THE EFFECTS OF OZONATION AND REFRIGERATOR STORAGE ON TOTAL PLATE COUNT, pH, AND PROTEIN CONTENT OF MILK

UNDERGRADUATE THESIS

By:
Ido Hosea Hasian Simorangkir
SIN. 175050107111063

ANIMAL SCIENCE PROGRAM
ANIMAL SCIENCE FACULTY
BRAWIJAYA UNIVERSITY
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This undergraduate thesis is one of the requirements to achieve Animal Science Bachelor degree at Animal Science Faculty Brawijaya University

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FOREWORD

Praised and gratitude from the author to God Almighty for His blessings and grace so the author can finish this undergraduate thesis by title **"The Effects of Ozonation and Refrigerator Storage on Total Plate Count, pH, and Protein Content of Milk"**. This undergraduate thesis was arranged as one of the requirements to achieve Bachelor Degree in Animal Science Faculty, Brawijaya University. Therefore, the author wants to express sincere thank to all individuals who already gave their contribution in forms of help, support and motivation. The author would like to express sincere thanks to:

1. Prof. Dr. Ir. Lilik Eka Radiati, MS., IPU as the supervisor who always give her time, suggestions and knowledge in guiding the author to complete this undergraduate thesis
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The author realize that there are some mistakes and imperfections in this undergraduate thesis, therefore the author really appreciates all critics and suggestions. Hopefully this undergraduate thesis will be useful for all parties to develop further development in animal science.

THE EFFECTS OF OZONATION AND REFRIGERATOR STORAGE ON TOTAL PLATE COUNT, PH, AND PROTEIN CONTENT OF MILK

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ABSTRACT

This research was conducted from August to September 2020 at Laboratory of Animal Products Technology, Animal Science Faculty, Brawijaya University, Malang. The purpose of this research was to determine the effect of ozonation on milk shelf life stored in refrigerator analyzed from microbiological and chemical quality of milk. The results of this research provide information on ozone as a process to prolong milk shelf life. The research method was Nested Experimental Design with 2 types of milk and 5 treatments nested in milk and continued by Duncan Multiple Range Test if there was significant difference. The results showed that milk types and refrigerator storage nested in milk had a highly significant effect ($P<0.01$) on TPC. Milk types and refrigerator storage nested in milk had a highly significant effect ($P<0.01$) on pH. Milk types had a highly significant effect ($P<0.01$) on protein content. Refrigerator storage nested in milk had a significant effect ($P<0.05$) on protein content. Suggestion for further research, it could be done a research about ozone half life in milk during storage.

Keywords: Cold storage, milk shelf life, ozonated milk

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SUMMARY

Milk is yellowish-white or bluish-white liquid resulted from mammalian udder gland secretion. The needs of milk in Indonesia continues to increase every year along with population growth and public awareness to consume food with complete nutritional content such as milk. Milk derived from dairy cows is a source of protein, fat, carbohydrates, minerals, and vitamins contained in perfect composition. The consumption of fresh milk also continues to increase where in 2017 the national consumption of cow's fresh milk reached 1.261.503 tons while the national fresh milk production only reached 928.108,13 tons which means the national fresh milk production was unable to fulfill the national needs and must be fulfilled by milk import. One of the things that cause national milk production unable to fulfill the national milk needs is the low quality of fresh milk produced from the local farmers. Milk produced from local farmers will be distributed first to the milk collection point and then sent to the village cooperative unit to

test the milk quality whether it is decent to be consumed or sent to the milk processing industry. One of milk quality indicators is bacteria contamination, the limit of bacteria contamination based on BSN (2011) are: *Total Plate Count* (TPC) 10^6 CFU/ml, *Staphylococcus aureus* 10^2 CFU/ml and *Enterobacteriaceae* 10^3 CFU/ml.

Various efforts are needed to extend the shelf life of milk and reduce the amount of bacterial contamination without damaging the milk nutritional quality, so far these efforts have been carried out by heating methods such as sterilization, Ultra High Temperature (UHT) and pasteurization. One of the compounds that are commonly used for sterilization in the food sector is ozone with the ozonation method, but ozone has not been widely applied in milk sterilization. Further research needs to be done on the effectiveness of ozone to eliminate pathogenic bacteria in milk. Ozone gas, the molecular triatomic form of oxygen has been widely researched and used in the food industry for tools surface cleaning and the treatment of raw materials. Ozone has a master bactericidal effect on both Gram-positive and Gram-negative, due to its high oxidation potential.

The objective of this research is to determine the effect of ozonation on milk shelf life stored in refrigerator analyzed from microbiological and chemical quality of milk.

This research was conducted from August to September 2020 at Laboratory of Animal Products Technology, Animal Science Faculty, Brawijaya University, Malang. The research method was Nested Experimental Design with 2 types of milk and 5 treatments nested in milk and continued by Duncan Multiple Range Test if there was significant difference. The results showed that milk types and refrigerator storage nested in milk had a highly significant effect ($P<0.01$) on TPC. Milk

types and refrigerator storage nested in milk had a highly significant effect ($P<0.01$) on pH. Milk types had a highly significant effect ($P<0.01$) on protein content. Refrigerator storage nested in milk had a significant effect ($P<0.05$) on protein content. Suggestion for further research, it could be done a research about ozone half life in milk during storage.

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LIST OF ABBREVIATIONS

PCA	: Plate Count Agar
ANOVA	: Analysis of Variance
DMRT	: Duncan's Multiple Range Test
TBTC	: Too Numerous To Count
pH	: Potential of Hydrogen
TPC	: Total Plate Count
LAF	: Laminar Air Flow
PP	: Pour Plate
CFU	: Colony Forming Unit
et al.	: et alia; et alii; et aliae
g	: gram
mL	: mililiter
%	: percent
^oC	: degree celcius

CHAPTER I

INTRODUCTION

1.1. Background

Milk is yellowish-white or bluish-white liquid resulted from mammalian udder gland secretion. Milk needs in Indonesia increases every year along with population growth and public awareness to consume food with complete nutritional content such as milk. Milk derived from dairy cows is a source of protein, fat, carbohydrates, minerals, and vitamins contained in perfect composition. The average composition of cow's milk contains 3.3% protein; 3.8% fat; 4.7% carbohydrate; 87.6% water; 0.76% vitamin; and mineral (Cahyaningtyas, et al., 2016). The consumption of fresh milk also increases where in 2017 the national consumption of cow's fresh milk reached 1.261.503 tons while the national fresh milk production only reached 928.108,13 tons (BPS, 2018) which means the national fresh milk production was unable to fulfill the national needs and must be fulfilled by milk import.

One of the things that cause national milk production unable to fulfill the national milk needs is the low quality of fresh milk produced from the local farmers. Milk produced from local farmers will be distributed first to the milk collection point and then sent to the village cooperative unit to test the milk quality whether it is decent to be consumed or sent to the milk processing industry. One of milk quality indicators is bacteria contamination, the limit of bacteria contamination based on BSN (2011) are: Total Plate Count (TPC) 10^6 CFU/ml, *Staphylococcus aureus* 10^2 CFU/ml and *Enterobacteriaceae* 10^3 CFU/ml. The level of bacteria contamination from local farmers tend to exceed the national standard limit mostly due to the bad sanitation of milking equipment such as inside the milk tanks, milk tank necks, milk buckets and milk filter cloths (Wicaksono and Sudarwanto, 2016). Milk is easily contaminated by bacteria because the perfect nutritional content of milk is good media for microorganisms growth, moreover, the high water content of milk which is about 87-88%, and milk pH which is close to neutral also causes milk preferred by bacteria (Cahyaningtyas, et al., 2016). Fresh milk only contain small amount of bacteria and should be stored in low temperature to keep its condition and prevent bacterial contamination. Milk spoilage in room temperature will happen in 4 hours and can be seen from the color and odor change. Cold storage and pasteurization are usually used to prolong milk shelf life (Nababan, et al., 2014). Fresh milk stored in 0-1°C will prolong its shelf life for one day (Putri, 2016).

Various efforts are needed to extend the shelf life of milk and reduce the amount of bacterial contamination without damaging the milk nutritional quality, so far these efforts have been carried out by heating methods such as sterilization, Ultra High Temperature (UHT) and pasteurization. Those various methods are aimed to reduce bacterial contamination in milk and also useful to extend the diversification of dairy products to increase milk consumption in the community (Hendrawati and Utomo, 2017). Pasteurized milk must be stored at temperature of 4°C to maximize its shelf life while the average shelf life of pasteurized milk in Indonesia is only 5-7 days (Kristanti, 2017).

One of the compounds that are commonly used for sterilization in the food sector is ozone with the ozonation method, but ozone has not been widely applied in milk sterilization. Further research needs to be done on the effectiveness of ozone to eliminate pathogenic bacteria in milk. Ozone gas, the molecular triatomic form of oxygen has been widely researched and used in the food industry for tools surface cleaning and the treatment of raw materials. Ozone has a master bactericidal effect on both Gram-positive and Gram-negative, due to its high oxidation potential (Couto, et al., 2016). Ozonation is the process of utilizing ozone (O_3) as a disinfectant to ensure the viability of food products because ozone can kill bacteria and viruses in water, meat, poultry, eggs, fish, fruits, vegetables and dry foods (Kusumawati, 2012). The use of ozone in the food sector is rated safe because it does not leave any residues. The mechanism of action of ozone in conducting sterilization is by attacking the surface layer of bacteria and oxidizing sulfhydryl from enzymes or oxidation with lipoproteins and lipopolysaccharides which are the largest layers of gram-negative bacteria. This will cause the breakdown of cell permeability to become lysis (Rusdi and Suliasih, 2002).

1.2. Problem

Based on the background description above, the problem of this research was how is the effect of ozonation and refrigerator storage on total plate count, pH, and protein content of milk

1.3. Objective

The objective of this research is to determine the effect of ozonation if ozone can reduce total plate count of milk therefore will slow down bacterial growth and slow down the decreases of pH and protein content during refrigerator storage.

1.4. Significance

1. The results of this research are expected to provide information in the animal science sector and academicians about the effect of ozonation on milk shelf life stored in refrigerator analyzed from Total Plate Count, pH and protein.
2. The results of this research are expected to provide information in the technology sector and milk processing industry to develop another method used for milk sterilization

1.5. Framework

Milk is yellowish-white or bluish-white liquid resulted from mammalian udder gland secretion. Milk is a food with great demand because of its high nutritional content as a source of carbohydrates, protein, and fat. However high water content and nutrition in milk is an ideal growth medium for bacteria that cause fresh milk prone to bacteria contamination and needs post-milking treatment to prevent contamination (Yudonegoro, et al., 2014).

Fresh milk only contain small amount of bacteria and should be stored in low temperature to keep its condition and prevent bacterial contamination. Milk spoilage in room temperature will happen in 4 hours and can be seen from the color and odor change. Cold storage and pasteurization are usually used to prolong milk shelf life (Nababan, et al., 2014). Fresh milk stored in 0-1^{o}C will prolong its shelf life for one day (Putri, 2016).

Ozonation is a technology using ozone (O_3) which has strong oxidizing natures to kill bacteria by entering the bacterial cell wall so the cell permeability changes and lysis occurs. General and safe ozonation are commonly used as a method to disinfect some food commodities such as vegetables, fruits, and water to eliminate contamination of pesticides, microorganisms and heavy metals (Asgar, et al., 2015).

Previous studies have shown that ozonation treatments can kill microorganisms in fresh milk. Ozonation at a temperature of 10C can kill microorganisms up to 85.35% and ozonation 10 minutes duration can kill microorganisms up to 94.50% (Rusdi and Suliasih, 2002). Ozonation for 10 minutes will produce the highest dissolved ozone, which means the reaction between ozone and bacteria is the most intensive and reduces the higher number of bacteria in milk (Kusumawati, 2012). The framework of this research can be seen in Figure 1.

1.6. Hypothesis

The hypothesis of this research is ozonation treatment on fresh milk will decrease total plate count of milk and slow down the bacterial growth during storage. Therefore, ozonation will slows down the decreases of pH and protein content of milk during storage.

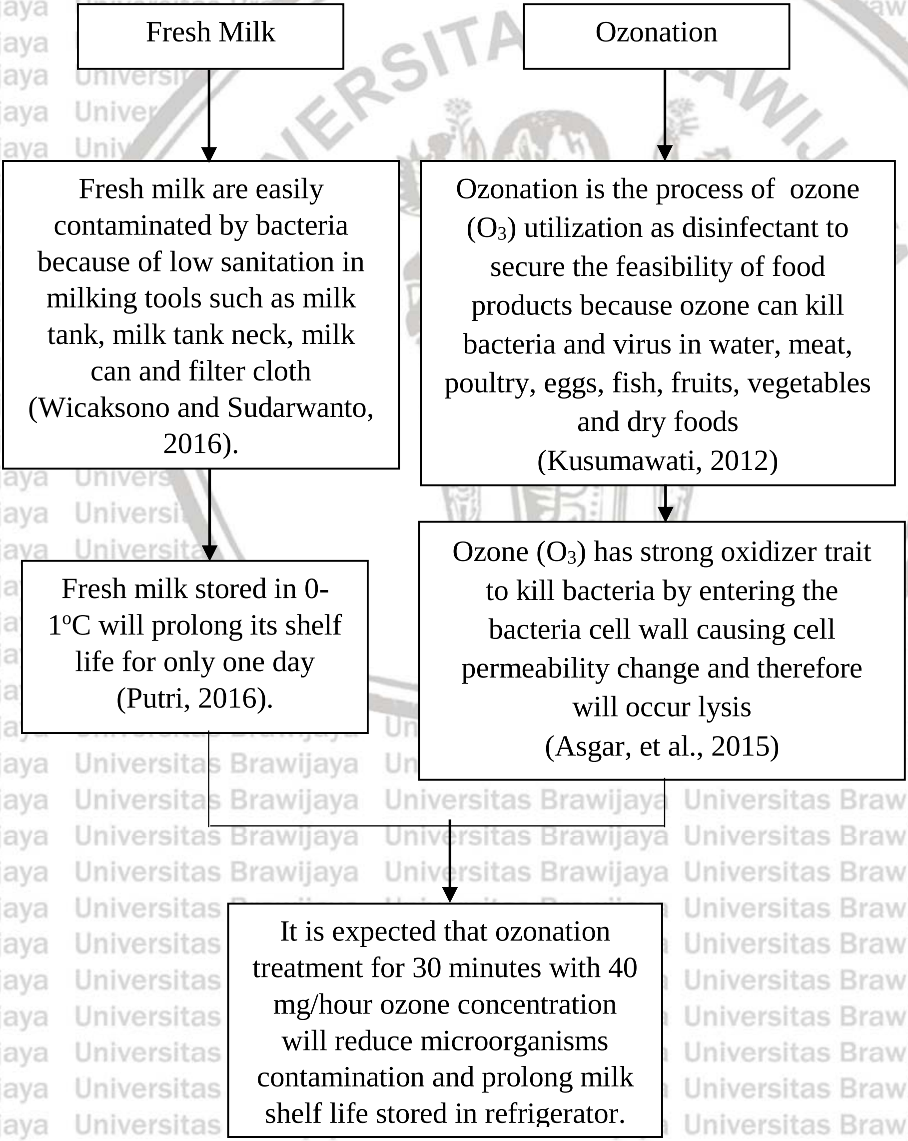

Figure 1. Framework Scheme

CHAPTER II

LITERATURE REVIEW

2.1. Milk

Milk is yellowish-white or bluish-white liquid containing high nutritional content resulted from mammalian udder gland such as cow, buffalo, horse, goat and camel (Usmiati and Abubakar, 2009). Fresh milk is liquid resulted from healthy and clean cow's udder, obtained by using good milking practice, with natural content without any addition or reduction and does not receive any treatment other than cold storage (BSN, 2011). Milk is a nearly perfect food that can be easily digested which means that the quality and quantity of milk production must be concerned to keep its nutritional content (Wicaksono and Sudarwanto, 2016).

The important components in milk are protein, fat, vitamin, mineral, lactose, enzymes and some species of microorganisms that give healthy effects as probiotics (Usmiati and Abubakar, 2009). Chemical composition in milk consist of 87,1% water, 3,4% protein, 3,9% fat, 4,9% carbohydrate, and 0,7% minerals (Thohari, et al., 2017). Fresh milk requirements based on BSN (2011) can be seen in Table 2.1.

Table 2.1. Fresh milk requirements

No.	Karakteristik	Satuan	Syarat
1	Berat Jenis (pada suhu 27,5^{O}C) minimum	g/ml	1,0270
2	Kadar lemak minimum	%	3,0
3	Kadar bahan kering tanpa lemak minimum	%	7,8
4	Kadar protein minimum	%	2,8
5	Warna, bau, rasa, kekentalan	-	Tidak ada perubahan
6	Derajat asam	^{O}SH	6,0-7,5
7	pH	-	6,3-6,8
8	Uji alkohol (70 %) v/v	-	Negatif
9	Cemaran mikroba, maksimum:		
	1. Total Plate Count	CFU/ml	1 x 10^{6}
	2. *Staphylococcus aureus*	CFU/ml	1 x 10^{2}
	3. *Enterobacteriaceae*	CFU/ml	1 x 10^{3}
10	Jumlah sel somatis maksimum	Sel/ml	4x10^{5}

*) Table continued in next page

	*) Continued table from previous page		
11	Residu antibiotika (Golongan Penisilin, Tetrasiklin, Aminoglikosida, Makrolida)	-	Negatif
12	Uji pemalsuan	-	Negatif
13	Titik beku	^{O}C	-0,520 s.d -0,560
14	Uji peroxidase	-	Positif
15	Cemaran logam berat, maksimum:		
	1. Timbal (Pb)	µg/ml	0,02
	2. Merkuri (Hg)	µg/ml	0,03
	3. Arsen (As)	µg/ml	0,1

Source: (SNI 3141.1: 2011)

2.2. Bacterial Contamination in Milk

One of the milk quality indicators is bacterial contamination, the limit of bacterial contamination in milk based on BSN (2011) are: Total Plate Count (TPC) 10^6 CFU/ml, *Staphylococcus aureus* 10^2 CFU/ml and *Enterobacteriaceae* 10^3 CFU/ml. The bacterial contamination level in milk from small farmers tend to be high because of low sanitation level such as the inside of milk tank, neck of milk tank, milk bucket and milk filter cloth (Wicaksono and Sudarwanto, 2016).

The high nutrition and water content in milk is prone to be used by bacteria as growth medium which will reduce the good benefits of milk and will spoil the milk quickly there is no good handling practices after milking (Yudonegoro, et al., 2014). Milk is preferred by bacteria because of its complete nutritional content with normal pH which is 6,6-6,8 and also its high water content around 87-88% (Murti, 2010).

2.3. Ozonation

Ozonation is the process of using ozone (O_3) as a disinfectant to ensure the quality of food products because ozone can kill bacteria and viruses in water, meat, poultry, eggs, fish, fruits, vegetables and dry foods (Kusumawati, 2012). Utilization of ozone at low concentrations between 0.01 ppm - 4.00 ppm is safe to apply in agriculture, health, the environment and industry sector (Haifan, 2017). The usage of ozone for antimicrobial purpose is already proven whether its on gram-positive bacteria, gram-negative bacteria, fungi and viruses, however the level of microbial inactivation can vary according to some factors such as organic matters, pH value and temperature (Munhōs, et al., 2019)

Ozone can be formed by using UV rays or dielectri barrier discharge plasma that will break oxygen molecules into two oxygen atoms, those oxygen atoms will react and form ozone (Yulianto, et al., 2019)

Ozone has a bactericidal effect due to its high oxidation potential (Couto, et al., 2016). Ozonation works by the ozone reacts with all cell protoplasms and acts as an oxidizer. Ozone will directly attack the surface layer of bacteria, namely conducting oxidation of sulfidrites from enzymes, or conducting oxidation with lipoproteins and lipopolysaccharides which are the largest layers of gram-negative bacteria. This will result in the breakdown of cell permeability defense and it will cause lysis (Rusdi and Suliasih, 2002).

2.4. Milk pH

pH (power of Hydrogen) value is the bond of hydrogen ions which dissociate in solution. Increasing the concentration of H+ (protons) will also increase the acidity of the material. A solution with a high pH indicates a low concentration of H+ (Lawrie, 2003).

Acidity is one of the parameters of milk quality. Milk acidity is caused by acidic compounds. Acids in milk are mostly lactic acid and various acidic compounds such as citric acid, amino acids and carbon dioxide that are soluble in milk (Kencanawati, et al., 2015). Acidity in milk occurs due to the formation of lactose from lactic acid by bacteria, so the longer period of time milk acidity will continue to increases due to the increasing number of bacteria that speeds up lactose fermentation to lactic acid (Nababan, et al., 2014).

BSN (2011) stated that the standard pH of fresh milk is 6.3-6.8. Milk pH that exceed 6.7 indicate the possibility that milk is produced from mastitis cow and if the pH value is below 6.5 then the milk may be colostrum or has been spoiled by bacteria (Soeparno, et al., 2011).

2.5. Protein

Protein is main macromolecule component used by organisms to synthesize new proteins based on their needs (Susanti and Hidayat, 2016). Protein is a vital ingredients for food because it provides essential amino acids needed for health and combined with wide functional properties such as to stabilize foams and emulsions (Singh, et al., 2014). Milk protein has high biological value which makes it a good source of amino acids with some functional properties and become a good source of protein diet (Padaga and Aulanni'am, 2017).

Duration of cold storage can decrease protein content in fresh milk because of microorganisms, where the microorganisms will decompose protein into metabolites such as indole, kadeverin, organic acids, CO_2, H_2S and sketol (Putri, 2016). Microorganisms will produce proteolitic enzymes to break protein down into oligopeptides and amino acids which will be used by the microorganisms as energy, this reaction will produce water and cause the protein content to decreases (Buckle, 2007).

CHAPTER III

MATERIALS AND METHODS

3.1. Research Location and Time

This research was conducted in August until September 2020 at Laboratory of Livestock Product Technology, Animal Science Faculty, Brawijaya University, Malang.

3.2. Research Materials

3.2.1. Fresh Milk

This research will use 3 L fresh milk obtained from Mr. Amin's farm, one of Malang local farmer located in Pujon, Malang.

3.2.2. Ozone Generator "Hanaco"

This research will use ozone generator "Hanaco" brand that can produce 400 mg/hour ozone for 1 L sample.

Previous research from Sari and Hadi (2014) shows that "Hanaco" ozone generator only produce 42,11 mg/hour of ozone.

3.2.3. Equipments and Materials

The equipments that will be used for this research are ozone generator, petrie dish, micropipette, incubator, autoclave, measuring tube, erlenmeyer, beaker glass, analytical balance, magnetic stirrer, vortex mixer, pH meter.

The main materials that will be used for this research are fresh milk from Mr. Amin's farm, aquadest, peptone, *Plate Count Agar* (PCA), buffer solution pH 4, buffer solution pH 7, fenolftalein 1%, potassium oxalate, NaOH 0,1N, formaldehide 40%.

3.3. Research Method

The research method was Nested Experimental Design with 2 types of milk and 5 treatments nested in milk.

Ozonated milk:

T_0 : Milk 0 minute after ozonation treatment

T_1 : Ozonated milk 24 hours after cold storage

T_2 : Ozonated milk 48 hours after cold storage

T_3 : Ozonated milk 72 hours after cold storage

T_4 : Ozonated milk 96 hours after cold storage

Fresh milk:

T_0 : Fresh milk without ozonation

T_1 : Fresh milk 24 hours after cold storage

T_2 : Fresh milk 48 hours after cold storage

T_3 : Fresh milk 72 hours after cold storage

T_4 : Fresh milk 96 hours after cold storage

3.4. Research Procedure

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

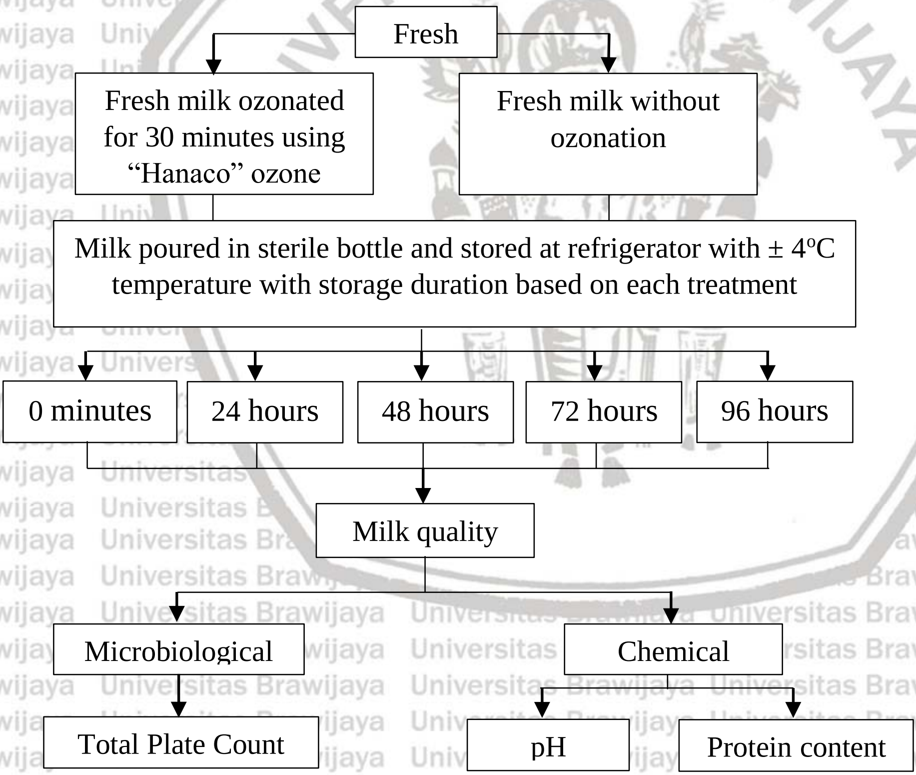

Figure 2. Research procedure

3.4.1. Fresh Milk Collecting

3 L Fresh milk collected from Mr. Amin's farm unit by using plastic bottles each containing 500 mL of milk for each treatment and delivered using cooling box to laboratory.

3.4.2. Ozonation

500 mL of fresh milk poured in erlenmeyer and wait until the milk temperature reaches 15ºC. 15ºC temperature is used because it has the longest half life ofo ozone (Lenntech, 2020). Half life of ozone in pH 7 water at different temperatures can be seen in Table 3.1. Insert the diffuser stone of ozone generator into the milk, set the timer for 30 minutes and turn on the ozone generator. The ozone generator will stop working immediately after 30 minutes based on the timer. The preparation of ozonation process can be seen in Figure 3.

Table 3.1. Half life of ozone in pH 7 water at different temperatures

Temperature (ºC)	Half life
15	30 minutes
20	20 minutes
25	15 minutes
30	12 minutes
35	8 minutes

Source : (Lenntech, 2020)

Figure 3. Ozone generator preparation

3.4.3. Storage

Ozonated milk and fresh milk poured into plastic bottle, locked tight and stored in refrigerator at ± 4°C temperature with duration based on each treatment before testing: 24, 48, 72 and 96 hours. For treatment 0 (T_0) will be tested without cold storage

3.5. Research Variables

3.5.1. Total Plate Count

Total Plate Count of milk will be counted by diluting milk sample then grow the sample in Plate Count Agar using pour plate method. The sample will be incubated at 37-40°C for 24 hours before calculating the Total Plate Count (Radiati, et al., 2019).

3.5.2. pH

The milk pH will be measured by using pH meter (Cable, 2005). The pH meter electrode will be calibrated first by using buffer solution with pH 4 and 7. Milk pH measured by dipping the pH electrode into the milk and the screen will show its pH.

3.5.3. Protein Content

Protein content will be measured by using formol titration method (Rohman and Sumantri, 2017). 10 mL of milk added with 20 mL aquadest; 0,4 mL saturated potassium oxalate solution and 1 mL fenolftalein indicator 1% then let it for 2 minutes before titrated with NaOH 0,1 N until it reaches standard color (standard color is the color resulted by adding 10 mL milk with 10 mL aquadest; 0,4 mL saturated potassium oxalate solution and one drop of rosanilin chloride indicator 0.01%). After the mixture reaches standard color, add 2 mL of 40% formaldehide solution and titrated with NaOH 0,1 N until it reaches standard color. Blank titration made by mixing 20 mL aquadest, 0,4 mL saturated potassium oxalate solution, 1 mL fenolftalein indicator 1% and 2 mL of 40% formaldehide solution and titrated with standar solution of NaOH.

Formol Titrant = Sample Titration Volume – Blank Titration Volume

Milk Protein Content = Formol Titrant x 1,83

3.6. Data Analysis

Obtained data will be analyzed statistically using Nested Experimental Design method and variance analysis. If there is significant result Duncan Multiple Range Test (DMRT) will be used to determine the difference in each treatment. The mathematical model is :

$$Y_{ij(k)} = \mu + \alpha_i + \beta_{j(i)} + \varepsilon_{k(ij)}$$

Where:

Y_{ij} = observation of A factor i-levek and B factor j-level with k replication

μ = general average

α_i = effects of A factor at i-level

$\beta_{j(i)}$ = effects of B factor at j-level nested on A factor at i level

$\varepsilon_{k(ij)}$ = effects of error

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. Ozonation : Milk tratment by infusing ozone (O_3) into milk to eliminate microorganisms.
b. *Total Plate Count* (TPC) : Method to count the amount of microorganisms in a sample.
c. Dilution : Process to decrease the concentration of a sample by mixing it with other solvent such as aquadest or peptone water.
d. Calibration : Act to adjust the instrument to provide more accurate results.
e. Titration : Slow addition of a solution with known concentration to determine the concentration of unknown solution.
f. Incubation : Process to incubate microorganisms in constant temperature to let the microorganisms grow

CHAPTER IV

RESULTS AND DISCUSSIONS

4.1. Total Plate Count (TPC)

The average amount of bacteria in ozonated milk and fresh milk stored in refrigerator are presented in Table 4.1 – Table 4.2.

Table 4.1. Average amount of bacteria in milk during refrigerator storage

Milk	Treatments	Average amount of bacteria (log CFU/ml)
Ozonated milk	T0 (without storage)	5.96 ± 0.03[a]
	T1 (24 hours)	6.01 ± 0.03[a]
	T2 (48 hours)	6.01 ± 0.02[a]
	T3 (72 hours)	6.78 ± 0.02[b]
	T4 (96 hours)	7.19 ± 0.01[c]
Fresh Milk	T0 (without storage)	6.05 ± 0.02[a]
	T1 (24 hours)	6.16 ± 0.02[b]
	T2 (48 hours)	6.24 ± 0.02[b]
	T3 (72 hours)	7.27 ± 0.07[c]
	T4 (96 hours)	8.06 ± 0.06[d]

Notes: Different superscripts (a-d) in the same column show highly significant difference ($P<0.01$). Superscript order shows the total bacteria amount from lowest to highest.

Table 4.2. Average amount of bacteria during refrigerator storage nested in milk

Treatments	Milk	Average amount of bacteria (log CFU/ml)
T0 (without storage)	Ozonated Milk	5.96 ± 0.03[a]
	Fresh Milk	6.05 ± 0.02[b]
T1 (24 hours)	Ozonated Milk	6.01 ± 0.03[a]
	Fresh Milk	6.16 ± 0.02[b]
T2 (48 hours)	Ozonated Milk	6.01 ± 0.02[a]
	Fresh Milk	6.24 ± 0.02[b]
T3 (72 hours)	Ozonated Milk	6.78 ± 0.02[a]
	Fresh Milk	7.27 ± 0.07[b]
T4 (96 hours)	Ozonated Milk	7.19 ± 0.01[a]
	Fresh Milk	8.06 ± 0.06[b]

Notes: Different superscripts (a-b) in the same column show highly significant difference ($P<0.01$). Superscript order shows the total bacteria amount from lowest to highest.

One of the milk quality indicators is bacterial contamination, the limit of Total Plate Count (TPC) in fresh milk based on BSN (2011) is 10^6 CFU/ml or 6 log CFU/ml. The bacterial contamination level in milk from small farmers tend to exceed the national standard because of low sanitation level such as the inside of milk tank, neck of milk tank, milk bucket and milk filter cloth (Wicaksono and Sudarwanto, 2016). The high nutrition and water content in milk is prone to be used by bacteria as growth medium which will reduce the good benefits of milk and will spoil the milk quickly there is no good handling practices after milking (Yudonegoro, et al., 2014).

Analysis of TPC in this research was carried out by growing it in PCA. The colonies' shape tend to be round or oval (Yousef and Carlstorm, 2003). The average amount of total bacteria in ozonated milk and fresh milk stored in refrigerator is presented in Table 4.1 – Table 4.2 while the statistical analysis is presented in appendix 4.

Analysis of variance showed that there is highly significant difference of total bacteria among treatment in ozonated and fresh milk. The average amount of bacteria in fresh milk from T0; T1; T2; T3; and T4 respectively is 6,05; 6,16; 6,24; 7,27; and 8,06 log CFU/ml while in ozonated milk the average amount of bacteria from T0; T1; T2; T3; and T4 respectively is 5,96; 6,01; 6,01; 6,78; and 7,19 log CFU/ml. The average amount of bacteria both in ozonated and fresh milk was increasing due to nutritional content of milk, milk neutral pH which is 6,6-6,8 and its high water content which is around 87-88% (Murti, 2010).

The result shows that the bacteria in fresh milk grow faster during storage compared to ozonated milk. It also can be seen from the ANOVA results where there is highly significant effect of cold storage on total bacteria in both ozonated and fresh milk, but the bacteria amount in ozonated milk had a high siginificant difference after 72 hours (T3) while the bacteria amount in fresh milk already had a significant difference after 24 hours (T1). This could be resulted because ozonated milk still contain ozone during the storage because of the ozone treatment as ozone has longer half life in low temperature based on Lenntech (2020) that ozone can be dissolved in water at 15^oC with pH 7 for 30 minutes, milk was ozonated at 15^oC and then stored at 4^oC that cause ozone to dissolved in milk longer and killed the bacteria for the first several hours. This result is similar to earlier research done by Genecya, et al., (2019) which shows that bacterial growth in ozonated milk during cold storage is faster than in fresh milk.

Ozone has a bactericidal effect due to its high oxidation potential (Couto, et al., 2016). Ozonation works by the ozone reacts with all cell protoplasms and acts as an oxidizer. Ozone will directly attack the surface layer of bacteria, namely conducting oxidation of sulfidrites from enzymes, or conducting oxidation with lipoproteins and lipopolysaccharides which are the largest layers of gram-negative bacteria. This will result in the breakdown of cell permeability defense and it will cause lysis (Rusdi and Suliasih, 2002).

4.2. pH Value

The average amount of pH value in ozonated milk and fresh milk stored in refrigerator are presented in table 4.3 – table 4.4.

Table 4.3. Average milk pH during refrigerator storage

Milk	Treatments	Average pH
Ozonated milk	T0 (without storage)	6,82 ± 0.10
	T1 (24 hours)	6.81 ± 0.07
	T2 (48 hours)	6.80 ± 0.05
	T3 (72 hours)	6.74 ± 0.04
	T4 (96 hours)	6.74 ± 0.08
Fresh Milk	T0 (without storage)	6.79 ± 0.02[c]
	T1 (24 hours)	6.64 ± 0.06[b]
	T2 (48 hours)	6.52 ± 0.04[ab]
	T3 (72 hours)	6.45 ± 0.07[a]
	T4 (96 hours)	6.43 ± 0.01[a]

Notes: Different superscripts (a-c) in the same column show highly significant difference (P<0.01). Superscript order shows the pH from lowest to highest.

Table 4.4. Average milk pH during refrigerator storage nested in milk

Treatments	Milk	Average pH
T0 (without storage)	Fresh Milk	6.79 ± 0.02
	Ozonated Milk	6,82 ± 0.10
T1 (24 hours)	Fresh Milk	6.64 ± 0.06[a]
	Ozonated Milk	6.81 ± 0.07[b]
T2 (48 hours)	Fresh Milk	6.52 ± 0.04[a]
	Ozonated Milk	6.80 ± 0.05[b]
T3 (72 hours)	Fresh Milk	6.45 ± 0.07[a]
	Ozonated Milk	6.74 ± 0.04[b]
T4 (96 hours)	Fresh Milk	6.43 ± 0.01[a]
	Ozonated Milk	6.74 ± 0.08[b]

Notes: Different superscripts (a-b) in the same column show highly significant difference (P<0.01). Superscript order shows the the pH from lowest to highest.

pH (power of Hydrogen) value is the bond of hydrogen ions which dissociate in solution. Increasing the concentration of H+ (protons) will also increase the acidity of the material. A solution with a high pH indicates a low concentration of H+ (Lawrie, 2003). pH indicates milk acidity, acidity is one of the parameters of milk quality. Milk acidity is caused by acidic compounds. Acids in milk are mostly lactic acid and various acidic compounds such as citric acid, amino acids and carbon dioxide that are soluble in milk (Kencanawati, et al., 2015). The average pH of milk stored in refrigerator is presented in Table 4.3 – Table 4.4 while the statistical analysis is presented in appendix 5.

The result shows that the pH value of fresh milk decreased rapidly during refrigerator storage while the pH value of ozonated milk was just slowly decreasing. The ANOVA result shows that there is highly significant effect of cold storage on pH value in fresh milk while there is no significant effect of cold storage on pH value in ozonated milk. The average pH value in fresh milk from T0; T1; T2; T3; and T4 respectively is 6,79; 6,64; 6,52; 6,45; and 6,43 while in ozonated milk the average amount of pH value from T0; T1; T2; T3; and T4 respectively is 6,82; 6,81; 6,80; 6,74; and 6,74. The pH value was decreasing during storage because of the increasing amount of total bacteria, acidity in milk occurs due to the formation of lactose from lactic acid by bacteria, so the longer period of time milk acidity will continue to increases due to the increasing number of bacteria that speeds up lactose fermentation to lactic acid (Nababan, et al., 2014).

4.3. Protein Content

The average amount of protein content in ozonated milk and fresh milk stored in refrigerator are presented in Table 4.5- table 4.6.

Table 4.5. Average protein content during refrigerator storage

Milk	Treatments	Average protein content (%)
Ozonated milk	T0 (without storage)	2.77 ± 0.11
	T1 (24 hours)	2.64 ± 0.06
	T2 (48 hours)	2.63 ± 0.09
	T3 (72 hours)	2.63 ± 0.12
	T4 (96 hours)	2.61 ± 0.08
Fresh Milk	T0 (without storage)	2.66 ± 0.19[c]
	T1 (24 hours)	2.57 ± 0.10[bc]
	T2 (48 hours)	2.47 ± 0.12[abc]
	T3 (72 hours)	2.39 ± 0.10[ab]
	T4 (96 hours)	2.29 ± 0.12[a]

Notes: Different superscripts (a-c) in the same column show significant difference ($P<0.05$). Superscript order shows the protein content from lowest to highest

Table 4.6. Average protein content during refrigerator storage nested in milk

Treatments	Milk	Average protein content (%)
T0 (without storage)	Fresh Milk	2.66 ± 0.19
	Ozonated Milk	2.77 ± 0.11
T1 (24 hours)	Fresh Milk	2.57 ± 0.10
	Ozonated Milk	2.64 ± 0.06
T2 (48 hours)	Fresh Milk	2.47 ± 0.12
	Ozonated Milk	2.63 ± 0.09
T3 (72 hours)	Fresh Milk	2.39 ± 0.10
	Ozonated Milk	2.63 ± 0.12
T4 (96 hours)	Fresh Milk	2.29 ± 0.12[a]
	Ozonated Milk	2.61 ± 0.08[b]

Notes: Different superscripts (a-b) in the same column show highly significant difference (P<0.01). Superscript order shows the protein content from lowest to highest.

Protein is a vital ingredients for food because it provides essential amino acids needed for health and combined with wide functional properties such as to stabilize foams and emulsions (Singh, et al., 2014). Milk protein has high biological value which makes it a good source of amino acids with some functional properties and become a good source of protein diet (Padaga and Aulanni'am, 2017). The average protein content of milk stored in refrigerator is presented in Table 4.5 – Table 4.6 while the statistical analysis is presented in appendix 6.

The result shows that the protein content of fresh milk decreased rapidly during refrigerator storage while the protein content of ozonated milk was just slowly decreasing. The

ANOVA result shows that there is significant effect of cold storage on protein content in fresh milk while there is no significant effect of cold storage on protein content in ozonated milk. The average protein content in fresh milk from T0; T1; T2; T3; and T4 respectively is 2,66; 2,57; 2,47; 2,39; and 2,29 while in ozonated milk the average amount of pH value from T0; T1; T2; T3; and T4 respectively is 2,77; 2,64; 2,63; 2,63; and 2,61. The protein content was decreasing during storage because of the increasing amount of total bacteria, bacteria will produce proteolitic enzymes to break protein down into oligopeptides and amino acids which will be used by the bacteria as energy, this reaction will produce water and cause the protein content to decreases (Buckle, 2007). Research done by Putri (2016) also confirms that cold storage will decrease milk protein content as bacteria will decompose protein into metabolites such as indole, kadeverin, organic acids, CO_2, H_2S and sketol.

CHAPTER V

CONCLUSIONS AND RECOMMENDATIONS

5.1. Conclusions

It was concluded that:

- Ozonation slows the bacteria growth during cold storage where the bacteria in ozonated milk had a highly significant increase after 72 hours of cold storage (T3) while bacteria in fresh milk already had a highly significant increase just after 24 hours of cold storage (T1).
- Ozonation prevent milk pH from decreasing significantly during cold storage where the pH of ozonated milk did not have any significant increase during cold storage while pH in fresh milk already had a highly significant decrease just after 24 hours of cold storage (T1).
- Ozonation prevent milk protein content from decreasing during cold storage where the protein content of ozonated milk did not have any significant increase during cold storage while protein content in fresh milk had a siginificant difference between T0 and T4.

5.2. Recommendations

Further research about ozone half life in milk is suggested to be done as the author found that the ozone odor still smell strongly until 72 hours of storage.

The usage of sterilized glass bottle as fresh milk container from farm and during storage will be better than the usage of plastic bottle to reduce contamination risks.

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APPENDIXES

Appendix 1. The procedure of milk pH assessment

According to Cable (2005), the procedure for pH value analysis of milk using pH meter is as follows:

1. Clean the electrode of pH meter using aquadest and wipe the electrode with tissue
2. Dip the electrode to buffer solution pH 4
3. Rinse the electrode with aquadest and wipe the electrode with tissue
4. Dip the electrode to buffer solution pH 7
5. Rinse the electrode with aquadest and wipe the electrode with tissue
6. Dip the electrode to the milk sample
7. Read the pH value on the monitor

Appendix 2. The procedure of milk protein content assessment

According to Rohman and Sumantri (2018), the procedure to measure protein content in milk is as follows:

Sample Titration

1. Pour 10 mL milk sample to erlenmeyer 125 mL.
2. add 20 mL aquadest,0,4 mL saturated potassium oxalate and 1 mL fenolftalein indicator 1%.
3. Let it for 2 minutes
4. Titrated with NaOH 0,1 N until it reaches standard color (standard color is the color resulted by adding 10 mL milk sample with 10 mL aquadest; 0,4 mL saturated potassium oxalate solution and one drop of rosanilin chloride indicator 0.01%)
5. Add 2 mL formaldehide 40% solution.
6. titrated with NaOH 0,1 N until it reaches standard color
7. Take note of NaOH 0,1 N volume needed to reach standard color.

Blank Titration

1. Mix 20 mL aquadest, 0,4 mL saturated potassium oxalate solution, 1 mL fenolftalein indicator 1% and 2 mL of 40% formaldehide solution.
2. titrated with NaOH 0,1 N until it reaches standard color
3. Take note of NaOH 0,1 N volume needed to reach standard color.

Formol titrant = Sample titration volume – Blank titration volume

Milk protein content = Formol titrant x 1,83

Appendix 3. Inoculant planting using pour plate method procedure

1. **Preparation of Plate Count Agar (PCA) as growth media**

   PCA were dissolved in aquadest with the dose of 22,5 grams PCA for every liter of aquadest, heated and homogenized with an ekectric stove until the solution become clear. Covered with cotton and aluminium foil, sterilized in autoclave.

2. **Preparation of peptone water as growth media**

   Peptone were dissolved in aquadest with the dose of 1 gram peptone for every liter of aquadest, heated and homogenized with an ekectric stove until the solution become clear. Covered with cotton and aluminium foil, sterilized in autoclave.

3. **Milk sample dilution**

   Sample dilution done in Laminar Air Flow (LAF). 1 mL of milk taken using micropipette and inserted into a reaction tube containing 9 mL of sterile peptone water, homogenized and the first dilution (10^{-1}) obtained. Taken 1 mL of the first dilution and inserted intp a reaction tube containing 9 mL of sterile peptone water, homogenized and the second dilution (10^{-2}) obtained. Dilution process continud until the sixth dilution (10^{-6}).

4. **Inoculant Planting**

   Inoculant planting done by using pour plate (PP) method in Laminar Air FLOW (LAF). 1 mL of the selected dilution inserted in a sterile petrie dish using micropipette then poured with 10-15 mL of sterile PCA solution and homogenized, wait until the media solidified and incubated at 37°C for 24 hour. Inoculant planting was done using the last 3 dilution (10^{-4}, 10^{-5}, and 10^{-6}).

5. **Calculating of total colonies**

Calculating done after the incubation process completed. Calculating was done manually using mica paper and permanent marker. Founded bacteria are marked and drawn on the mica paper.

Guidelines to calculate bacteria :

PP = $\frac{1}{dilution\ factor}$ x total colonies (colonies counted are colonies within 30-300 range)

a. If total colonies are all <30, then counted using the lowest dilution factor.
b. If total colonies are all >300, then counted using the highest dilution factor.
c. If there is one colony within 30-300, counted using that dilution factor.
d. If there are 2 colonies within 30-300, made a comparison between dilution factor, if the comparison result >2, counted using the lowest dilution factor, if the comparison ≤2, made an average between the 2 diluton factor.
e. If all 3 colonies are within 30-300, made a group and compared, choose the group with comparison result closest to 2, if that comparison result >2, counted using the lowest dilution factor in that group, if the comparison ≤2, made an average between the 3 diluton factor.

Appendix 4. Data and statistical analysis of milk Total Plate Count

Milk	T	R	10^{-4}	10^{-5}	10^{-6}	CFU/ mL	LOG CFU/ mL
Ozo nated	0	1	96	10	5	$9,6 \times 10^5$	5,98
		2	86	11	2	$8,6 \times 10^5$	5,93
		3	91	10	4	$9,1 \times 10^5$	5,96
	1	1	95	32	1	$9,5 \times 10^5$	5,98
		2	106	22	1	$1,1 \times 10^6$	6,04
		3	100	27	0	$1,0 \times 10^6$	6,00
	2	1	106	8	3	$1,1 \times 10^6$	6,04
		2	103	10	5	$1,0 \times 10^6$	6,00
		3	104	9	4	$1,0 \times 10^6$	6,00
	3	1	TNTC	63	3	$6,3 \times 10^6$	6,80
		2	TNTC	58	4	$5,8 \times 10^6$	6,76
		3	TNTC	60	3	$6,0 \times 10^6$	6,78
	4	1	TNTC	157	10	$1,6 \times 10^7$	7,20
		2	TNTC	150	12	$1,5 \times 10^7$	7,18
		3	TNTC	148	19	$1,5 \times 10^7$	7,18
Fresh	0	1	107	7	4	$1,1 \times 10^6$	6,04
		2	118	40	5	$1,2 \times 10^6$	6,08
		3	113	24	4	$1,1 \times 10^6$	6,04
	1	1	140	15	3	$1,4 \times 10^6$	6,15
		2	138	10	2	$1,4 \times 10^6$	6,15
		3	145	13	2	$1,5 \times 10^6$	6,18
	2	1	167	12	4	$1,7 \times 10^6$	6,23
		2	181	18	0	$1,8 \times 10^6$	6,26
		3	174	15	2	$1,7 \times 10^6$	6,23
	3	1	TNTC	217	20	$2,2 \times 10^7$	7,34
		2	TNTC	157	37	$1,6 \times 10^7$	7,20
		3	TNTC	189	29	$1,9 \times 10^7$	7,28
	4	1	TNTC	TNTC	102	$1,0 \times 10^8$	8,00

		2	TNTC	TNTC	117	$1,2 \times 10^8$	8,08
		3	TNTC	TNTC	126	$1,3 \times 10^8$	8,11

Notes : T = Treatment
R = Replication

Milk	Storage Treatment	Replication			S Total	M Total
		1	2	3		
Ozonated	T0	5.98	5.93	5.96	17.87	
	T1	5.98	6.04	6.00	18.02	
	T2	6.04	6.00	6.00	18.04	
	T3	6.8	6.76	6.78	20.34	
	T4	7.2	7.18	7.18	21.56	95.83
Fresh	T0	6.04	6.08	6.04	18.16	
	T1	6.15	6.15	6.18	18.48	
	T2	6.23	6.26	6.23	18.72	
	T3	7.34	7.2	7.28	21.82	
	T4	8	8.08	8.11	24.19	101.37
Total						197.20

Notes : S Total = Storage Treatment Total
M Total = Milk Total

a. Correction Factor (CF)

$$CF = \frac{(197.20)^2}{2x5x3}$$
$$= 1296.261$$

b. Sum of square total

$$SS\ T = (5,98^2 + \ldots + 8,11^2) – 1296.261$$
$$= 14.11$$

c. Sum of square milk

$$SS\ M = \frac{95.83^2 + 101.37^2}{3 \times 5} - 1296.261$$

$= 1.02305$

d. Sum of square storage nested in ozonated milk

$$SS\ S\text{-Ozonated} = \frac{17.87^2 + \cdots + 21.56^2}{3} - \frac{95.83^2}{3 \times 5}$$

$= 3.790106667$

e. Sum of square storage nested in fresh milk

$$SS\ S\text{-Fresh} = \frac{18.16^2 + \cdots + 24.19^2}{3} - \frac{101.37^2}{3 \times 5}$$

$= 9.27584$

f. Sum of square storage nested in milk

SS S-M = SS S-Ozonated + SS S-Fresh

= 3.790106667 + 9.27584

= 13.06595

g. Sum of square error

SS E = SS T – SS M – SS S-M

= 14.11 - 1.02305 - 13.06595

= 0.02

Analysis of Variance (ANOVA)

SV	df	SS	MS	Fscore	F0,05	F0,01
Milk	1	1,023	1,0230	857,307	5,32	11,26
(S-M)	8	13,0659	1,6332	1368,64	2,45	3,56
Error	20	0,02	0,0011			
Total	29	14,11				

Conclusion : Fscore > F0.01 means that there is highly significant difference of total plate count between milk. There is highly significant effect of refrigerator storage duration nested in milk on total plate count

Further test was carried out using Duncan's Multiple Range Test (DMRT)

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

$= \sqrt{\frac{0,00119}{3}}$

$= 0.0199444$

LSR 1% $= (0.01 \text{ ; df error}) \times S_X$

1% of Duncan's Multiple Range Test (DMRT) critical table

P	2	3	4	5
SSR 1%	4,024	4,197	4,312	4,395
LSR 1%	0,0802	0,0837	0,086	0,0876

Ozonated Milk Notation

Treatments	Average	Notation
T0	5.96 ± 0.03	a
T1	6.01 ± 0.03	a
T2	6.01 ± 0.02	a
T3	6.78 ± 0.02	b
T4	7.19 ± 0.01	c

Fresh Milk Notation

Treatments	Average	Notation
T0	6.05 ± 0.02	a
T1	6.16 ± 0.02	b
T2	6.24 ± 0.02	b
T3	7.27 ± 0.07	c
T4	8.06 ± 0.06	d

T0 Notation

Milk	Average	Notation
Ozonated	5.96 ± 0.03	a
Fresh	6.05 ± 0.02	b

T1 Notation

Milk	Average	Notation
Ozonated	6.01 ± 0.03	a
Fresh	6.16 ± 0.02	b

T2 Notation

Milk	Average	Notation
Ozonated	6.01 ± 0.02	a
Fresh	6.24 ± 0.02	b

T3 Notation

Milk	Average	Notation
Ozonated	6.78 ± 0.02	a
Fresh	7.27 ± 0.07	b

T4 Notation

Milk	Average	Notation
Ozonated	7.19 ± 0.01	a
Fresh	8.06 ± 0.06	b

Appendix 5. Data and statistical analysis of milk pH

Milk	Treatment	Replication			T	M
		1	2	3	Total	Total
Ozonated	T0	6,77	6,93	6,75	20,45	
	T1	6,81	6,74	6,88	20,43	
	T2	6,85	6,75	6,80	20,40	
	T3	6,70	6,75	6,77	20,22	
	T4	6,69	6,83	6,71	20,23	101.73
Fresh	T0	6,79	6,81	6,77	20,37	
	T1	6,67	6,58	6,68	19,93	
	T2	6,49	6,56	6,50	19,55	
	T3	6,38	6,51	6,46	19,35	
	T4	6,42	6,44	6,44	19,30	98.5
Total						200.23

Notes : T Total = Treatment Total
M Total = Milk Total

a. Correction Factor (CF)

CF $= \frac{(200.23)^2}{2x5x3}$

$= 1336.46$

b. Sum of square total

SS T $= (6.77^2 + ... + 6.44^2) - 1336.46$

$= 0.70$

c. Sum of square milk

SS M $= \frac{101.73^2 + 98.5^2}{3\ x\ 5} - 1336.46$

$= 0.34863$

d. Sum of square storage nested in ozonated milk

$$SS\ S\text{-}Ozonated = \frac{20.45^2 + \cdots + 20.23^2}{3} - \frac{101.73^2}{3 \times 5}$$

= 0,016935

e. Sum of square storage nested in fresh milk

$$SS\ S\text{-}Fresh = \frac{20.37^2 + \cdots + 19.30^2}{3} - \frac{98.5^2}{3 \times 5}$$

= 0,268933

f. Sum of square storage nested in milk

SS S-M = SS S-Ozonated + SS S-Fresh
= 0,016935 + 0,268933
= 0,28587

g. Sum of square error

SS E = SS T – SS M – SS S-M
= 0.70 – 0.34863 – 0.28587
= 0.07

Analysis of Variance (ANOVA)

SV	df	SS	MS	Fscore	F0,05	F0,01
Milk	1	0,3486	0,3486	104,154	5,32	11,26
(S-M)	8	0,2858	0,0357	10,6756	2,45	3,56
Error	20	0,07	0,0033			
Total	29	0,70				

Conclusion : Fscore > F0.01 means that there is highly significant difference of pH value between milk. There is highly significant effect of refrigerator storage duration nested in milk on pH value

Further test was carried out using Duncan's Multiple Range Test (DMRT)

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

$$= \sqrt{\frac{0.00335}{3}}$$

$$= 0,033403$$

LSR 1% = (0.01 ; df error) x S_X

1% of Duncan's Multiple Range Test (DMRT) critical table

P	2	3	4	5
SSR 1%	4,024	4,197	4,312	4,395
LSR 1%	0,0802	0,0837	0,086	0,0876

Ozonated Milk Notation

Treatments	Average	Notation
T0	6,82 ± 0.10	a
T1	6.81 ± 0.07	a
T2	6.80 ± 0.05	a
T3	6.74 ± 0.04	a
T4	6.74 ± 0.08	a

Fresh Milk Notation

Treatments	Average	Notation
T0	6.79 ± 0.02^{c}	c
T1	6.64 ± 0.06^{b}	b
T2	6.52 ± 0.04^{a}	ab
T3	6.45 ± 0.07^{a}	a
T4	6.43 ± 0.01^{a}	a

T0 Notation

Milk	Average	Notation
Fresh	6.79 ± 0.02[c]	a
Ozonated	6,82 ± 0.10	a

T1 Notation

Milk	Average	Notation
Fresh	6.64 ± 0.06[b]	a
Ozonated	6.81 ± 0.07	b

T2 Notation

Milk	Average	Notation
Fresh	6.52 ± 0.04[a]	a
Ozonated	6.80 ± 0.05	b

T3 Notation

Milk	Average	Notation
Fresh	6.45 ± 0.07[a]	a
Ozonated	6.74 ± 0.04	b

T4 Notation

Milk	Average	Notation
Fresh	6.43 ± 0.01[a]	a
Ozonated	6.74 ± 0.08	b

Appendix 6. Data and statistical analysis of milk protein content

Milk	Treatment	Replication			T Total	M Total
		1	2	3		
Ozonated	T0	2,7	2,72	2,9	8,32	
	T1	2,71	2,61	2,6	7,92	
	T2	2,53	2,7	2,66	7,89	
	T3	2,51	2,64	2,74	7,89	
	T4	2,63	2,53	2,68	7,84	39.86
Fresh	T0	2,52	2,59	2,87	7,98	
	T1	2,46	2,61	2,64	7,71	
	T2	2,53	2,54	2,33	7,40	
	T3	2,28	2,42	2,48	7,18	
	T4	2,27	2,18	2,41	6,86	37.13
Total						76.99

Notes : T Total = Treatment Total
M Total = Milk Total

a. Correction Factor (CF)

$$CF = \frac{(76.99)^2}{2x5x3}$$
$$= 197.582$$

b. Sum of square total

$$SS\ T = (2.7^2 + ... + 2.41^2) – 197.582$$
$$= 0.81$$

c. Sum of square milk

$$SS\ M = \frac{39.86^2 + 37.13^2}{3\ x\ 5} – 197.582$$
$$= 0.24843$$

d. Sum of square storage nested in ozonated milk

SS S-Ozonated $= \frac{8.32^2 + \cdots + 7.84^2}{3} - \frac{39.86^2}{3 \times 5}$

= 0,05156

e. Sum of square storage nested in fresh milk

SS S-Fresh $= \frac{7.98 + \ldots + 6.86^2}{3} - \frac{37.13^2}{3 \times 5}$

= 0,256373333

f. Sum of square storage nested in milk

SS S-M = SS S-Ozonated + SS S-Fresh
= 0,05156 + 0,256373333
= 0,30793

g. Sum of square error

SS E = SS T – SS M – SS S-M
= 0.81 – 0.24843 – 0.30793
= 0.25

Analysis of Variance (ANOVA)

SV	df	SS	MS	Fscore	F0,05	F0,01
Milk	1	0,2484	0,2484	19,9595	5,32	11,26
(S-M)	8	0,3079	0,0384	3,0925	2,45	3,56
Error	20	0,25	0,0124			
Total	29	0,81				

Conclusion : Fscore > F0.01 means that there is highly significant difference of protein content between milk. Fscore > F0.05 means that there is significant effect of refrigerator storage duration nested in milk on protein content.

Further test was carried out using Duncan's Multiple Range Test (DMRT)

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

$$= \sqrt{\frac{0.01245}{3}}$$

$$= 0{,}064412$$

LSR 1% = (0.01 ; df error) x S_X

5% of Duncan's Multiple Range Test (DMRT) critical table

P	2	3	4	5
SSR 5%	2,95	3,097	3,19	3,255
LSR 5%	0,1900	0,1994	0,2054	0,2096

1% of Duncan's Multiple Range Test (DMRT) critical table

P	2
SSR 1%	4,024
LSR 1%	0,259193362

Ozonated Milk Notation

Treatments	Average	Notation
T0	2.77 ± 0.11	a
T1	2.64 ± 0.06	a
T2	2.63 ± 0.09	a
T3	2.63 ± 0.12	a
T4	2.61 ± 0.08	a

Fresh Milk Notation

Treatments	Average	Notation
T0	2.66 ± 0.19	c
T1	2.57 ± 0.10	bc
T2	2.47 ± 0.12	abc
T3	2.39 ± 0.10	ab
T4	2.29 ± 0.12	a

T0 Notation

Milk	Average	Notation
Fresh	2.66 ± 0.19	a
Ozonated	2.77 ± 0.11	a

T1 Notation

Milk	Average	Notation
Fresh	2.57 ± 0.10	a
Ozonated	2.64 ± 0.06	a

T2 Notation

Milk	Average	Notation
Fresh	2.47 ± 0.12	a
Ozonated	2.63 ± 0.09	a

T3 Notation

Milk	Average	Notation
Fresh	2.39 ± 0.10	a
Ozonated	2.63 ± 0.12	a

T4 Notation

Milk	Average	Notation
Fresh	2.29 ± 0.12	a
Ozonated	2.61 ± 0.08	b

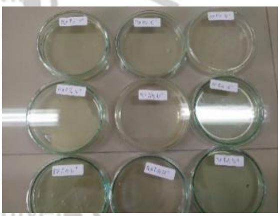
Appendix 10. Research documentation

Milk pH assessment

Formol titration results

Formol titration process

Formol titration process



Petrie dishes after 24 hours incubation

Inoculant planting process in LAF

Ozonation process

Ozonation Preparation