found in all parts of pineapple so that the use of pineapple extract becomes more efficient and effective.

The research framework is explained in the form of a schematic as shown.

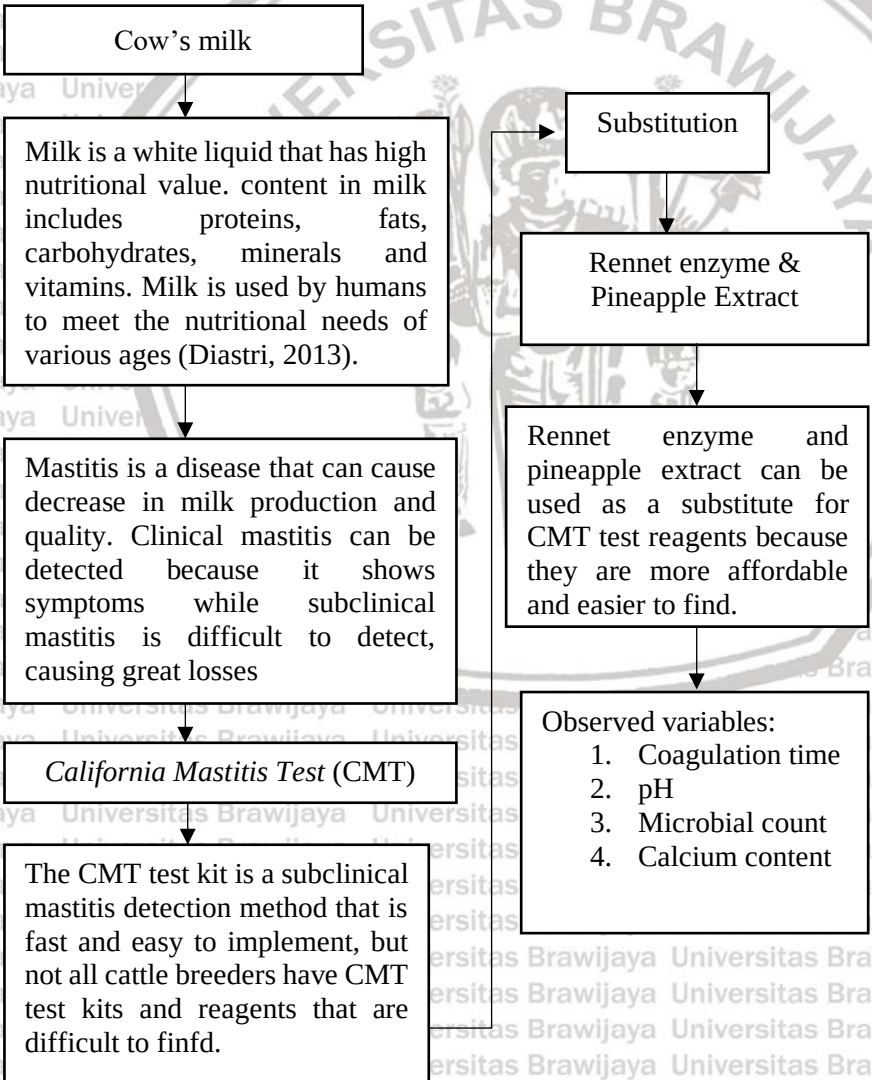

Figure 1. Framework

1.6. Hypothesis

The hypothesis in this study is that rennet enzyme and pineapple extract can show reaction towards subclinical mastitis milk therefore can be used as reagents to indicate subclinical mastitis milk.

CHAPTER II
LITERATURE REVIEW

2.1. Milk

Milk is a source of animal protein needed for human growth and health, because milk contains high quality nutritional value. Milk contains protein, fat, carbohydrates, minerals and vitamins which are substances needed by humans. Milk is a white liquid produced by mammals and obtained by milking. Whole milk has a slightly sweet taste due to the presence of lactose and low Cl levels. (Diastari and Agustina, 2013).

The kinds and numbers of bacteria will be different from different milk groups, according to SNI (2011) the total amount of bacterial contamination is around 1 X 10^6 CFU / ml, in addition to low bacteria, milk must be free of impurities, have a normal odor, and be free from spores and microorganisms that can cause disease. Milk is said to have good quality or A grade quality if it has a number of microbes not more than 10,000 / mL, milk with good quality or quality B has a number of microbes between 100,000 - 1,000,000 / ml, while milk with poor quality or quality C if the number of microbes is more from 1,000,000 / ml. Generally, the low quality of milk is due to the high number of microbes, especially pathogenic bacteria. As a result of the high number of pathogenic microbes resulting in decreased milk quality which can also be caused by factors of poor environmental sanitation, less sterile equipment, dirty cages and lack of cleanliness of udders in the milking process (Khoirani, 2015).

According to SNI No. 31441.1: 2011 concerning the quality requirements of fresh milk, fresh milk

which is good for consumption must meet the requirements in terms of nutritional content as well as food safety. There are conditions for contamination, maximum microbial content in milk, antibiotic residue content and a predetermined maximum metal contamination. To obtain good fresh milk, efforts must be made to minimize the opportunities for microbes to contaminate milk by taking into account several factors that can affect the quality of the milk, such as cage sanitation, worker health and hygiene, cleaning of dairy equipment, maintaining the purity of fresh milk and the most important thing is animal health and hygiene (Navyanti and Adriyani, 2015).

Riyanto et al., (2016) stated that mastitis is classified into two category, namely clinical and subclinical. Clinical mastitis can be detected through the physical appearance of milk such as; milk contaminated by blood, thickened milk and broken. colour abnormalities that include milky white, reddish white and pale white. Clinical mastitis disease will display a difference in the quality of the milk colour to become reddish due to the presence of blood or mixed with pus. Normal milk has an odour that does not change or deviate, which is a characteristic smell of milk. Changes occur from the normal aroma if the bacterial content exceeds the maximum limit, which is more than $1x10^6$ CFU/ml in milk and quality of milk according to pH of normal milk is around 6.3 to 6.8 (SNI,

2011). Milk with pH value more than 6.7 usually comes from cow that is sick, especially mastitis. Subclinical mastitis milk has the pH ranging from 6.3 and 7.2. The study also showed a relationship between the increase in the pH value of milk which was directly proportional to the increase in the degree of mastitis.

2.2. Mastitis

Mastitis is an inflammation that occurs in the internal tissue of the mammary glands or udder which is characterized by physical and chemical changes in milk and is accompanied or without pathological effects of the mammary glands and is a disease that causes a lot of losses in dairy farms around the world. Mastitis can be caused by trauma or physiological disorders, but the economic disadvantage of this disease is often caused by bacterial infections including Staphylococus aureus, Streptococus agalactiae. This bacterium is of particular concern because it is the main pathogen causing mastitis in dairy cows (Pratomo et al., 2013).

Subclinical mastitis is a disease that is very detrimental to the economy of the dairy farming industry. Economic losses such as loss of quality of milk, cow culling problematic and treatment costs. Subclinical mastitis often worries the dairy farming industry, moreover, subclinical mastitis infection in quarters can develop into clinical mastitis which has the potential to increase the number of new infections in livestock (Ayano et al., 2013; Wicaksono and Sudarwanto, 2016).

Mastitis can be divided into two, namely clinical mastitis and subclinical mastitis. Clinical mastitis includes acute and chronic mastitis. Acute mastitis is characterized by swollen udder, heat, redness, pain to touch and changes in function. Cattle do not want to eat while the changes in milk are thick, clotted, the color changes to yellow, reddish or has red spots. whereas chronic mastitis is characterized by cattle that look healthy, udder feels hard when touched and wrinkles like a drooping nipple. Meanwhile, subclinical mastitis is an inflammation of the udder without finding any symptoms in udder and milk such as healthy-looking cattle, normal body temperature, normal looking udder, milk that does not clot and milk color that does not show any change (Suryowardojo, 2012).

2.3. Rennet Enzyme

Enzyme is a protein that acts as a catalyst for biological reactions (biocatalyst). The use of enzymes is currently growing rapidly, especially in the food processing industry, such as the use of the enzyme renin which is used to coagulate milk in the cheese-making process (Mustakim et al., 2012). Renet enzyme or also often referred to as renin or chymosin is a proteolytic enzyme that is produced from the stomach of an animal.

Commercial renin enzymes are generally obtained from the abomasum or fourth stomach of calves that only consume milk from their mother. When calves eat other foods besides milk, the enzyme renin or chymosin will no longer be secreted by the body. Renin is a protease enzyme that can hydrolyze hemoglobin as effectively as pepsin and trypsin. Renin enzyme derived from calves will work effectively against cow's milk (Ginting, 2015).

Chymosin secreted in the abomasum of young ruminants belongs to the aspartic proteinase group. Aspartic proteinase contained in the stomach is secreted in the abomasum mucus in the form of a zymogen and acts as a coagulant and digest milk. The younger the animals are fed milk, the more procymosin is produced. Calves that are less than 3 months old and consume only milk have a prokymosin content of 70-80% (Moschopoulou, 2011).

Rennet is an acidic protease enzyme, which is an enzyme that has an active site on two carboxyl groups. Rennet also contains other protease enzyme namely pepsin. Protease is an enzyme added to milk to hydrolyse casein, particularly kappa casein, which stabilizes the formation of coagulation preventing micelles. Renin works to coagulate milk through two stages of reaction, namely enzymatically and non-enzymatically. Rennet enzyme will destabilize the casein micelles. Renin breaks the specific bond between phenylalanine and methionine, destroying the carbohydrate-rich (glycoprotein) portion to form para-k-casein. The remaining casein cannot maintain the stability of the micelles due to the loss of the acidic part of the molecule. Then the k-caseins approach each other and unite by hydrophobic bonds, forming a three-dimensional network that doubles as the liquid phase of the milk. Rennet does not remove calcium from micelles, resulting in the formation of tough and elastic calcium-phospho-caseinate. Lactic acid bacteria are able to degrade casein into amino acids through a complex proteolytic system involving protease enzymes, peptidases, amino acid-carriers and peptide-carriers (Miskiyah et al., 2011).

2.4. Bromelain Enzyme

Pineapple is a fruit that can be easily found, so its use in this study is very effective and efficient. In pineapples, there is an acid called citric acid which can produce clots in milk. Bromelain enzyme is a protease enzyme that is able to hydrolyze peptide bonds in proteins into smaller molecules. Bromelain enzyme is present in all pineapple plant tissues. About half of the protein in pineapples contains the protease bromelain. Pineapple is a source of protease with high concentrations in ripe fruit (Rakhmah and Point, 2016; Purwaningsih, 2017).

Enzymes derived from plants are an alternative to replace the use of conventional enzymes. Bromelain is present in all parts of pineapple as a fruit, stems, tubers, crown and skin (Komansilan et al., 2019).

Bromelain is a type of sulfhydryl protease enzyme capable of hydrolysing peptide bonds in proteins or polypeptides into smaller molecules, namely amino enzymes. In general, amino acids are obtained as a result of protein hydrolysis, either using enzymes or acids. Amino acids function, among others, are building blocks of protein: including enzymes, the basic framework of a number of important compounds in metabolism (especially vitamins, hormones, and nucleic acids), and binders of important metals needed in enzymatic reactions. Bromelain is a protease enzyme that is able to break down proteins. The working process of the bromelain enzyme is to break down proteins into amino acids (Nur et al., 2017).

2.5. pH Value

The pH scale has a number of 1 to 14, acids have a lower scale, namely 0 to 7, while alkaline have a higher scale, namely 7 to 14, then a pH of 7 indicates a neutral position. Normally pH in milk can be caused by the presence of casein, buffer, phosphate, and citrate. In addition, the increase and decrease in pH resulted from the conversion of lactose to lactic acid by microbial enzymatic activity (Diastari and Agustina, 2013).

The addition of enzymes or acids in cheese making aims to reduce the pH to 4.5 - 5.4 where this pH is the isoelectric point of casein in milk (Budiman et al., 2017).

Renin enzyme is the most stable in the pH range 5.3 - 6.3 but has relatively better stability at pH 2.0. This enzyme has a very high resistance to acids. Renin can maintain its full activity at pH 1.6. However, at high pH, renin undergoes irreversible changes (Ginting, 2015).

The acidity level of milk or pH according to the Indonesian National Standard (SNI, 2011) is 6.3 - 6.8. If the number of bacteria contained in milk is high, it can affect the pH of the milk because the more bacteria, the more milk lactose will be converted into lactic acid so that the milk turns sour. The more bacteria contaminate the milk, the lower the quality of the milk will be. The increase and decrease in the pH of milk can be caused by the conversion from lactose to lactic acid by microbes and enzymatic activity (Pramesthi et al., 2015).

Rennet enzyme is the most stable in the pH range 5.3 - 6.3 but has relatively better stability at pH 2.0. This enzyme has a very high resistance to acids. Renin can maintain its full activity at pH 1.6. However, at high pH, renin undergoes

irreversible changes. The addition of different pineapple extracts can produce different pH values for each treatment (Ginting, 2015; Jaya and Didik, 2009).

2.6. Coagulating Time

Proteolytic enzymes produced by bacteria can cause clots in milk. This enzyme usually works in three stages, namely the absorption of the enzyme into the casein particles, then followed by a change in the state of the casein particles due to the action of the enzyme and then depositing the changed casein as a calcium salt or complex salt. Calcium ions in milk are needed for the deposition process. If there is a deviation, the milk can turn liquid or even too thick due to the milking and livestock factors (Diastari and Agustina, 2013).

2.7. Number of Microbes

The causes of damage to milk include microbial contamination. Milk microbes can come from outside the udder that enter through the nipple after milking or during the milking process. Some indications that can be used to measure the quality of milk are by looking at the total bacteria and pH of fresh milk after milking (Pramesthi et al., 2015).

The high milk content in milk makes it a very appropriate medium for the breeding of both pathogenic and non-pathogenic bacteria that can reduce the quality of milk. One of the bacteria that often contaminates fresh cow's milk is Staphylococcus aureus. Staphylococcus aureus is a Gram-positive bacterium that can cause toxicity in humans and mastitis in dairy cattle. Standardization of milk quality

according to BPOM RI (2008) is a maximum microbial contamination of 1 X 106 CFU / ml (Wibowo, et al., 2015).

One way to detect the number of microbes is by means of the TPC (Total Plate Count) test in the laboratory. TPC testing is intended to show the number of microbes contained in a material by counting the number of bacterial colonies grown on agar media (Yunita, et al., 2015).

2.8. Calcium Content

Milk is a source of essential minerals needed by the body. The two main minerals contained in milk are calcium and phosphorus. Calcium is an essential mineral needed for various body functions such as bone formation, blood clotting, catalysts for biological reactions and regulating muscle reactions. Calcium has levels varying between 107 - 133 mg / 100 g in fresh milk. There are several factors that can cause differences in the composition of fresh milk, namely genetic factors, age and the environment in which cows live (Taufik et al., 2018).

Rennet coagulation in milk is usually divided into 3 stages. The first stage involves the enzymatic hydrolysis of the k-kasein, the second stage refers to the aggregation and so-gel transition of the micelles and the third stage involves the aging syneresis and structural rearrangements of the formed gel. In the cheese industry calcium chloride is usually added to milk because it improves the texture and yield of cheese curd. This practice has the effect of reducing the rennet coagulation time. The addition of calcium lowers the milk pH, which in turn increase the rate of the enzymatic reaction (Sandra et al., 2012).

The method that can be used to determine the amount of calcium levels is by using Atomic Absorption Spectrophotometry (AAS) because AAS is very appropriate for

the analysis of substances at low concentrations with high enough accuracy. Thus, the method chosen for the determination of calcium levels is AAS. This method used has high sensitivity (detection limit is less than 1 ppm) (Saputri and Afrila, 2017).

CHAPTER III
MATERIALS AND METHODS

3.1. Location and Time

The research was conducted at the Laboratory of Microbiology, Faculty of Animal Science, Brawijaya University Malang to test pH, clotting rate and TPC (Total Plate Count) tests. CMT testing is carried out at the sampling point. Testing of calcium content is carried out at the Chemistry Laboratory, Faculty of Mathematics and Sciences, Brawijaya University. The research was conducted in January - April 2021.

3.2. Materials and Equipment

The research material was the effect of the addition of commercial rennet enzyme and pineapple extract on cattle subclinical mastitis milk towards pH, clotting rate, total microbes and calcium content. Here are the tools and materials needed:

1. Materials:
    a) Subclinical mastitis cow milk
    b) Rennet enzyme
    c) Pineapple Extract
    d) Testing:
        1) CMT: reagent, sample
        2) pH: Sample, aquadest, pH 4 buffer solution, and pH 7 buffer solution.
        3) AAS Test: sample
        4) Total Plate Count (TPC) : sample, peptone, spiritus, aquades, alcohol 70%.

2. Materials:
   a. Subclinical mastitis milk sample: sterile sample bottle 50ml
   b. Pineapple Extract: blender, strainer, container
   c. Cow's milk test:
      1) California Mastitis Test: paddle
      2) pH: pH meter, beaker glass, and dry tissue.
      3) Clotting Time: Timer
      4) Total Plate Count (TPC): Petri Dish, test tube, micropipette, cotton, aluminum foil, sterile gauze, bunsen, test tube rack, measuring cup, beaker glass, Erlenmeyer, spatula, PCA (Plate Count Agar), autoclave, water bath, scale, incubator, and refrigerator
      5) Calcium Content: AAS test equipment

3.3. Method

The method used was experimental research with the basic principles of making cheese is to agglomerate milk. Data obtained from direct observation. Data processing used the experimental method nested complete randomized design (CRD) with 2 types of subclinical mastitis milk, 7 treatments and 2 replications. The variables tested included pH, clotting rate, number of microbes and calcium levels.

Table 1. Research Data Tabulation Model

Treatment	Subclinical Mastitis Rate	
	PA = (+++) & (++++)	PB = (+) & (++)
T0	R1	R1
	R2	R2
T1	R1	R1
	R2	R2
T2	R1	R1
	R2	R2
T3	R1	R1
	R2	R2
T4	R1	R1
	R2	R2
T5	R1	R1
	R2	R2
T6	R1	R1
	R2	R2

Treatments:

T0 : Subclinical Mastitis Milk (*Control*)

T1 : subclinical mastitis milk + 1% Pineapple Extract

T2 : subclinical mastitis milk + 2% Pineapple Extract

T3 : subclinical mastitis milk + 4% Pineapple Extract

T4 : subclinical mastitis milk + 1% Rennet Enzyme + 1% Pineapple Extract

T5 : subclinical mastitis milk + 2% Rennet Enzyme + 2% Pineapple Extract

T6 : subclinical mastitis milk + 4% Rennet Enzyme + 4% Pineapple Extract

3.4. Research Variables

The variables observed in this study were:

1. pH using a pH meter (Wijaya, 2015)
2. Coagulation time using a stopwatch.
3. The number of microbes using the TPC (Total Plate Count) test (Wicaksono and Mirnawati, 2016).
4. Analysis of calcium content using the AAS method.

3.5. Research Procedure

Sampling was conducted in Brau hamlet, Batu city. Taking milk samples in the morning milking. Milk samples from each dairy cow were tested for CMT on the spot to ensure that the cow's milk was subclinical mastitis milk then put into a sterile sample bottle and then taken to the microbiology laboratory, treated and examined for pH, clotting rate, microbial count and calcium level.

The procedure of this research can be seen in Figure 2.

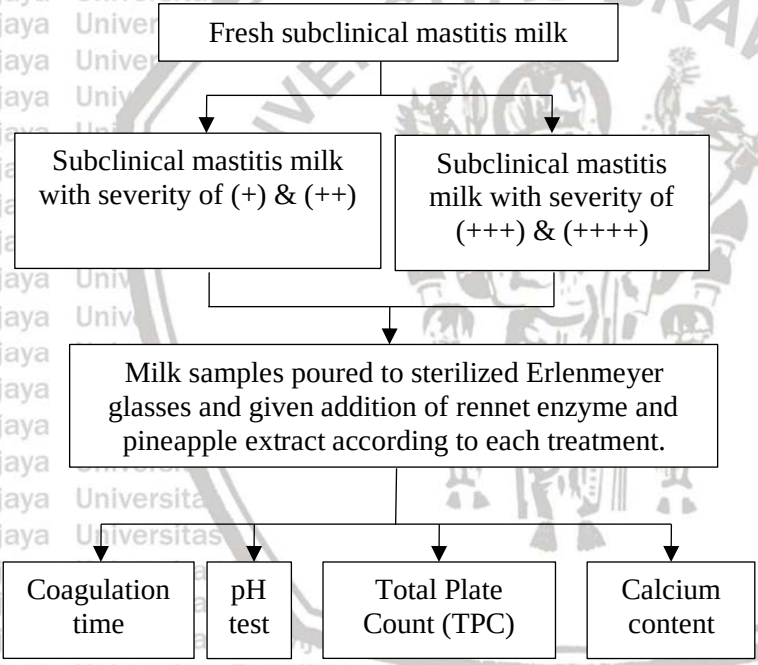

Figure 2. Research Procedure

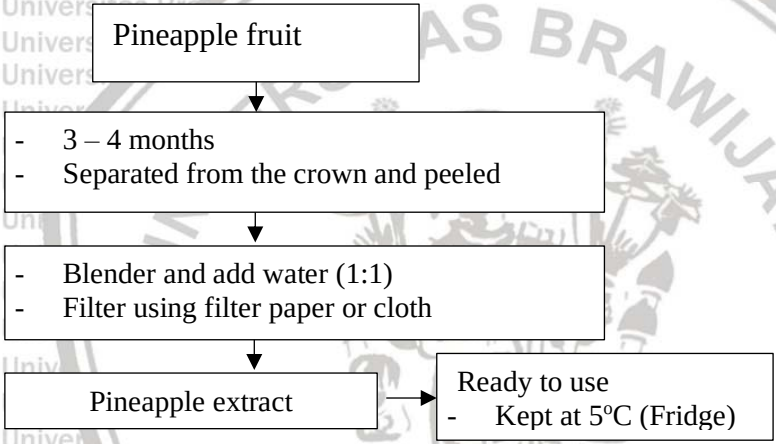

Figure 3. Procedure of making pineapple extract (Jaya and Didik, 2009)

3.6. Data Analysis

The data obtained from each variable were analyzed statistically using analysis of variance to determine whether or not there was a significant effect on the parameters of the Nested Completely Randomized Design (CRD) mathematical model treatment. If there is a real or very real effect, then to find out the difference between treatments, proceed with Duncan's Multiple Range Test (DMRT). The formula for CRD is:

$$Yijk = \mu + \tau i + \varepsilon ijk$$

Where:

Yijk = response treatment to-i replication to – j
μ = general average
τi = effect of treatment – i
εijk = effect of error
i = treatment 0,1,2,3,4,5,6
k = replication 1,2

Duncan's Multiple Range Test (DMRT) will be calculated using the following formula:

$$S\bar{x} = \sqrt{\frac{MS_E}{r}}$$

Where:

MS_E = middle square of error
r = replication

3.7. Terminology

a. Milk : Subclinical mastitis milk
b. Total Plate Count (TPC) : Method to count the number of microorganisms in a sample.
c. Atomic Absorption Spectrophotometry (AAS) : Method used to calculate calcium content
d. Mastitis : Subclinical mastitis

CHAPTER IV
RESULTS AND DISCUSSION

4.1. Research Results Towards Coagulating Time

Based on the results of the research that has been done, the results of the clotting rate of sub-clinical mastitis milk that have been given rennet enzyme and pineapple extract with certain concentrations can be seen in Table 2.

Table 2. Average Clotting Rate

Treatment (clotting rate)	M_A = (+++) & (++++)	M_B = (+) & (++)
T0	Until coagulate	Until coagulate
T1	55.00 ± 0.00^{ef}	57.5 ± 0.71^{ef}
T2	54.50 ± 0.71^{ef}	55.5 ± 0.71^{ef}
T3	52.50 ± 0.71^{e}	46 ± 1.41^{e}
T4	6.23 ± 0.33^{abc}	19.5 ± 0.71^{abcd}
T5	8.05 ± 0.07^{abcd}	14 ± 1.41^{abc}
T6	4.03 ± 0.04^{ab}	9.5 ± 0.71^{ab}
Average	25.76 ± 25.50	28.86 ± 22.68

Note: superscripts (a – f) in the same column indicate significant difference ($P<0,01$).

The results of the analysis of variance showed that the treatment of rennet enzyme and pineapple extract gave a very significant difference ($p < 0.01$) on the clotting rate of subclinical mastitis cow's milk. Table 2 shows that the higher the concentration of rennet enzyme and pineapple extract given, the faster the clotting rate in subclinical mastitis cow's milk. T0 is the control variable so that the clotting rate calculation process runs well. The sample of milk used in this study was subclinical mastitis milk with 2 different levels of severity, namely M_A which is a sample of subclinical mastitis milk with severity of (+++) and (++++) and M_B is subclinical mastitis milk with severity of (+) and (++).

Hadley (1935) stated that the detection of mastitis with rennet enzyme is based on the principle that rennet enzyme easily causes normal milk casein fractions to clot. Milk that is not normal or mastitis has a lower amount of casein and calcium than normal milk, so it doesn't clot quickly. Another factor that affects the failure of mastitis milk to clot is an increase in pH value. In this study, the milk samples used were samples of subclinical mastitis milk with a pH ranging from 6.4 to 6.7 where the pH is still included in the milk quality standards set by the Indonesian National Standard (SNI, 2011), which is between 6.3 - 6. , 7, and the treatment given to the sample was pineapple extract with a concentration of 1%, 2% and 4% for T1, T2 and T3 while T4, T5 and T6 is given rennet enzyme and pineapple extract with 1%, 2% and 4% and the result shows that subclinical mastitis milk given the treatments still coagulate and this might likely be due to the changes in pH value after giving the treatments.

Clotting time based on the research shows that between treatments T1 to T6 shows different results in terms of clotting time. T1, T2 and T3 is milk sample given pineapple extract as reagent while T4, T5 and T6 is milk sample given rennet enzyme and pineapple extract as reagent, because the amount of reagent given is different based on the amount thus resulting in different clotting time. T1, T2 and T3 with treatments given pineapple extract each 1%, 2% and 4% shows clotting time results between 40 – 57 minutes and T4 to T6 which is given rennet enzyme and pineapple extract with 1%, 2% and 4% concentration shows clotting time between 4 to 20 minutes. In this case, the best treatment is T6 which is subclinical mastitis milk that is given 4% rennet enzyme and 4% pineapple extract

and then homogenized and waited until coagulation shows which mark the clotting time that is the average of 4 minutes.

Tabel 2 shows a significant difference in treatment results. The difference shown might likely be due to the reaction between the milk that is given rennet enzyme and pineapple extract. According to Rakhmah and Titik (2016), the increase of protein in cheese which is given higher pineapple extract concentration aside from the acidity that can help coagulate protein is also because pineapple contains bromaelaine enzyme. Bromelaine enzyme is a protease enzyme contained in pineapple which can help hydrolise protein into amino acid. This is shown in the conducted research that milk sample which already shows sign of acidity in the pH value and added rennet enzyme and pineapple extract resulted in milk coagulation. The coagulant reacts directly with casein and milk coagulation is influenced by factors such as chemical composition, milk acidity, somatic cell count as well as the coagulating enzyme source and concentration.

4.2. Research Results Towards pH Values

Table 3., shows that the analysis results towards treatments provide a very significant difference ($p < 0.01$) on the pH of subclinical mastitis milk clotting. Table 3., shows that the higher the concentration of the enzyme added, the lower the pH in the milk. Data and analysis are presented in appendix 6. According to (Bittante, 2011) the acid produced will lower the pH and as a result casein will clot until it forms a curd, this is in accordance with the research that has been done. The concentration of rennet enzyme and pineapple extract added as treatment was 1%, 2% and 4% indicating that the higher the concentration added, the pH of the milk will be more acidic, this

is in accordance with (Wardhani, 2018) that the higher the acid concentration will accelerate to reach the working conditions of the enzyme, the protease activity during coagulation is influenced by the acidity of milk.

Table 3. Average pH Value

Milk	Average
M_A = (+++) & (++++)	$6{,}53 \pm 0{,}11^{b}$
M_B = (+) & (++)	$6{,}39 \pm 0{,}03^{a}$

Treatment (pH)	M_A = (+++) & (++++)	M_B = (+) & (++)
T0	6.70 ± 0.01^{fg}	6.42 ± 0.03^{a}
T1	6.50 ± 0.04^{abcde}	6.43 ± 0.01^{abcde}
T2	6.49 ± 0.01^{abcd}	6.42 ± 0.01^{abcdef}
T3	6.45 ± 0.01^{ab}	6.35 ± 0.04^{abcdefg}
T4	6.69 ± 0.01^{f}	6.40 ± 0.01^{ab}
T5	6.43 ± 0.00^{abc}	6.39 ± 0.01^{abc}
T6	6.43 ± 0.07^{a}	6.37 ± 0.04^{abcd}
Average	$6.53 \pm 0{,}11^{b}$	6.39 ± 0.03^{a}

Notes: superscript (a – b) shows indicates there is a difference (P<0,05) between milk samples. Superscripts (a – f) in the same column indicate significant difference (P<0,01) between treatments.

In Table 3., P0 as the control variable shows different results with the milk samples that have been given treatment 1 to 3, namely the addition of rennet enzyme and pineapple extract with different concentrations according to the treatment. The addition of rennet enzyme and pineapple extract with different concentrations showed a very significant difference

when compared with the control variable. While M_A and M_B show different pH values because M_A is subclinical mastitis milk with (+++) and (++++) severity while M_B is subclinical mastitis milk with severity of (+) and (++) which shows that the pH of each milk is different. The difference of pH in M_A and M_B milk is due to the different severity of subclinical mastitis this can be confirmed through the CMT test which is carried out before the milk sample is taken. The difference in the level of mastitis also means that the number of bacteria found in milk is different. The number of bacteria in milk can affect the physical and chemical characteristics of milk such as the pH value. This is in accordance with Pramesthi et al. (2015) that the number of bacteria contained in milk is still high, which affects the acidity level or pH of the milk. The more bacteria, the more milk lactose is converted into lactic acid so that the milk turns sour.

The addition of rennet enzyme and pineapple extract can reduce the pH of the milk because of the acid content contained. The presence of chymosin in rennet enzyme and citric acid in pineapple extract causes coagulation. According to Rakhmah and Titik (2016), pineapple contains citric acid which can help coagulate protein, pineapple also contains bromelain enzyme. Bromelain enzyme is a protease enzyme contained in pineapple that can help hydrolyze protein into amino acids. The protease enzyme is an enzyme that is quite stable, because it is resistant to a rather extreme pH and ambient temperature. This property causes the protease enzyme to be easily isolated by a

relatively simple method. The protease enzyme in the form of the bromelain enzyme can be isolated from pineapple (*Ananas comosus*), where bromelain is distributed to all parts of the plant, both in the fruit, stalk, skin and stem of pineapple (Soehartono 1992; Marji 2018).

The pH between M_A and M_B showed difference, namely ($P < 0.05$) and showed a significant difference, namely ($P < 0.01$) between treatments. This could be because the amount of concentration added was different between treatments. The higher the concentration added will affect the amount of substrate that is transformed. The pH of M_A and M_B milk before being treated were 6.7 and 6.4, after being treated, the results showed that the addition of rennet enzyme and pineapple extract according to the treatment concentration affected pH and clotting rate. The treatment with the highest concentration given was T3, which is the addition of 4% each of rennet enzyme and pineapple extract resulted in the lowest pH and the fastest clotting time. Bromelain enzyme is active at pH 6.5 or in the pH range 6 to 8. With the catalyst speed will increase with increasing enzyme concentration. The high concentration of enzymes will affect the number of substrates that are transformed (Chairunnisa 1985; Marji 2018).

4.3. Research Results Towards TPC (*Total Plate Count*)

Total plate count (TPC) is a method to determine the total heterogenous microorganisms in samples. According to Palawe and Jumliani (2018), TPC is a method that can be used to calculate the number of microbes in foodstuffs. The plate count method (TPC) is the most widely used method in analysis, because colonies can be seen directly without using a microscope. The TPC value used to determine the quality of

milk in terms of total microorganisms. the data analysis showed that total microorganisms of subclinical mastitis milk is not significantly different. Table 4 presents the average of TPC. Data and analysis are presented in appendix 7.

Table 4. Average of TPC.

Treatment (TPC) (Log CFU/ml)	M_A = (+++) & (++++)	M_B = (+) & (++)
T0	7.94 ± 0.07	7.73 ± 0.08
T1	7.05 ± 0.32	6.52 ± 1.59
T2	6.42 ± 1.59	6.34 ± 0.15
T3	7.10 ± 0.02	7.06 ± 0.69
T4	7.75 ± 0.11	7.24 ± 0.62
T5	7.68 ± 0.05	7.07 ± 0.39
T6	7.79 ± 0.01	6.82 ± 0.54
Average	7.39 ± 0,69	6.97 ± 0.70

Notes: sample between milk and treatments did not show any significant difference on the number of bacteria.

SNI (2011) stated that the maximum microbial contamination found in milk through the TPC test is $1x10^6$ CFU / ml or equivalent to 6 (Log_{10} CFU/ml), this shows that in terms of microbial contamination, the milk is not suitable for drinking because based on the results of conducted research, it shows that the average amount of TPC in milk M_A was 7,39 (Log_{10} CFU / ml) and resulted in a higher microbial population than the population in M_B milk with a population mean of 6,97 (Log_{10} CFU / ml). According to Zain and Bambang (2017) TPC can provide an overview of the overall microbiological conditions of the microorganisms contained in the product including bacteria, molds and yeasts.

The high average number of TPC for milk samples indicates that there is high microbial activity in milk. The activity produced by these microbes can also affect the taste of milk and the pH of the milk because the bacteria that arise as a result of contamination and bacterial growth cause the fermentation of lactose into lactic acid. According to Navyanti

and Retno (2015), the activity of bacteria in milk varies depending on the type and group, such as *Streptococcus lactis*, with its varieties being able to break down lactose into lactic acid. *Bacillus coagulans* and *Bacillus calidolactics* are known to produce lactic acid. The presence of lactic acid can cause a decrease in the pH of the milk. When the pH of milk reaches the isoelectric point of milk protein, the protein can clot.

The high number on the average of TPC shows that there is an increase in the number of cells which shows that it causes an infection of the udder which also causes a decrease in milk lactose. Olives et al., (2020) stated that since the decrease of lactose content is associated to a linear increase in Somatic Cell Count (SCC), milk acidity is reduced in milk with high SCC, which affects the whole coagulation process. The effect of SCC, lactose, and pH as markings of udder infection on milk composition, coagulation properties and curd firming parameters. Results shows that SCC, pH, and lactose affects contemporarily and independently, milk quality and coagulation properties.

4.4. Research Result Towards Calcium Content

Milk that is not of good quality or is damaged will break or clot. According to Aritonang (2017), the water mantle that surrounds casein in damaged milk is in an unstable state which results in casein agglomeration by heat or acid. The cause of clotting is the presence of calcium ions so that the calcium caseinate is deposited. Naturally, calcium phosphocaseinate cannot be precipitated by calcium ions. In order to settle, these compounds must be sensitive to calcium ions which can convert K - casein into paracasein. Beux et al., (2017) stated that coagulant reacts directly with casein and milk coagulation is

influenced by other factors such as pH value or milk acidity, somatic cell count, chemical composition, as well as the coagulating enzyme source and concentration. Casein is a phosphoprotein that is characterized by acids cluster and stabilized by calcium ions and phosphate.

Table 5. Average Calcium Content

Treatment (Calcium Content)	MA = (+++) & (++++)	MB = (+) & (++)
T0 (Mg/Kg)	115.08 ± 21.10^{d}	147.90 ± 6.01^{d}
T1 (Mg /L)	31.47 ± 1.48^{a}	25.29 ± 7.05^{ab}
T2 (Mg /L)	39.86 ± 6.13^{ab}	20.01 ± 3.68^{a}
T3 (Mg /L)	$47.01 \pm 0{,}91^{abc}$	30.44 ± 4.44^{abc}
T4 (Mg /Kg)	135.66 ± 26.76^{de}	156.54 ± 5.59^{de}
T5 (Mg /Kg)	$135{,}88 \pm 18.17^{def}$	207.28 ± 17.47^{def}
T6 (Mg /Kg)	$135{,}97 \pm 31.86^{def}$	177.79 ± 22.58^{def}
Average	91.56 ± 49,58	109.32 ± 78.20

Notes: superscripts (a – f) in the same column indicate significant difference (P<0,01) in terms of calcium content between treated samples.

Calcium content and somatic cells affect the milk production as much as 99%. Milk productions decrease due to the damaged udder that is caused by mastitis bacteria. Subclinical mastitis causes milk production to decrease up to 15% (Haerah, 2015; Wulansari et al., 2017). Table 5 shows that the average calcium content in M_A for T0 is 115.08 ppm, this shows that this number is far below The Ministry of Health of Republic Indonesia (2005), the calcium content for fresh cow's milk is 143 mg / 100 mL of milk or the same as 0.143%. This is because the milk used for M_A is fresh cow's milk milked from cows with subclinical mastitis with a severity (++) and (+++),

whereas in M_B milk, the average T0 calcium level is 147,90 ppm which indicates that the calcium level is still within reach. T1, T2 and T3 is a treatment with the addition of pineapple extract while T4, T5 and T6 is treated with the addition of rennet enzyme and pineapple extract so that the resulting results are very significantly different. In T1, T2 and T3, the clotting that occurs in the sample is not too solid so that when calculating the calcium level using Atomic Absorption Spectrophotometry (AAS) method, it does not need to be destroyed which means that the calculated calcium is the dissolved calcium.

The results of the calcium level test using the AAS method showed very significant differences ($P < 0.01$) between treatments. This is due to the difference in treatment and the amount of concentration given to each treatment. The analysis showed that the treatment of rennet enzyme and bromelain enzyme contained in the pineapple extract is proteolytic and had a very significant effect on the total calculated calcium levels. The use of rennet enzyme and pineapple extract at the same time as much as 1%, 2% and 4% causes the formation of curd and the higher the concentration given, the more calcium is trapped so that when it is tested, the calcium levels also increase. Whereas with milk that was only given the addition of pineapple extract treatment, namely the T1, T2 and T3 treatment, the clotting reaction that occurred did not form curd but also showed an increase in the amount of calcium along with

the increase in extract concentration which is 1%, 2% and 4%. pineapple extract added.

(a) (b)

Figure 4. Treated samples: a. Sample treated with rennet enzyme and pineapple extract (T4, T5 and T6), b. Sample with the addition of pineapple extract only (T1, T2 and T3).

Table 5. shows the difference in units in the results of the calcium content test, which is (mg/kg) and (mg/l). This is because T0 as the control sample is calculated as a sample with liquid – viscous characteristics. T4 , T5 and T6 milk samples that were given the addition of rennet enzyme and pineapple extract treatment with an increasing volume of concentration showed the results of increased calcium levels which resulted in a sediment such as tofu or called curd so that when the test was carried out the curd that were formed were destructed first so that the calcium content was able to be calculated, while the T1, T2 and T3 sample which was only given pineapple extract did not produce curd as firm as T4, T5 and T6 sample so that it was not necessary to do the destruction.

When the milk was taken for sample, the CMT test result showed that the cow had subclinical mastitis with severity of (++++) and the cow was still being milked while pregnant. This can affect the milk pH because there might colostrum mixed with the milk and also the mineral content in milk, one of which is calcium. This is in agreement with Oka et al., (2017) that calcium levels in milk can change because the content of minerals such as calcium and phosphorus can be affected by the lactation period, namely in the early stages of lactation, milk minerals become lower because they are used for milk synthesis. With the addition of rennet enzyme and pineapple extract which contain calcium, it can affect the amount of calcium present in milk after being treated, this can explain an increase in the results of the calcium level test that has been done.

CHAPTER V
CONCLUSIONS AND SUGGESTIONS

5.1. Conclusion

From the research that has been done, it can be concluded that:

- Rennet enzyme and pineapple extract can be used to detect cows with subclinical mastitis disease. T3 showed the results of the fastest clotting time with the addition of 4% rennet enzyme and 4% pineapple extract.
- The pH results for T0 as control sample show that cow's milk with subclinical mastitis with severity (+) & (++) has an average of 6.4 pH while cow's milk with subclinical mastitis with severity (+++) & (+++) has 6.7 pH after each treatment, the pH test results showed a decrease in pH this is due to the acid content contained in the pineapple extract and the rennet enzyme. The analysis showed that the treatment of proteolytic enzymes had a significant effect on the pH of the milk.
- Total Plate Count (TPC) test results show that the number of bacteria contained in the two milk samples has an average that is above the SNI standard (2011) which is 1 x 10_6 CFU / ml or equivalent to 6 (Log_{10} CFU/ml). The average number of microbes at M_A was 7.39 (Log_{10} CFU / ml) and M_B 6.97 (Log_{10} CFU / ml).
- Rennet enzyme and pineapple extract showed differences in calcium content based on each treatment. The higher concentration is given to the milk, the higher calcium content is found within the milk.

5.2. Recommendations

Further research that is suggested to be done as the author found that there should be color changes to indicate the reaction of rennet enzyme and pineapple extract towards subclinical mastitis milk, and to add treatment using only rennet enzyme to clarify the result also use normal milk as treatment for negative control. to compare between substances.

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ATTACHMENT

Attachment 1. Procedure of California Mastitis Test (CMT).

According to Priono et al. (2016) The CMT test is carried out by adding the CMT reagent to milk in a 1: 1 ratio. The paddle is slowly shaken for 10-15 seconds to homogenize the reagent and the milk in the paddle. The test results are - (negative) if the mixture of milk and CMT reagent remains homogeneous, + (positive 1) a little sediment is formed, ++ (positive 2) the precipitate is clearly visible, +++ (positive 3) the mixture thickens immediately, and ++++ (positive 4) forms a lot of gel. Result is given a score of 0, + is given a value of 1, ++ is given a score of 2, +++ is given a score of 3 and ++++ is given a score of 4 to facilitate data analysis.

Attachment 2. Procedure of Coagulation Time

1. Clean and sterilize the equipment that is going to be used.
2. Pour milk sample into each beaker glass according to treatment number.
3. Add rennet enzyme and pineapple extract to each beaker glass filled with milk sample according to treatment, in this case:
   - T0 : Subclinical Mastitis Milk (*Control*)
   - T1 : subclinical mastitis milk + 1% Pineapple Extract
   - T2 : subclinical mastitis milk + 2% Pineapple Extract
   - T3 : subclinical mastitis milk + 4% Pineapple Extract
   - T4 : subclinical mastitis milk + 1% Rennet Enzyme + 1% Pineapple Extract
   - T5 : subclinical mastitis milk + 2% Rennet Enzyme + 2% Pineapple Extract
   - T6 : subclinical mastitis milk + 4% Rennet Enzyme + 4% Pineapple Extract
4. Mix gently until homogenized.
5. Observe and write the time (minute) when coagulation has formed.

Attachment 3. Procedure of pH Analysis

According to Wijaya and Yunianta (2015), the pH meter is turned on and stabilized 15 – 30 minutes before using and calibrated using pH 4 and pH 7 buffer solution. The pH meter electrode is immersed into 10 ml of sample and waited to show a stable number. Then the electrode is removed and rinsed with distilled water. The pH value was measured in 2 replications for each sample.

Attachment 4. Procedure of Total Plate Count (TPC)

According to Wicaksono and Mirnawati (2016), Microbiological quality testing of milk was carried out using the plate count method with the pour plate method. From each sample of milk which is a 100-dilution stage, a decimal dilution is carried out by mixing 1 ml of milk into 9 ml of 0.1% BPW (Buffered Peptone Water). The decimal dilution continues until dilution 10^{-5}, 10^{-4} and 10^{-3}. Then inoculant planting is carried out by inserting 1 ml into a sterile petri dish that has been previously labeled according to the dilution numbers 10^{-5}, 10^{-4} and 10^{-3}. Next, 10 – 15 ml of plate count agar (PCA) (44-46 ° C) was poured into each petri dish and then homogenized. After the agar media solidified, the petri dishes were put in an incubator at 32 ° C in an inverted position for 24 hours, then the number of bacterial colonies that grew could be read and counted.

Guidance of Calculating Microbe (Soestyaningsih and Azizah, 2020)

The number of bacterial colonies from the sample is calculated using the formula:

$$\text{Colony / ml} = \textit{Total Colony } X \frac{1}{\textit{Dilution Factor}}$$

There are some things to consider when calculating the number of colonies of bacteria from the samples are:

1. The petri dish selected and counted are those containing colony between 30 – 300 CFU / ml. if the colony count per sample is more than 300 CFU / ml it is categorized as too numerous to count (TNTC).
2. Several colonies joined into one or one series of colony chains that are bound as a line are counted as one colony

3. Colonies that grow to cover more than half the area of a petri dish, are not referred to as colonies but as spreaders

If the comparison between the number of bacteria from the consecutive dilutions between the larger dilution and the previous dilution is <2, the results are averaged. However, if the result is ≥ 2, then use the number of microbes from the previous dilution (the smallest dilution).

Attachment 5. Procedure of Atomic Absorption Spectroscopy (AAS).

Atomic Absorption Spectrometry (AAS) method is based on the principle of light absorption by atoms. The atoms will absorb light at a certain wavelength, depending on the characteristic of the particle. Light at this wavelength has enough energy to change the electronic level of an atom for which the electronic transition of an atom is specific. By absorbing an energy, the atom is energized to the level of excitation (Gandjar, 2007).

According to Saputri and Ayu (2017), atomic absorption spectrometry is used because it is very appropriate for the analysis of substances at low concentration with high enough accuracy. Thus, the method chosen for the determination of calcium content is the atomic absorption spectrometry method (AAS), the method is also used because it has a high sensitivity (detection limit is less than 1 ppm).

The procedure is according Saputri and Ayu (2017):

1. 5ml of the sample after destruction is put into a 25 ml flask using pipettes and filled with aquabidest to the mark.
2. Then the absorbance is measured using an atomic absorption spectrophotometer which has been conditioned and the method is set in which the calcium concentration is determined at a wavelength of 422 nm - 423 nm with an air-acetylene flame.
3. The absorbance value obtained must be within the range of the calibration curve for the calcium standard solution.

4. The calcium concentration in the sample is determined based on the regression line equation of the calibration curve.

Attachment 6. Data and Statistic Analysis of Coagulating Time

Milk	Treatment	Repetition		Total Treatment	Average ± SD
		1	2		
Milk A	0	0	0	0	0 ± 0.00
	1	55	55	110	55.00 ± 0.00
	2	55	54	109	54.50 ± 0.71
	3	53	52	105	52.50 ± 0.71
	4	6.46	6	12.46	6.23 ± 0.33
	5	8	8.1	16.1	8.05 ± 0.07
	6	4	4.05	8.05	4.03 ± 0.04
Total Repetition		181,46	181.46	179.15	360.61
Milk B	0	0	0	0	0 ± 0.00
	1	58	57	115	57.5 ± 0.71
	2	56	55	111	55.5 ± 0.71
	3	47	45	92	46 ± 1.41
	4	20	19	39	19.5 ± 0.71
	5	13	15	28	14 ± 1.41
	6	10	9	19	9.5 ± 0.71
Total Repetition		204	200	404	

a. Correction Factor (CF)

$$CF = \frac{(360.61+404)^2}{2 \times 7 \times 2}$$

$$= \frac{(764.61)^2}{28}$$

$$= 20879.59$$

b. Sum of Square Total

$$SStotal = (0^2+6.46^2+8^2+\ldots\ldots\ldots\ldots\ldots+45^2) - 20879.59$$

$$= 15208.157$$

c. Sum of Square of Total Milk

$SS_M = (\frac{360.61^2 + 404^2}{7\ x\ 2}) - CF$

= 67.239

d. Sum of Square of Milk A

$SS_{MA} = (\frac{0^2 + 12.46^2 + 16.1^2 + \ldots\ldots + 105^2}{2}) - (\frac{360.61^2}{7\ x\ 2})$

$= (\frac{35485.264}{2}) - (\frac{130039.57}{14})$

= 17742.632 – 9288.54

= 8454.09

e. Sum of Square Milk B

$SS_{MB} = = (\frac{0^2 + 39^2 + 28^2 + \ldots\ldots + 92^2}{2}) - (\frac{404^2}{7\ x\ 2})$

$= (\frac{36676}{2}) - (\frac{163216}{14})$

= 18338 – 11658.29

= 6679.71

f. Sum of Square Treatment

$SSt = SS_{MA} + SS_{MB}$

= 8454.09 + 6679.71

= 15133.805

g. Sum of Square Error

SSe = SStotal – SSt – SSm

= 15208.157 – 15133.805 – 67.239

= 7.11

Analysis of Variance
(ANOVA)

SV	df	SS	MS	Fcount	F0,05	F0,01
Milk	1	67.239	67.24	0.0533	5.99	13.75
Treatment	12	15133.805	1261.15	2482.56	3.58	6.37
Error	14	7.11	0.51			
total	27					

Notes: Fscore > F0.01, means that there is a highly significant difference between treatments and no difference between milk samples.

Further test was carried out using Duncan's Multiple Range Test (DMRT) to determine the effect of rennet enzyme and pineapple extract given to the milk samples towards coagulation time.

$$Sx = \frac{\sqrt{MS_E}}{r}$$
$$= \sqrt{\frac{0,51}{2}}$$
$$= 0.504$$

LSR 1% = (t0.01 ; df error) x Sx
1% of DMRT critical table

Duncan Table	**2**	**3**	4	5	6	7
SSR 1%	4.21	4.391	4.508	4.591	4.654	4.703
LSR 1%	2.12	2.21	2.27	2.3138	2.34555037	2.37024568

Notation Milk	Treatment	Average	Notation
MA = (++) & (+++)	T0	Until coagulate	a
	T1	55.00 ± 0.00	b
	T2	54.50 ± 0.71	b
	T3	52.50 ± 0.71	b
	T4	6.23 ± 0.33	a
	T5	8.05 ± 0.07	a
	T6	4.03 ± 0.04	a
MB = (+) & (++)	T0	Until coagulate	a
	T1	57.5 ± 0.71	b
	T2	55.5 ± 0.71	b
	T3	46 ± 1.41	b
	T4	19.5 ± 0.71	a
	T5	14 ± 1.41	a
	T6	9.5 ± 0.71	a

Attachment 7. Data and Statistic Analysis of pH

Milk	Treatment	Repetition		Total	Average ± SD
		1	2		
Milk A	0	6.69	6.7	13.39	6.70 ± 0.01
	1	6.47	6.52	12.99	6.50 ± 0.04
	2	6.48	6.5	12.98	6.49 ± 0.01
	3	6.45	6.44	12.89	6.45 ± 0.01
	4	6.68	6.69	13.37	6.69 ± 0.01
	5	6.45	6.45	12.9	6.43 ± 0.00
	6	6.38	6.48	12.86	6.43 ± 0.07
Total Repetition		45,60	45.60	45.78	91.38
Milk B	0	6.4	6.44	12.84	6.42 ± 0.03
	1	6.39	6.4	12.79	6.43 ± 0.01
	2	6.38	6.39	12.77	6.42 ± 0.01
	3	6.34	6.4	12.74	6.35 ± 0.04
	4	6.43	6.42	12.85	6.40 ± 0.01
	5	6.41	6.42	12.83	6.39 ± 0.01
	6	6.37	6.32	12.69	6.37 ± 0.04
Total Repetition		44,72	44.72	44.79	89.51

a. Correction Factor (CF)

$$CF = \frac{(91.38+89.51)^2}{2 \times 7 \times 2}$$

$$= \frac{(180.89)^2}{28}$$

$$= 1168.61$$

b. Sum of Square Total

$$SStotal = (6.69^2+6.68^2+6.45^2+\ldots\ldots\ldots\ldots\ldots+6.32^2) - 1168.61$$

$$= 0.30$$

c. Sum of Square of Total Milk

$SS_M = (\frac{91.38^2 + 89.51^2}{7\ x\ 2}) - CF$

$= 0.12$

d. Sum of Square of Milk A

$SS_{MA} = (\frac{13.39^2 + 13.37^2 + 12.9^2 + \ldots\ldots + 12.89^2}{2}) - (\frac{91.38^2}{7\ x\ 2})$

$= (\frac{1193.2112}{2}) - (\frac{8350.3044}{14})$

$= 596.6056 - 695.450314$

$= 0.16$

e. Sum of Square Milk B

$SS_{MB} = = (\frac{12.84^2 + 12.79^2 + 12.77^2 + \ldots\ldots + 12.69^2}{2}) - (\frac{89.51^2}{7\ x\ 2})$

$= (\frac{1144.5977}{2}) - (\frac{8012.0401}{14})$

$= 572.29885 - 572.288579$

$= 0.01$

f. Sum of Square Treatment

$SSt = SS_{MA} + SS_{MB}$

$= 0.16 + 0.01$

$= 0.17$

g. Sum of Square Error

$SSe = SStotal - SSt - SSm$

$= 0.30 - 0.17 - 0.12$

$= 0.01$

Analysis of Variance
(ANOVA)

SV	df	SS	MS	Fcount	F0,05	F0,01
Milk	1	0.125	0.12	9.05	5.99	13.75
Treatment	12	0.166	0.01	18.14	3.58	6.37
Error	14	0.01	0.00			
Total	27					

Notes : Fcount > F0.01 means that there is a highly significant difference between treatments and Fcount > 0,05 means that there is a difference between sample milk A and milk B.

Further test was carried out using Duncan's Multiple Range Test (DMRT) to determine the difference in milk A and milk B towards pH value.

$$Sx = \frac{\sqrt{MS_E}}{r}$$

$$= \sqrt{\frac{0.001}{2 \; x \; 7}}$$

$$= 0.007$$

LSR 5% = (t0.05 ; df error) x Sx

Duncan Table	**2**
SSR 5%	3.261
LSR 5%	0.02

Notation

Milk	Average	Notation
MA = (++) & (+++)	6.53 ± 0.11	b
MB = (+) & (++)	6.39 ± 0.03	a

Duncan's Multiple Range Test (DMRT) to check on the different effect of rennet enzyme and pineapple extract given to the milk samples towards pH value.

$Sx = \frac{\sqrt{MS_E}}{r}$

$= \sqrt{\frac{0.001}{2}}$

$= 0.02$

LSR 1% = (t0.01; df error) x Sx

Duncan Table	**2**	**3**	4	5	6	7
SSR 1%	4.21	4.39	4.51	4.59	4.65	4.70
LSR 1%	0.082	0.086	0.088	0.090	0.091	0.092

Notation

Milk	Treatment	Average	Notation
MA = (++) & (+++)	T0	6.70 ± 0.01	b
	T1	6.50 ± 0.04	a
	T2	6.49 ± 0.01	a
	T3	6.45 ± 0.01	a
	T4	6.69 ± 0.01	b
	T5	6.43 ± 0.00	a
	T6	6.43 ± 0.07	a
MB = (+) & (++)	T0	6.42 ± 0.03	a
	T1	6.43 ± 0.01	a
	T2	6.42 ± 0.01	a
	T3	6.35 ± 0.04	a
	T4	6.40 ± 0.01	a
	T5	6.39 ± 0.01	a
	T6	6.37 ± 0.04	a

Attachment 8. ***Data and Statistic Analysis of TPC (Total Plate Count)***

Milk	Treatment	Repetition		Total	Average ± SD
		1	2		
Milk A	0	7.89	7.99	15.88	7.94 ± 0.07
	1	6.82	7.28	14.10	7.05 ± 0.32
	2	7.54	5.30	12.85	6.42 ± 1.59
	3	7.08	7.11	14.19	7.10 ± 0.02
	4	7.83	7.67	15.50	7.75 ± 0.11
	5	7.71	7.64	15.35	7.68 ± 0.05
	6	7.79	7.78	15.57	7.79 ± 0.01
Total Repetition		52,66	52.66	50.77	103.44
Milk B	0	7.78	7.67	15.45	7.73 ± 0.08
	1	5.40	7.64	13.04	6.52 ± 1.59
	2	6.45	6.23	12.68	6.34 ± 0.15
	3	7.54	6.57	14.11	7.06 ± 0.69
	4	7.67	6.8	14.47	7.24 ± 0.62
	5	6.79	7.34	14.13	7.07 ± 0.39
	6	7.20	6.44	13.64	6.82 ± 0.54
Total Repetition		48,83	48.83	48.69	97.52

a. Correction Factor (CF)

$$CF = \frac{(103.44+97.52)^2}{2 \times 7 \times 2}$$

$$= \frac{(200.96)^2}{28}$$

$$= 1442.29$$

b. Sum of Square Total

$$SStotal = (7.89^2+7.83^2+7.71^2+\ldots.+14.11^{2)} - 1442.29$$

$$= 13.91$$

c. Sum of Square of Total Milk

$SS_M = (\frac{103.44^2 + 97.52^2}{7\ x\ 2}) - 1442.29$

$= 1.25$

d. Sum of Square of Milk A

$SS_{MA} = (\frac{15.88^2 + 15.5^2 + 15.35^2 + \ldots\ldots + 14.19^2}{2}) - (\frac{103.44^2}{7\ x\ 2})$

$= (\frac{1535.675}{2}) - (\frac{10699.11}{14})$

$= 767.8376 - 764.22$

$= 3.62$

e. Sum of Square Milk B

$SS_{MB} = = (\frac{15.45^2 + 14.47^2 + 14.13^2 + \ldots\ldots + 14.11^2}{2}) - (\frac{97.52^2}{7\ x\ 2})$

$= (\frac{1363.764}{2}) - (\frac{9510.40}{14})$

$= 681.8729 - 679.31$

$= 2.56$

f. Sum of Square Treatment

$SSt = SS_{MA} + SS_{MB}$

$= 3.62 + 2.56$

$= 6.17$

g. Sum of Square Error

$SSe = SStotal - SSt - SSm$

$= 13.91 - 6.17 - 1.25$

$= 6.49$

Analysis of Variance
(ANOVA)

SV	df	SS	MS	Fcount	F0,05	F0,01
Milk	1	1.250	1.25	2.43	5.99	13.75
Treatment	12	6.174	0.51	1.11	3.58	6.37
Error	14	6.49	0.46			
Total	27					

Notes : Fcount < 0.05, therefore shows that there is no difference in the effect of milk and treatments on toward Total Plate Count (TPC).

Attachment 9. Data and Statistic Analysis of Calcium Content

Milk	Treatment	Repetition		Total	Average ± SD
		1	2		
Milk A	0 (mg/kg)	100.16	130	230.16	115.08 ± 21.10
	1 (mg/l)	30.42	32.51	62.93	31.47 ± 1.48
	2 (mg/l)	35.52	44.19	79.71	39.86 ± 6.13
	3 (mg/l)	47.65	46.36	94.01	47.01 ± 0,91
	4 (mg/kg)	116.73	154.58	271.31	135.66 ± 26.76
	5 (mg/kg)	123.03	148.72	271.75	135.88 ± 18.17
	6 (mg/kg)	158.5	113.44	271.94	135.97 ± 31.86
Total Repetition		612,01	669.8	1281.81	
Milk B	0 (mg/kg)	152.15	143.65	295.8	147.90 ± 6.01
	1 (mg/l)	30.27	20.3	50.57	25.29 ± 7.05
	2 (mg/l)	22.61	17.41	40.02	20.01 ± 3.68
	3 (mg/l)	27.3	33.58	60.88	30.44 ± 4.44
	4 (mg/kg)	152.59	160.49	313.08	156.54 ± 5.59
	5 (mg/kg)	194.93	219.63	414.56	207.28 ± 17.47
	6 (mg/kg)	193.76	161.82	355.58	177.79 ± 22.58
Total Repetition		773,61	756.88	1530.49	

a. Correction Factor (CF)

$$CF = \frac{(1281.81+1530.49)^2}{2 \times 7 \times 2}$$

$$= \frac{(2812.3)^2}{28}$$

$$= 282465.40$$

b. Sum of Square Total

$$SStotal = (100.16^2+116.73^2+123.03^2+...+33.58^2) - 282465.40$$

$$= 113653.84$$

c. Sum of Square of Total Milk

$SS_M = \left(\frac{1281.81^2 + 1530.49^2}{7 \times 2}\right) - 282465.40$

$= 2208.63$

d. Sum of Square of Milk A

$SS_{MA} = \left(\frac{230.16^2 + 271.31^2 + 271.75^2 + \ldots\ldots + 94.01^2}{2}\right) - \left(\frac{128.81^2}{7 \times 2}\right)$

$= \left(\frac{293533.917}{2}\right) - \left(\frac{1643036.88}{14}\right)$

$= 146766.958 - 117359.77$

$= 29407.18$

e. Sum of Square Milk B

$SS_{MB} = \left(\frac{295.8^2 + 313.08^2 + 414.56^2 + \ldots\ldots + 60.88^2}{2}\right) - \left(\frac{1530.49^2}{7 \times 2}\right)$

$= \left(\frac{491679.156}{2}\right) - \left(\frac{2342399.64}{14}\right)$

$= 245839.578 - 167314.26$

$= 78525.318$

f. Sum of Square Treatment

$SSt = SS_{MA} + SS_{MB}$

$= 29407.18 + 78525.318$

$= 107932.50$

g. Sum of Square Error

$SSe = SStotal - SSt - SSm$

$= 113653.84 - 107932.50 - 2208.63$

$= 3512.71$

Analysis of Variance
(ANOVA)

SV	df	SS	MS	Fcount	F0,05	F0,01
Milk	1	2208.63	2208.63	0.25	5.99	13.75
Treatment	12	107932.50	8994.37	35.85	3.58	6.37
Error	14	3512.71	250.91			
Total	27					

Notes : Fcount > F0.01 means that there is a highly significant difference between treatments towards calcium content.

Further test was carried out using Duncan's Multiple Range Test (DMRT) to determine the effect of rennet enzyme and pineapple extract given to the milk samples towards calcium content.

$Sx = \frac{\sqrt{MS_E}}{r}$

$= \sqrt{\frac{250.91}{2}}$

$= 11.201$

LSR 1% = (t0,01 ; df error) x Sx

1% of DMRT critical table

Duncan Table	**2**	**3**	4	5	6	7
SSR 1%	4.21	4.39	4.51	4.59	4.65	4.70
LSR 1%	47.155	49.182	50.492	51.422	52.128	52.677

Notation

Milk	Treatment	Average	Notation
MA = (++) & (+++)	T0	115.08 ± 21.10	b
	T1	31.47 ± 1.48	a
	T2	39.86 ± 6.13	a
	T3	47.01 ± 0,91	a
	T4	135.66 ± 26.76	b
	T5	135.88 ± 18.17	b
	T6	135.97 ± 31.86	b
MB = (+) & (++)	T0	147.90 ± 6.01	b
	T1	25.29 ± 7.05	a
	T2	20.01 ± 3.68	a
	T3	30.44 ± 4.44	a
	T4	156.54 ± 5.59	b
	T5	207.28 ± 17.47	b
	T6	177.79 ± 22.58	b

Attachment 10. Calcium Content Analysis Report

KEMENTERIAN PENDIDIKAN DAN KEBUDAYAAN
UNIVERSITAS BRAWIJAYA FAKULTAS MIPA
JURUSAN KIMIA
Jl. Veteran, Malang 65145, Jawa Timur, Indonesia. Telp +62-341-575838, fax : +62-341-554403
http://kimia.ub.ac.id, e-mail:kimia_UB@ub.ac.id

LAPORAN HASIL ANALISIS

NO : 20210011/R.1/T.1/R.1/TT.150803/2021

1. Data Konsumen
   Nama : Asiah Putri Humairo
   Instansi : Fakultas Peternakan Universitas Brawijaya
   Alamat : Jl. Veteran Malang
   Telepon : 08115422998
   Status : Mahasiswa S-1
   Keperluan Analisis : Uji Kuantitas
2. Sampling Dilakukan Oleh : Konsumen
3. Identifikasi Sampel
   Nama Sampel : ***Susu Mastitis***
   Wujud : Cair
   Warna : Putih
   Bau : Tidak Ada Bau
4. Prosedur Analisis : Dilakukan oleh Unit Analisis dan Pengukuran Jurusan Kimia FMIPA Universitas Brawijaya Malang
5. Penyampaian Laporan Hasil Analisis : Diambil Langsung
6. Tanggal Terima Sampel : 27 Januari 2021
7. Data Hasil Analisis :

No	Kode	Parameter	Hasil Analisis		Metode Analisis	
			Kadar	Satuan/ppm	Pereaksi	Metode
1.	**P0U1**	Ca	100,16 ± 0,31	mg/kg	HNO_3	AAS
2.	**P0U2**	Ca	130,00 ± 0,21	mg/kg	HNO_3	AAS
3.	**P1U1**	Ca	116,73 ± 0,16	mg/kg	HNO_3	AAS
4.	**P1U2**	Ca	154,58 ± 0,15	mg/kg	HNO_3	AAS
5.	**P2U1**	Ca	123,03 ± 0,19	mg/kg	HNO_3	AAS
6.	**P2U2**	Ca	148,72 ± 0,14	mg/kg	HNO_3	AAS
7.	**P3U1**	Ca	158,50 ± 0,16	mg/kg	HNO_3	AAS
8.	**P3U2**	Ca	113,44 ± 0,15	mg/kg	HNO_3	AAS

Catatan:
1. Hasil analisis ini adalah nilai rata-rata pengerjaan analisis secara duplo,
2. Hasil analisis ini hanya berlaku untuk sampel yang kami terima dengan kondisi sampel saat itu.

Malang, 02 Februari 2021

Mengetahui,
Ketua Jurusan Kimia,

Masruri, S.Si., M.Si., Ph.D.
NIP. 19731020 200212 1 001

Ketua Unit Analisis dan Pengukuran,

Moh. Farid Rahman, S.Si., M.Si.
NIP. 19700720 199702 1 001

KEMENTERIAN PENDIDIKAN DAN KEBUDAYAAN
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LAPORAN HASIL ANALISIS

NO : 20210021/R.1/T.1/R.1/TT.150803/2021

1. Data Konsumen
   - Nama : Asiah Putri Humairo
   - Instansi : Fakultas Peternakan Universitas Brawijaya
   - Alamat : Jl. Veteran Malang
   - Telepon : 08115422998
   - Status : Mahasiswa S-1
   - Keperluan Analisis : Uji Kuantitas
2. Sampling Dilakukan Oleh : Konsumen
3. Identifikasi Sampel
   - Nama Sampel : ***Susu Mastitis***
   - Wujud : Cair
   - Warna : Putih
   - Bau : Tidak Ada Bau
4. Prosedur Analisis : Dilakukan oleh Unit Analisis dan Pengukuran Jurusan Kimia FMIPA Universitas Brawijaya Malang
5. Penyampaian Laporan Hasil Analisis : Diambil Langsung
6. Tanggal Terima Sampel : 02 Februari 2021
7. Data Hasil Analisis :

No	Kode	Parameter	Hasil Analisis		Metode Analisis	
			Kadar	Satuan	Pereaksi	Metode
1.	P0U3	Ca	152,15 ± 0,18	mg/kg	HNO_3	AAS
2.	P0U4	Ca	143,65 ± 0,18	mg/kg	HNO_3	AAS
3.	P1U3	Ca	152,59 ± 0,13	mg/kg	HNO_3	AAS
4.	P1U4	Ca	160,49 ± 0,05	mg/kg	HNO_3	AAS
5.	P2U3	Ca	194,93 ± 0,05	mg/kg	HNO_3	AAS
6.	P2U4	Ca	219,63 ± 0,09	mg/kg	HNO_3	AAS
7.	P3U3	Ca	193,76 ± 0,03	mg/kg	HNO_3	AAS
8.	P3U4	Ca	161,82 ± 0,20	mg/kg	HNO_3	AAS

Catatan:
1. Hasil analisis ini adalah nilai rata-rata pengerjaan analisis secara duplo,
2. Hasil analisis ini hanya berlaku untuk sampel yang kami terima dengan kondisi sampel saat itu.

Malang, 18 Februari 2021

Mengetahui,
Ketua Jurusan Kimia,

Masruri, S.Si., M.Si., Ph.D.
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Ketua Unit Analisis dan Pengukuran,

Moh. Farid Rahman, S.Si., M.Si.
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LAPORAN HASIL ANALISIS

NO : 20210079/R.1/T.1/R.1/TT.150803/2021

1. Data Konsumen
   - Nama : Asiah Putri Humairo
   - Instansi : Fakultas Peternakan Universitas Brawijaya
   - Alamat : Jl. Veteran Malang
   - Telepon : 08115422998
   - Status : Mahasiswa S-1
   - Keperluan Analisis : Uji Kuantitas
2. Sampling Dilakukan Oleh : Konsumen
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   - Nama Sampel : ***Susu Mastitis***
   - Wujud : Cair
   - Warna : Putih
   - Bau : Ada Bau
4. Prosedur Analisis : Dilakukan oleh Unit Analisis dan Pengukuran Jurusan Kimia FMIPA Universitas Brawijaya Malang
5. Penyampaian Laporan Hasil Analisis : Diambil Langsung
6. Tanggal Terima Sampel : 24 Maret 2021
7. Data Hasil Analisis :

No	Kode	Parameter	Hasil Analisis		Metode Analisis	
			Kadar	Satuan	Pereaksi	Metode
1.	A1U1	Ca	30,42 ± 0,04	mg/L	HNO_3	AAS
2.	A1U2	Ca	32,51 ± 0,03	mg/L	HNO_3	AAS
3.	A2U1	Ca	35,52 ± 0,04	mg/L	HNO_3	AAS
4.	A2U2	Ca	44,19 ± 0,02	mg/L	HNO_3	AAS
5.	A3U1	Ca	47,65 ± 0,04	mg/L	HNO_3	AAS
6.	A3U2	Ca	46,36 ± 0,04	mg/L	HNO_3	AAS
7.	B1U1	Ca	30,27 ± 0,02	mg/L	HNO_3	AAS
8.	B1U2	Ca	20,30 ± 0,04	mg/L	HNO_3	AAS
9.	B2U1	Ca	22,61 ± 0,04	mg/L	HNO_3	AAS
10.	B2U2	Ca	17,41 ± 0,04	mg/L	HNO_3	AAS
11.	B3U1	Ca	27,30 ± 0,04	mg/L	HNO_3	AAS
12.	B3U2	Ca	33,58 ± 0,03	mg/L	HNO_3	AAS

Catatan:
1. Hasil analisis ini adalah nilai rata-rata pengerjaan analisis secara duplo,
2. Hasil analisis ini hanya berlaku untuk sampel yang kami terima dengan kondisi sampel saat itu.

Malang, 26 April 2021

Mengetahui,
Ketua Jurusan Kimia,

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Ketua Unit Analisis dan Pengukuran,

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NIP. 197007201997021001

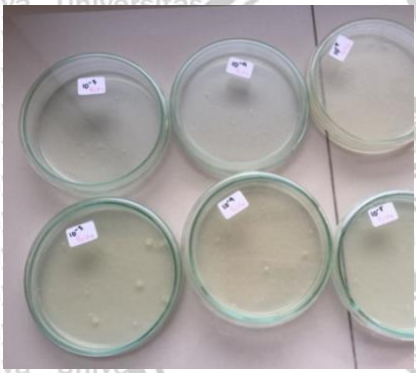
Attachment 11. Documentation

a. Subclinical mastitis milk before the addition of rennet enzyme and pineapple extract.

b. Formed curd after the addition of rennet enzyme and pineapple extract (T1 – T3).

c. After the addition of pineapple extract as treatment (T4 – T6).

d. Inoculum planting for Total Plate Count.



e. Petri dish after incubation

f. pH analysis using digital pH meter.