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Physical, Chemical, and Microbiological Quality of Fresh Cow's Milk in Different Packaging Types During Storage

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A B S T R A C T

Milk is a livestock product with high nutritional content, recognized as a source of complete and balanced nutrition for humans, as it contains essential carbohydrates, proteins, fats, minerals, and vitamins. Improper milk handling can also cause a short shelf life and low selling prices, which ultimately will also reduce the income of farmers as milk producers. Good packaging can protect the product from microbial contamination and prevent the oxidation process caused by light and oxygen. The type of packaging certainly affects the physical, chemical, and microbiological quality of fresh cow's milk during storage. The purpose of this study was to determine the physical, chemical, and microbial quality of fresh cow's milk in different packaging during storage. This study used a completely randomized design factorial pattern consisting of 2 (two) factors: the type of packaging and storage time. The observed variables were colour, pH, water content, protein content, and total microbial count. The data obtained were analyzed using ANOVA (analysis of variance); if significant results were found ($P < 0.05$), they were further analyzed using Duncan's multiple range test. The results showed that the type of aluminum pouch packaging was able to inhibit microbial growth due to its airtight and light-resistant properties, which allowed it to maintain temperature quality during storage. Milk packaged in aluminium packaging of size 7.6×10^4 cfu/ml, which is smaller than milk in glass and plastic bottles. Furthermore, milk packaged in aluminium has the highest protein content during storage, namely 3.302%.

Contribution to Sustainable Development Goals (SDGs):
SDG 2: Zero Hunger
SDG 3: Good Health and Well Being
SDG 12: Responsible Consumption and Production

1. INTRODUCTION

1.1. Research Background

Milk is one of the livestock products with a high nutritional content, known as a complete and balanced source of nutrition for humans. It contains essential carbohydrates, proteins, fats, minerals, and vitamins that are vital for human health. Fresh milk is a liquid that comes from the udders of healthy and clean cows, which is obtained by proper milking, whose natural content is not reduced or added to anything, and has not received any treatment except cooling [1]. Milk has a high nutritional value because it contains essential chemical elements needed by the body, including high protein and fat. The main components of milk are water (87.9%), protein (3.5%), fat (3.5-4.2%), vitamins and minerals (0.85%) [2]. Its high nutritional value makes milk an excellent medium for microorganisms to grow and develop, so that, in a very short time, the milk becomes unfit for consumption if not handled appropriately. An essential aspect in assessing the quality of milk, which reflects the level of consumer acceptance, is the relationship between its physical, chemical, and microbiological properties [3].

The physical properties of milk indicate the condition of the milk that can be tested using the five senses, such as the color of the milk. The color of milk can change from one color to another. This depends on the breed of livestock, the type of feed, the amount and nature of fat, solids, and color-forming compounds [4]. According to Susilorini [5], the chemical properties of milk are related to its acidity level. The content of various acidic compounds (citric acid, complex phosphoric acid, amino acids, dissolved carbon dioxide) affects the chemical properties of milk. In contrast, the microbiological properties of milk indicate the level of contamination of milk by microorganisms. In addition, the pH value of milk between 6.5 and 6.6 is a very favorable condition for microorganisms because a pH close to neutral (pH 6.5-7.5) is optimal for bacterial growth, which can lead to milk spoilage. The activity of microorganisms is primarily responsible for milk spoilage.

Improper milk handling can also result in a short shelf life and low selling prices, which ultimately reduce the income of farmers as milk producers [6]. Microbial growth in milk can compromise the quality and safety of the milk, as indicated by changes in taste, aroma, color, consistency, and appearance. The type of packaging has a significant impact on the quality of milk during storage. The milk processing industry continues to strive to develop technology to extend the shelf life of milk and its processed products. Packaging technology plays a crucial role in product shelf life.

Good packaging can protect the product from microbial contamination and prevent oxidation due to light and oxygen [7][8]. Various types of packaging materials are used to maintain the quality and extend the shelf life of pasteurized milk, including glass bottles, cardboard boxes, and plastic packaging. Each type of packaging material has its advantages and disadvantages. When selecting packaging, consideration must be given to the possibility of component migration from the packaging to the product. Several factors influence migration, such as contact time, temperature, fat content, surface area, and volume ratio. For dairy products, packaging selection is based on the permeability of the dominant microbial content that causes damage. Factors that must be considered when selecting packaging for fresh milk include compatibility with the dairy product, chemical inertness, non-toxicity, ability to protect the product from impact and physical damage, maintaining sanitation, and protection from light and odor. Based on this, the type of packaging certainly affects the physical, chemical, and microbiological quality of fresh cow's milk during storage..

1.2. Research Objective

This study aimed to determine the physical, chemical, and microbiological quality of fresh cow's milk in different packaging during storage.

2. MATERIALS AND METHODS

The research was conducted from March to August 2025 at the Agricultural Laboratory of Warmadewa University. Milk samples were taken from the Margo Utama milking farm in Kalibaru, Bajuwangi, East Java.

The equipment used in the research included petri dishes, Erlenmeyer flasks, measuring cups, pH meters, incubators, ovens, autoclaves, hot plate stirrers, test tubes, stirrers, micropipettes, analytical scales, Bunsen burners, and colony counters. The materials used in this research were cow's milk from the Margo Utama Kalibaru Bajuwangi East Java dairy farm, 0.1% peptone water buffer (BPW), plate count agar (PCA), Lowry A, and Lowry B.

The samples used were cow's milk from the Margo Utama milking farm in Kalibaru, Bajuwangi, East Java. Fresh cow's milk samples were packaged in their respective packaging types and stored at 4°C. Samples were observed according to treatment and observation variables.

The research design used was a Completely Randomized Design (CRD) with a factorial pattern.

Factor 1 encompasses the type of packaging, comprising three levels: glass bottles (P1), plastic bottles (P2), and aluminum pouches (P3). Factor 2 includes storage durations of 1 day (L1), 2 days (L2), 3 days (L3), and 4 days (L4). Each treatment consists of three replications, resulting in 36 experimental samples.

The variables observed in this study included physical properties, such as pH; chemical properties of fresh milk, including water content and protein content; and microbiological properties, specifically total plate count (TPC).

Color

The color of milk is observed sensorily using the senses, and the data is displayed descriptively.

pH

The pH value was measured using a pH meter and calibrated with buffer solutions of pH 4 and 7. The pH meter cathode was inserted into the sample and left in place until the digital measurement no longer changed. The pH meter cathode was rinsed with distilled water and dried before being used again.

Water content

Determination of water content is based on the difference in weight of the sample before and after drying. The porcelain cup to be used is first dried for 1 hour at a temperature of 105°C. The dried cup is then cooled in a desiccator for 30 minutes and weighed until its weight is constant. The sample to be analyzed is weighed to a mass of 2 grams in the cup, then dried in an oven at a temperature of 100-105°C for 5 hours or until it reaches a constant weight. The weight remains constant. Water content is calculated using the formula:

Water content (%)=((BC))/((BA)) x100%

Information :

A: weight of empty cup (grams)

B : weight of cup + sample (grams)

C: weight of cup + sample after drying (grams)

Protein Content

Analysis of dissolved protein levels using the Lowry-Follin Chiocalteau Phenol method (Apriyantono, 1989) with the formula:

$x = (y – a) / b$

y = sample absorbance

a and b = the determination of the standard curve created

% Dissolved Protein = (x[mg/ml]× fp × 100 %) / [mg/ml]) sample

a. *Total Microbes*

A total of 10 g of chicken meat sample was placed in a sterile heat-resistant Erlenmeyer flask. A total of 90 mL of sterile diluent solution was added to the aseptic Erlenmeyer flask. The sample was then crushed with a Stomathcer for 120 seconds, resulting in a sample with a dilution of 10^{-1}. 1 mL of the sample solution was taken aseptically with a pipette, then put into a test tube containing 9 mL of sterile diluent solution to produce a dilution of 10^{-2}, then 1 mL of the 10-2 dilution was taken and put into a test tube containing 9 mL of sterile diluent solution to produce a dilution of 10-3 to a dilution of 10^{-6}. For each selected dilution, 1 mL of the sample was pipetted aseptically into a sterile Petri dish in duplicate and added with 1 mL of sterile PCA (Plate Count Agar). After the agar media solidifies, the petri dish is incubated in an inverted position in an incubator at 37°C for 2 days.

The data obtained were analyzed using ANOVA (analysis of variance); if there were results that had a significant effect ($P < 0.05$), they were further analyzed using Duncan's multiple range test [9].

3. RESULTS AND DISCUSSION

Statistical analysis revealed that different types of packaging had a highly significant effect ($P < 0.01$) on the protein content and total microbial count of fresh cow's milk. Storage time had a significant impact ($P < 0.01$) on pH, protein content, and total microbial count. The interaction between the type of packaging and storage time had a highly significant effect ($P < 0.01$) on the total microbial count of fresh cow's milk. The significance of the type of packaging and storage time for fresh cow's milk is evident in Table 1. The pH value, water content, protein content, and total microbial count of fresh cow's milk are presented in Table 2.

Table 1. Significance of packaging type and storage time of fresh cow's milk

Observation Variables	Treatment		Interaction
	Packaging Type	Storage Period	
pH	ns	**	ns
Water content	ns	ns	ns
Protein Content	**	**	ns
Total Microbes	**	**	**

Information :

ns =No significant effect (P>0.05)

* =Significantly affected (P<0.05)

** =Very significant effect (P<0.01)

The results of the study showed that the type of packaging did not affect the pH value of fresh cow's milk. Fresh cow's milk packaged in glass bottles, plastic, and aluminum pouches had pH values that were not significantly different, ranging from 6.633 to 6.388 Table 2). This is because pH changes are primarily triggered by bacterial growth in the milk during storage. The type of packaging only plays a role in protecting against oxygen, light, and microbial contamination after the packaging process, and therefore does not directly alter the pH. The storage period showed a significant difference in the pH value of fresh cow's milk. Fresh cow's milk had the lowest pH on the 4th day of storage, which was 6,350. The results showed that the longer the storage period, the lower the pH of fresh cow's milk became. The pH value of cow's milk decreased during storage due to the activity of lactic acid-producing bacteria such as Streptococcus thermophilus and Lactobacillus spp [10]. These bacteria convert lactose in milk into lactic acid, which increases acidity and lowers the pH value of milk [11].

The water content of fresh cow's milk does not differ significantly between different types of packaging and storage periods. Packaging aims to maintain the quality of the milk by protecting it from contamination, exposure to light, and oxygen. As long as the milk does not undergo significant dilution or evaporation, and the packaging can protect the milk from extreme environmental conditions, the water content of fresh milk will remain relatively constant and is not significantly affected by the type of packaging used. The water content of fresh milk remains relatively stable during standard storage, as it is determined by the milk itself when it is milked and does not change significantly without evaporation or contamination by other fluids [12].

The results of the study showed that the treatment type and storage time had a significant effect on the protein content of fresh cow's milk, but the interaction did not have a significant impact. Aluminum pouches produced the highest protein content of 3.302%, which was significantly different from the protein content of fresh cow's milk packaged in glass bottles and plastic bottles. This is because vacuum aluminum foil packaging is airtight and opaque, thus inhibiting the protein degradation process due to microbial and enzyme activity triggered by air and light [13]. The mechanism in fresh milk is that packaging with airtight and light-resistant properties (such as aluminum pouches) is more effective in preventing oxidation and the growth of proteolytic microorganisms that can reduce protein content. Meanwhile, glass bottles that are translucent and not airtight allow for a quicker increase in water content and protein damage. Plastic bottles usually have a better effect than glass but are still less impermeable than vacuum aluminum foil [14]. During storage, the protein content of fresh cow's milk decreases. Fresh cow's milk on the 4th day of storage has the lowest protein content of 2.359%. This decrease in protein content is caused by the activity of microorganisms that develop during storage, which can damage milk protein through various biochemical and enzymatic reactions [15].

Based on the research results, cow's milk packaged in aluminum pouches produced the lowest total microbial count, namely 7.6 x 104 cfu/ml (Table 2). Aluminum pouch packaging generally has properties that are light-tight, airtight, and resistant to penetration of microorganisms from the external environment. This makes the growth of microbes in milk during storage can be minimized compared to other packaging materials, such as plastic bottles or glass bottles, which are more easily penetrated by light and air [16]. Thus, aluminium pouch packaging effectively inhibits microbial contamination and the growth of bacteria that can damage milk and reduce its quality. This is also in line with the fact that milk in aluminum pouch packaging tends to have the highest protein content and better maintained quality during storage, due to minimal microbial interference.

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Table 2. pH value, water content, protein content, and total microbes of fresh cow's milk

Day Observation	pH	Water content (%)	Protein Content (%)	Total Microbes (cfu/ml)
Packaging Type				
Glass Bottle	6.375 a	86.415 a	2.381 b	1.0 x105 b
Plastic Bottles	6.388 a	86.174 a	2.262 b	5.9 x105 a
Aluminum Pouch	6.363 a	86.669 a	3.302 a	7.6 x104 c
Day 1	6.833 a	85.540 a	2.858 a	4.3 x104 d
Day 2	6.433 a	87.364 a	2.999 a	6.1 x104 c
Day 3	6.383 ab	84.887 a	2.378 b	3.4 x105 b
Day 4	6.350 b	87.887 a	2.359 b	8.8 x105 a
Interaction				
Glass Bottles ; Day 1	6.385 a	86.245 a	2.560 a	4.8 x104 b
Glass Bottles ; Day 2	6.480 a	87.262 a	2.773 a	6.1 x104 b
Glass Bottles ; Day 3	6.360 a	83.842 a	2.038 a	1.8 x105 ab
Glass Bottle ; Day 4	6.325 a	88.311 a	2.150 a	2.3 x105 ab
Plastic Bottles: Day 1	6.350 a	86.556 a	2.394a	1.4 x105 ab
Plastic Bottles: Day 2	6.390 a	86.712 a	2.513 a	4.9 x104 b
Plastic Bottles: Day 3	6.400 a	84.357 a	2.033 a	2.7 x106 a
Plastic Bottles: Day 4	6.395 a	87.070 a	2.108 a	6.7 x106 a
Aluminium Foil: Day 1	6.310 a	83.816 a	3.617 a	1.2 x104 c
Aluminium Foil: Day 2	6.410 a	88.117 a	3.710 a	7.4 x104 b
Aluminium Foil : Day 3	6.360 a	86.461 a	3.061 a	8.3 x104 b
Aluminium Foil : Day 4	6.310 a	88.279 a	2.818 a	4.2 x105 ab

Note: Mean values followed by the same letter in the same column indicate no significant difference in the 5% DMRT test.

The study's results showed an increase in total microbes during storage. On the 4th day, the total microbes in fresh cow's milk reached 8.8 x10^5 cfu/ml. However, this number of microbes was still below the threshold. The indicator of bacterial contamination in fresh cow's milk is Total Plate Count (TPC) 1 x 10^6 CFU/ml, according to SNI Number 3141.1 of 2011. The increase in the number of microbes in fresh cow's milk during storage is caused by several factors, particularly the activity of microorganisms that remain alive in the milk after milking. Even though milk is stored in cold conditions (for example, at a refrigerator temperature of 4°C), some lactic acid bacteria and other microorganisms can still grow, albeit at a slower rate than at room temperature. This microbial growth causes the total number of microbes to increase over time during storage [17].

In this study, the interaction between packaging type and storage time had a highly significant effect ($P < 0.01$) on the total microbial count of fresh cow's milk. Aluminum pouch packaging effectively inhibited microbial growth during storage. This occurs because aluminum pouch packaging is airtight, light-proof, and has a good barrier against the penetration of microorganisms from outside [18]. Thus, milk packaged in aluminum pouches is better protected from external microbial contamination, and also, microbes in the milk cannot grow optimally during storage, especially at low temperatures. In contrast, other packaging, such as plastic bottles or glass bottles, is less impermeable, allowing faster microbial growth during the same storage time.

4. CONCLUSION

The results of the study showed that the aluminum pouch packaging type was able to inhibit microbial growth because it had airtight and light-resistant properties, so that it could maintain temperature quality during storage. Milk packaged in aluminum packaging has total microbe namely 7.6 x 10^4 cfu/ml, which is smaller than milk in glass and plastic bottles. Furthermore, milk packaged in aluminum has the highest protein content during storage, namely 3.302%.

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