Total bacteria and milk nutrition using ZnO doped N thin film with visible light to maintain milk quality

M Z Asror1, M I Bsatoham2, N I Pratiwi2 and H Susanto2
1 Department of Animal Science, Faculty of Animal and Agricultural Sciences, Diponegoro University, Indonesia
2 Department of Physics, Faculty of Science and Mathematic, Diponegoro University Indonesia
email zainulasror28@gmail.com

Abstract. Milk is susceptible to microbial contamination due to its high nutrient contents. The presence of microbial contamination can damage the nutrients in milk such as protein, fat and lactose. Therefore, there is a need to keep milk quality. ZnO doped N thin film can kill pathogenic bacteria. The aim of this research is to investigate the number of bacteria and milk nutrition using ZnO doped N thin films with visible light to maintain milk quality. Milk samples were obtained from smallholder dairy farm in Central Java. Fresh milk was poured in a beaker glass, then ZnO doped with N thin films and illuminated with visible light. The testing scheme was carried out for 0, 60, 120 and 180 minutes in the refrigerator. Total bacteria was calculated using Total Plate Count (TPC) method. Milk nutrition received using lactoscan (MMC, Bulgaria). The conclusion of this research is ZnO doped N thin film with visible light could reduce total bacteria by 95.2%, Coliform by 66%, S. aureus by 94.4% and milk nutrition in accordance with Indonesian National Standards of fresh milk. Suggestions from this study, it is necessary to apply ZnO doped N thin films in food technology as an effort to maintain milk quality.

1. Introduction
Cow milk is a food that has very important nutrients for a human such as protein, lactose, fat, and mineral [1]. The high level of nutrient content in cow milk gives a chance for the growth of pathogenic bacteria. Generally, dairy farmers in Indonesia are folk farmers who are still traditional. The milk quality produced by folk farms has not been in line with the standard as the total bacteria 5 x 10^6 CFU/mL and the total contamination of coliform 229,76 MPN/mL [2,3].

The high level of bacterial contamination can cause the degradation of milk quality (protein, lactose, and fat). This is due to the non-hygiene handling process of milking and post-milking. Sources of bacterial contamination in milk are affected by non-sterile equipment, sanitation during milking and post-milking. Milk has a standard based on SNI (Indonesian National Standard) that is the maximum bacterial total of 1x10^6 CFU/mL, protein 2.8%, fat 3%, lactose 4%, and density 1.024 [4]. On the other hand, the milk quality that is not convenient with SNI will be rejected by the dairy industry. This can cause losses economically. Therefore, that needs to solve in reducing bacterial contamination in order to maintain the milk quality.

Today nanotechnology has grown such as photocatalysts that are interesting because of the efficiency and the cost relatively low [5]. The photocatalyst is an efficient and cheap method of bacterial degradation. ZnO is one of the most popular photocatalysts because it has high, inexpensive and non-toxic photoactivity [6]. ZnO can be active under UV light. Therefore ZnO must be doped with nitrogen so that it can be active under visible light. ZnO doped N under visible light will produce reactive oxygen species (ROS) such as OH-, O2-, O2, H2O2 which can exist as antibacterial [7]. In previous studies,
photocatalysts can reduce the contamination of pathogens bacteria such as *Staphylococcus aureus* and *E. coli* [8]. Thus, this study aimed to know the effect of ZnO doped N thin film on bacterial contamination in milk. The results of this study could provide a method for maintaining the quality of milk and to ensure food safety from bacterial contamination.

### 2. Material and Method

#### 2.1 Materials

All chemicals were reagent grade and used without further purification. Zinc acetate dehydrate (Zn(COOCH$_3$)$_2$.2H$_2$O as precursor of ZnO, isopropanol ((CH$_3$)$_2$CHOH), monoethanolamine (C$_2$H$_7$NO) as a solvent and stabilizer, urea (CH$_4$N$_2$O) as doping and fresh milk.

#### 2.2 Fabrication of N doped ZnO thin film

Synthesis of doped ZnO N nanoparticles was carried out by sol-gel method with Zn (CH$_3$COO)$_2$·2H$_2$O precursors as a source of Zn and isopropanol (IPA: (CH$_3$)$_2$CHOH), monoethanolamine (MEA: HOCH$_2$CH$_2$NH$_2$), and urea (CH$_4$N$_2$O) as a nitrogen source. Making ZnO nanoparticles by dissolving Zinc Asetat into propanol and monoethanolamine solutions at room temperature with a concentration of 0.3 M Zinc Asetat with a 1:1 molar ratio and then sterile for 1 hour. The result was a clear white solution. The next process was coating on a glass substrate with a temperature of 450°C. Before deposition, glass substrates were cleaned by the Radio Corporation of America (RCA) method. In this method, glass substrates were cleaned by acetone and methanol for 10 min. An ultrasonic cleaner was used to wash dust and oil out. Then, we immersed the glass slide into the double-distilled water for 8 min and dried the substrates off using a compressor gun. The obtained solutions were sprayed onto glass substrates for 1 hour at 450°C. Furthermore, the films were annealed at 450°C for 1 hour.

#### 2.3 Testing process

This research was carried out on a laboratory scale. Milk samples were obtained from smallholder dairy farm in Central Java. The testing process was carried out on a lab scale. 500 ml fresh milk were poured in a beaker glass. Then ZnO doped with N thin films placed on top and illuminated with visible light. The testing scheme was carried out for 0, 60, 120 and 180 minutes in the refrigerator. The total bacteria data was taken at 0, 60, 120 and 180 minutes while milk quality was taken at 0 and 180 minutes.

#### 2.4 Total plate count analysis

The test of total bacteria uses the TPC method (Total Plate Count) based on Indonesian National Standard (SNI) [9]. The sample of milk is dissolved with BPW 0.1%. Then it is diluted with $10^{-3}$, $10^{-4}$, $10^{-5}$ levels dilution. 1 mL of suspension was taken at $10^{-5}$ level dilution and inserted into a petri dish. Then add Plate Count Agar (PCA) then incubate at 37°C for 24 hours [10]. Calculate the number of colonies with the following formula

$$\text{Total Bacteria} = \frac{\text{Number of Colonies}}{\text{Dilution level factor}}$$
2.5 Milk quality analysis

The testing of dairy components in this study was performed with Lactoscan (MCC, Bulgaria). The testing includes protein, fat, lactose and density of milk. The pH of milk was measured with pH meter 5011 (Ezdo Gondo, Chinese Taipei). The milk sample was poured 50 ml into glassware, then the pH meter was dipped into milk and the result of pH recorded. Each sample was duplicated and the result calculated on average. The test of somatic cells in milk using the breed method [11].
3. Results and discussion

3.1 Bacterial analysis

The total bacteria in milk before treatment was $2.5 \times 10^6$ CFU/mL. It shows that the milk samples from the dairy farmers have high bacterial contamination. The high levels of bacterial contamination in milk are closely related to hygiene and sanitation during the handling of milking. Based on Hassan et al. [12] sources of bacterial contamination in milk are influenced by non-sterile equipment, sanitation during milking and post-milking treatment.

Based on figure 3 shows the effect of the thin film of ZnO doped N on the total bacteria in milk. The degradation of total bacteria increased in time to 0, 60, 120 and 180 minutes. At 0 minute the total bacteria was $2.5 \times 10^6$ CFU/mL, at 60 minutes was $1.1 \times 10^6$ CFU/mL at 120 minutes was $0.5 \times 10^6$ CFU/mL and at 180 minutes was $0.16 \times 10^6$ CFU/mL or the percentage of amount degradation of 120 minutes was 95.2%. Furthermore, based on figure 3 shows the effect after ZnO doped N thin film treatment for 180 minutes, Coliform bacteria decreased by 66% and S. aureus was 94.4%. It shows that the treatment of ZnO doped N thin film for 120 minutes could affect the decrease in the number of bacteria in milk. This is because of the existing of photocatalysts that can decrease total bacteria. Photocatalysts of ZnO doped N are capable of producing hydroxyl radicals (OH-) under visible light that can kill bacteria. According to Prasanna and Vijayaraghavan [13] photocatalyst N doped ZnO has antibacterial activity so it can decrease bacteria. The result of total bacteria was according to SNI of fresh milk. Total fresh milk bacteria maximum limit is $1 \times 10^6$ CFU/mL [4].

The photocatalyst mechanism of killing bacteria, when ZnO is photo-induces by solar light with photonic energy ($h_\nu$) equal or greater than excitation energy ($E_g$) $e^-$ from the filled valence band (VB) are promoted to an empty conduction band (CB). This photoinduced process produces electron-hole ($e^-/h^+$) pairs. The photoexcited $e^-_{\text{CB}}$ can be reacted with surface adsorbed oxygen to produce superoxide radical anions which subsequently converted to hydroxyl radicals [14, 15, 16].

$$\text{ZnO-N} \rightarrow h^+_{\text{VB}} + e^-_{\text{CB}} \quad (1)$$
$$e^-_{\text{CB}} + \text{O}_2 \rightarrow \text{.O}_2 \quad (2)$$
$$\text{O}_2^{\text{ads}} + \text{H}_2\text{O} \rightarrow \text{.OH} + \text{OH}^- \quad (3)$$

Figure 3. Schematic diagram of reaction mechanism of ZnO doped N thin film with visible light for 0, 60, 120 180 minutes.
H₂O → OH\textsubscript{ads} + H⁺ 

\text{(4)}

OH\textsubscript{ads} + h\textsuperscript{+}\textsubscript{VB} → .OH

\text{(5)}

OH → organic contaminants → degradation products + CO₂ + H₂O

\text{(6)}

The h\textsuperscript{+}\textsubscript{VB} that react with the adsorbed hydroxyl anion will form hydroxyl radicals. The hydroxyl radicals as a result of the transfer of an electron from the valance band (VB) to the conduction band (CB) will be capable of damaging the cell wall and can decompose the cellular materials [8]. Less oxidative species such as superoxide anions and singlet oxygen (O₂\textsuperscript{1}) are believed to be responsible for the antibacterial action during the visible light induced photocatalysis [17, 18].

![Figure 4. Schematic diagram of reaction mechanism of ZnO doped N thin film with visible light kills bacteria [8].]

| Test          | Standard Quality [4] | Before Storage | After Storage | Percentage Decrease |
|---------------|-----------------------|----------------|---------------|---------------------|
| Total Bacteria cfu/ml | 1x10\textsuperscript{6} | 2.5x10\textsuperscript{6} | 1.2x10\textsuperscript{7} | 95.2%               |
| *Coliform* MPN/ml | 20                    | 24             | 8             | 66%                |
| *S. Aureus* cfu/ml | 1x10\textsuperscript{2} | 3.06x10\textsuperscript{3} | 0.17x10\textsuperscript{3} | 94.4%              |

3.2 Milk Quality Analysis

Milk quality on ZnO doped N thin film treatment showed in table 2. The data showed that milk quality in accordance with Indonesian national standard of fresh milk.

The protein of milk before treatment was about 2.90% while after treatment was 2.90%. The lactose of milk before treatment was 5.03% dropped to 4.0%. The fat of milk before treatment was 3.90% become 3.80 and the density of milk did not change. The test results of protein, lactose, fat and milk density was still in accordance with SNI. According to SNI [4], standards for fresh milk are protein 2.8%, lactose 4%, fat 3%, and density 1.024 g / mL. It shows that photocatalysts do not damage the milk component. This is because ZnO doped N photocatalysts can decrease bacteria that become a cause of...
damage to the milk component. The result of the pH test before treatment was 6.8 and after treatment becomes 6.4. The thin-film of ZnO doped N could kill bacteria while not changing the pH of the milk. Based on the table 2, the result of somatic cells test before treatment was 530.100 cells/ml and after treatment was 321.590 cells/ml. There were decrease in somatic cells by 95.2%. The results showed that the milk produced was better than SNI. According to SNI [4] standard fresh milk for somatic cells is at least 400.000 cells/ml. During the storage process, somatic cell decline was due to photocatalyst N-ZnO that able to kill cells. According to Sirelkhatim et al. [19] ZnO photocatalysts produce radical hydroxyl (OH-) that can kill cells and damage DNA.

### Table 2. Result of milk quality analysis.

| Test       | Standard Quality [4] | Before Storage | After Storage | Unit |
|------------|-----------------------|----------------|---------------|------|
| Protein    | Min 2,8, 2,90         | 2,90           | 2,90          | %    |
| Lactose    | 4                     | 5,03           | 4,08          | %    |
| Fat        | Min 3, 3,90           | 3,80           |               | %    |
| Density    | 1,024, 1,026          | 1,026          |               | g/ml |
| Somatic Cell | 400,000               | 530,100        | 321,590       | cell/ml |
| pH         | 6,3 – 6,8             | 6,8            | 6,4           | -    |

4. Conclusion
ZnO doped N thin film with visible light for 180 minutes could reduce bacteria in milk such as total bacteria by 95.2%, *coli* by 66% and *S. aureus* by 94.4%. Furthermore, ZnO doped N thin film with visible light produced milk with quality parameters such as protein, lactose, fat, density, somatic cell and milk pH which were in accordance with Indonesian National Standards for milk. Thus, ZnO doped N is one of the effort to maintain milk quality and food safety.

5. References
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