Effect of ozonation and pasteurization on total microorganism, pH and density whole milk and skim milk during cold storage

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Abstract. Aim of this research was to evaluate the differences in physicochemical characteristics and stability during cold storage of whole and skim milk from ozonation as compared to pasteurization. In this current research, descriptive methods was employed consist of two control samples (untreated skim and whole milk) and four treatments: (1) pasteurized skim milk, (2) ozonated skim milk, (3) pasteurized whole milk and (4) ozonated whole milk. Ozonation was carried out for 650 seconds at 0.36 L/min for whole milk and 5.01 L/min for skim milk, meanwhile, pasteurization process was performed at 72 °C for 15 seconds. The reduction of microorganisms after treatment of pasteurized skim milk, ozonated skim milk, pasteurized whole milk and ozonated whole milk, respectively were 2.97, 2.16, 3.09 and 1.7 log cycle. During storage, ozonated samples, had increased density faster than pasteurized samples, ozonated skim milk also has largest increase in growth microorganisms, meanwhile, ozonated whole milk has the fastest decreased of pH than other samples. Therefore, ozonation treatment can be used as milk pre-treatment to reduce microorganisms, however, this treatment must be carried out with other preservation process.

Keywords: Milk, Ozonation, Pasteurization and Storage

1. Introduction
Milk is a food that provides complete nutrition because it has complex nutritional values that other foods do not have. Milk is consist of complete components which are a water and soluble components such as carbohydrates, fats, proteins and some other minor components: vitamins, minerals and enzymes. Each of components has influence of functional character of milk.

Milk is also one of perishable food so that it has a high risk of damage during storage. Milk also has high water activity and neutral pH, this makes milk be a suitable media for growth of microorganisms which causes a decrease in quality and stability of milk [3]. Some of the microorganisms in milks are pathogenic but some of them are decomposing microorganisms that form enzymes which can decrease quality and stability during storage [3].

Thermal process is one of conventional applied for maintain milk stability by reducing microorganisms. Pasteurization is low temperature heat treatment process (under 100 °C) which aim to reduce microorganisms in milk. Based on its purpose, pasteurization has significant influence in
stability, physicochemical properties and shelf life of milk. However, this process has considerable influence on sensory properties and nutritional value of milk. This process can also occur structural changes in milk fat, protein and other components [9].

There is alternative process to reduce microorganisms in milk by using substances that can reduce and inactivate microorganisms without change sensory properties and decrease nutritional value in milk [3]. Ozone, peroxide and CO₂ are some substances that can reduce microorganisms in milk, limit enzyme production by microorganisms during storage and have low effect at sensory properties changes.

Ozone (O₃) is triatomic oxygen formed by added free radical oxygen to molecular oxygen [7]. Ozone has antimicrobial ability because of its oxidation potential [1]. Antimicrobial ability in ozone is also based on high biocidal effectiveness and has broad and active antimicrobial spectrum to destroy bacteria, fungi, viruses, protozoa and spores [6]. Although ozone have oxidising properties, ozone is approved by US Food and Drug Administration (FDA) for use in food and has been applied in many food in aimed for reduce microorganisms.

Research on the effects of ozonation has also been carried out recently. Rojek observed that pressurized ozone in 5–25 g/L for 5–25 minutes in skim milk can reduce 2.4 \( \log_{10} \) cfu/cm² [10]. Calvacante also obtained that bubbling ozonation in raw milk for 15 minutes reduced 0.5–1 \( \log_{10} \) cycle total microorganisms, and reduced Enterobacteriaceae, psychrotrophic bacteria, Staphylococcus sp and molds respectively 0.96; 0.13; 1.02 and 0.48 \( \log_{10} \) cycle. Calvacante was obtained that ozonation process for 15 minutes in raw milk didn’t change physicochemical parameters as expected before [3].

2. Materials and methods

2.1. Milk samples
The whole milk was collected from Faculty of Animal Husbandry, Padjadjaran University. For the skim milk sample, whole milk is preheated to 60 °C and transferred to supply tank of cream separator for skimming process. Skimming process done when the level of milk fat is 0%. The samples were then separated into 2 control samples (Untreated skim milk and whole milk) and 4 treatments; (A) Pasteurized whole milk; (B) Ozonated whole milk; (C) Pasteurized skim milk; (D) Ozonated skim milk. After treatment process, each samples was packed in glass bottle and stored at 4 °C for 14 days. Total microorganisms, pH value and density was measured during storage.

2.2. Ozonation process
Two liters of milk, each sample was placed in tubular tank. Before ozonation process milk was refrigerated until the temperature reaches 3 °C. Ozonation was carried out for 650 seconds at 0.36 L/min for whole milk sample and 5.01 L/min for skim milk sample. Flow rate that been used in this study was obtained from the results of an evaluation process in order to determine the ozonation that which can reduce the number of microorganisms that are equivalent to pasteurization process.

2.3. Pasteurization process
The pasteurization process that used in this study was High Temperature Short Time (HTST). 2 liters of each samples was heated at 72 °C for 15 seconds. During the pasteurization, the temperatures was measured regularly using a thermometer.

2.4. Analysis of total microorganisms
Total microorganisms was evaluated by Total Plate Count method. Each samples were diluted into sterile BPW 0.1% (Buffer Peptone Water). Sample was inoculated on media PCA (Plate Count Agar) and incubated at 24 ± 1 °C for 48 hours. Total microorganisms was count in the media and calculated using the standard equation.
2.5. **Analysis of pH value**

pH values of untreated skim milk, untreated whole milk, pasteurized whole milk, pasteurized skim milk, ozonated skim milk was measured by using pH meter (Digiticez DW 01). Electrodes of pH meter was dipped into samples to be analyzed. The temperature of the sample is also measured during the pH measurement.

2.6. **Analysis of density**

Density of each samples were determined using an instrument (Lactoscan Milk Analyzer MCC50 Serial 0403). Lactoscan test the sample with an ultrasonic sensor. Sample will be reflected in transducer and with help of pulse generator the wavelength will shown on the oscilloscope and will be obtained and compared with the standard according to time interval and amplitude.

3. **Results and discussion**

3.1. **Total microorganisms**

![Graph](image-url)

**Figure 1.** (a) Total microorganisms of skim milk sample during storage (b) Total microorganisms of whole milk sample during storage.

Before treatment, the counts of total microorganisms untreated whole milk and skim milk were 7.45 log_{10} and 7.06 log_{10} cfu/ml. High bacterial load is normally caused by poor sanitation at milking process [5]. Sample was then treated according to the code of the sample. Pasteurization treatment can reduced respectively 3.09 and 2.97 log cycles total microorganisms in whole milk and skim milk. While, ozonation treatment can reduced total microorganisms by 1.7 log cycles for whole milk sample and 2.16 log cycles for skim milk sample.

There was a difference number in total reduction of microorganisms between ozonated whole milk and ozonated skim milk samples that caused by difference in fat content and organic matters. Patil verified effect of ozonation process on *E. coli* in different orange juices (varying amount of organic matter) [8]. The authors concluded that organic matter can influence the effectiveness of ozone, which was more efficient in low amount of organic matters juices. Fat content in whole milk also decreases the efficiency of ozone for the reduction of *S. aureus* [4].

During storage, total microorganisms in the sample tend to increase with the length of storage time. Pasteurized skim milk sample had a smaller increase of 0.79 log cycles compared to ozonated skim milk and untreated skim milk. While pasteurized whole milk sample increased by
0.95 log cycles, greater than the increase in ozonated whole milk samples by 0.88 log cycles and smaller when compared to untreated whole milk which increased by 2.18 log cycles.

3.2. pH value

The untreated whole milk and skim milk sample that used in this study had a pH value of 6.87 and 6.84, respectively. Sample was then given ozonation and pasteurization treatment based on the selected sample code. Pasteurization and ozonation process in milk has no significant effect on the pH changes where respectively pasteurized skim milk, pasteurized whole milk, ozonated skim milk and ozonated whole milk sample had pH value of 6.84, 6.85, 6.76 and 6.84. It was observed that tensioactive addition (ozone) did not altered the milk physicochemical properties [2]. Calvacante also verified that ozonation process did not change the physicochemical parameters [3]. pH of the samples during storage tend to decreased because of lactose degradation by lactic acid bacteria such as *Streptococcus thermophilus*, *Lactobacillus lactis* and *Lactobacillus thermophilus*. Degradation causing a decrease in pH and an increase on acidity in milk). An increase in milk acidity can also be caused by presence of compound that have acidic properties such as complex phosphate, amino acids and citric acids [12].

There was no significant difference (P > 0.05) between changes of pH among pasteurized skim milk and ozonated skim milk during storage. Based on figure 2 a, slope of pasteurized skim milk, ozonated skim milk, and untreated skim milk curve were respectively 0.017, 0.025 and 0.131. These slope values states that pasteurized skim milk sample was a sample with the slowest rate change in pH. Significant difference (P < 0.05) in changes of pH was observed among pasteurized whole milk sample and ozonated whole milk sample. Based on figure 2 b, slope of pasteurized whole milk, ozonated whole milk and untreated whole milk were 0.011, 0.157 and 0.201. These slope values states that rate of pH change of ozonated whole milk sample was faster than pasteurized whole milk sample, and even almost the same as untreated whole milk sample. Rate of pH changes were depends on the total number of microorganisms in each sample, the greater total number of microorganisms in the sample, the faster rate of milk spoilage [12].

![Figure 2](image-url)
3.3. Density

Figure 3. (a) Density of skim milk sample during storage (b) Density of whole milk sample during storage.

The density of untreated whole milk and skim milk that were used as sample are 1.024 g/ml and 1.031 g/ml, respectively. Samples was then given pasteurization and ozonation treatment based on the selected sample code. Samples that have been given treatment, either pasteurization or ozonation treatment have increased density. Pasteurization can change milk density because evaporation of sample which causes milk to lose water, this causes the total solid in milk increase and will affect the increase of density. Ozonation process can cause fat oxidation in milk that causes decreasing of fat levels and affect density of ozonated milk samples.

There was significant difference between density changes during storage on pasteurized skim milk sample and ozonated skim milk sample. Based on figure 3 a, slope of curve density changes on pasteurized skim milk, ozonated skim milk and untreated skim milk were 0.0001, 0.0002 and 0.0004, respectively. Increase in the number of microorganisms, especially protein breaking microorganisms can increase the yield of secondary metabolites in the form of N₂ which tend to increase the density value [12]. Density changes in pasteurized whole milk sample and ozonated whole milk sample showed no significant difference. Untreated whole milk sample had the highest slope value among other skim milk sample, this shows that untreated whole milk has faster change in density. Ueda stated that density changes might be caused by change in fat physical structure which cause an increase in fat density that increase sample density during storage [11].

4. Conclusion

In this research, ozone treatment can reduce microorganisms by 1.7 – 2.2 log cycles in raw milk sample. This reduction of microorganisms is lower due to pasteurization treatment. However, during storage both samples, either skim milk and whole milk, did not show a significant difference in total microorganisms changes. Complementary, there is no significant changes in physicochemical properties after ozonation process, however during storage ozonated sample tend to have faster spoil rate than pasteurized milk. Therefore, ozonation treatment can be used as milk pre-treatment to reduce microorganisms, however, this treatment must be carried out with other preservation process.

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