The influence of ozone dosage, exposure time and contact temperature of ozone in controlling food quality (case study: tofu)

Eva Fathul Karamah1,*, Rana Rezeki Najeges1, Muhammad Zaki Zahirisyah1
1Department of Chemical Engineering, Universitas Indonesia, Kampus UI Depok 16424, Indonesia
* corresponding author: eva@che.ui.ac.id

Abstract. Tofu is one of the common foods consumed by the people of Indonesia, therefore to be classified as safe and feasible to be consumed, certain quality standard is needed. This research is about the effect of the dose of ozone in the ozonated water as well as the exposure time and contact temperature of ozone in controlling quality of Tofu. The quality standard of Tofu is detected from the decrease in the number of Escherichia coli bacteria and total aerobic mesophyll bacteria, the increase of the pH value to the decrease in the amount of protein in Tofu. The study was conducted by testing the productivity of ozonator, testing the ozone solubility in water, and storing Tofu in the ozonated water. 50 gram of Tofu was carried out for 40, 80, 120 minutes at 8°C, 37°C incubator temperature, and room temperature (25°C) with ozone dosage variation and storage in refrigerator temperature (8°C) for 7 days. The result shows that the highest ozone dosage (0.28 mg/L) can eliminate bacteria up to 1 log cfu/g. The best temperature to store tofu in ozonated water is the lowest amongst all, which is 8°C. The longer the tofu being exposed with ozone; the higher number of bacteria will be eliminated.

Keywords: Aerobic Mesophilic, Bacteria, Ozonated Water, Tofu, Escherichia coli

1. Introduction
Tofu belongs to the food group that is very easily damaged because the composition of tofu that contain lots of protein around 8% and water content (Aw) of 0.89-0.99. This causes tofu to be suitable media for the growth of microbes so the tofu is susceptible to decay by bacterial decay. High bacterial population levels have a negative impact on the quality of tofu because bacteria that grow and multiply will produce by-products that will change the quality of tofu [1]. In addition to high enough water content (80%) and protein number (15%), tofu damage is also caused by rancidity due to oxidation or hydrolysis of components from tofu. Microbial growth in food products is influenced by intrinsic and extrinsic factors. Intrinsic factors include acidity (pH), water activity (aw), equilibrium humidity (Eh), nutritional content, biological structure, and antimicrobial content. Extrinsic factors include storage temperature, relative humidity, and the type and amount of gas in the environment [2]. The evaluated performance parameters in this research are divided into 2 performance parameters, namely microbiology and physical. In the microbiological performance parameter, it consists of the number of bacteria Escherichia coli (E. coli) and Total Mesophil Aerobic in CFU (Colony Forming Unit)/gram, protein in tofu. Meanwhile, for physical performance parameters, can be seen from the pH value in the tofu. The independent variables in this experiment are the dose of ozone, duration of contact, contact temperature and ozone dosage dissolved in ozonated water. Variation of soluble ozone dosage was obtained by varying the water temperature when dissolving ozone (5°C and 10°C). In this research,
The authors hope to know the effectiveness of ozone, especially the ozonated water in maintaining the quality of tofu. Based on the formulation of the problem, the purpose of this research is knowing the effect of ozonated water contact duration on the number of TBMA and E. coli, pH and protein content contained in tofu and knowing the effect of ozone-related water contact temperature on the number of TBMA and E. coli, pH and protein content contained in tofu.

2. Material and Methods
This study will be conducted with the initial step by testing the ozone solubility. This step is required to give an outlook of the ability of ozone that is soluble in water and will give the general outlook about which range of variations should be done in the further study. Then, the study will further discuss about finding the optimum operating conditions using ozonation based on the bacterial degradation percentage. The tofu used in the study is in 50 grams, the type of ozonator used is commercial middle scale named Aquasuper, the ozone concentration of solubility is 0.28 mg/L. The total liquid volume used is 2 L. The tofu is obtained from the tofu factory located at Lenteng Agung. In this study, food preservation test done with several variations. There is a variation in the duration of contacts of ozonated water with tofu, variations in temperature of contacted ozonated water contacts with tofu and variation of dosage of ozonated water used in maintaining the quality of tofu. Variations on the duration of contact are made by contacting ozonated water with tofu for 40, 80, and 120 minutes. Sample controls of tofu (blanko) are also observed as a reference to tofu that is not being contacted with un-ozonated water. After contact, the tofu is stored in the refrigerator (temperature 8°C) for 7 days and observed the quality change every 2 days interval. Contact duration 40, 80, and 120 minutes were taken based on the results of study of Sotelo et al. [3], on the analysis of ozone decomposition in water.

3. Result and Discussion
3.1. Tofu Initial Characterization
The purpose of this study is to know the initial characterization of tofu and obtained the microbiological character. Tests conducted include five parameters to be compared that is TBMA (Total Aerobic Mesophilic), E. coli, pH and Protein. The presence of aerobic mesophyll bacteria is considered an indicator microbe in assessing the level of sanitation in foods [1]. The amount of TBMA contained in the tofu can indicate whether it is safe to or not to eat. As can be seen in table 1, the initial characterization of tofu from 5 parameters has already met the qualification of the standard tofu quality for consumption (SNI). According to the SNI Document 01-3142-1998 [4] on the quality requirements of tofu, the minimum amount of protein in tofu is 9%. In this study, protein content in tofu has a protein amount of 12.27%, This indicates that the tofu still has a protein content in accordance with the quality requirements to be eaten. Judging from the microbiological quality, the tofu used as the sample in this study has known to be a medium that is suitable to be a place of bacterial growth.

In the terms of acidity, the pH value possessed by tofu before being treated is 5.55. The figure shows that tofu is used in the category of weak acidic foods. Foods with a pH value above 4.6 belong to the
category of weak acid foods [1]. Whereas, foods with pH below 4.6 are categorized as acidic foods. Foods that include weak acids are a type of food that is more likely to be contaminated by bacteria. At pH between 4.6-9, many types of bacteria can multiply rapidly. So it can be concluded that tofu used as a sample in this study has conditions that are suitable for breeding bacteria.

Judging from the microbiological quality, know that used as a sample in this study has a number of TBMA as much as $10^7$ cfu/gr or 7 log cfu/gr. According to Cataldo [5], this number makes food classified as quite satisfactory (B). Food category "enough" has the characteristics of the number of TBMA above from $(>) 10^6$ cfu/gr. The number of TBMA categories (A) satisfactory is $>10^4$ cfu/gr, (B) is quite satisfactory, amounting to $>10^5-10^6$, and (C) is dangerous amounting to above $10^8$ cfu/gr. The number of bacteria is affected by the condition of the tofu. Results The number of bacteria is affected by the condition of the tofu. With a pH of 5.55; moisture content 83%; and protein amount 12.27%; tofu is a medium that is very suitable to be used as a place for bacterial growth.

The value of the tofu water content contained before being treated was 83%. This figure shows that tofu has a large water content. The high water content in tofu is caused by the amount of water involved in the process of making tofu. Due to high water content, tofu can be a medium for bacterial growth. The higher the water content, the greater the likelihood of bacterial growth in the tofu. In general, the durability of food ingredients has a close relationship with the water content contained. The water content in food ingredients also determines the feasibility of consumer acceptance and the durability of the ingredients.

Signs of damage to tofu are marked by mucus and the smell of tofu is damaged. Bacteria that damage it are lactic acid bacteria in the form of Streptococcus, coliform group, gram-shaped gram-negative psychotropic group, and the dominant gram-positive bacteria found in fresh tofu.

3.2. Water Initial Characterization
The purpose of this study is to know the initial characterization of water to known the microbiological character. Water quality characterization is done by sampling tap water in the Intensification Process Laboratory, Department of Chemical Engineering, University of Indonesia.

| No | Parameter                  | Value  | Unit          | Measurement method       |
|----|----------------------------|--------|---------------|--------------------------|
| 1  | Escherichia coli           | <1.8   | APM/100mL     | 18-Mikro-24/MU/SMM-AAS   |
| 2  | Total mesophyllic aerobic  | $2.8 \times 10^4$ | CFU/mL | SNI 3554-2015          |
| 3  | pH                        | 5.6    | -             | pH meter                |

From table 2, it can be seen that the large number of E. coli bacteria indicates that the water is within the polluted range because it has exceeded the standard quality threshold of 1.8 CFU/mL with a threshold safe limits 1 CFU/mL. Also known from the results that we have from the laboratory for initial characterization, the ozone dissolved in water will first react with contaminants or disinfect the E. coli and TBMA bacteria contained in tap water before it is finally dissolved in water and used to disinfect the bacteria contained in the tofu.

3.3. Effect of Contact Duration of Ozonated Water to the Quality of Tofu
In this study, food preservation test done with a variation in the duration of contacts of ozonated water with tofu. Variations on the duration of contact are made by contacting ozonated water with tofu for 40, 80, and 120 minutes as stated in the method above.

3.3.1. Effect of Contact Duration of Ozonated Water to Total Mesophilic Aerobic Bacteria. In this subchapter will be discussed about the effect of contact duration on TBMA. The initial number of TBMAs in tofu before being contacted with ozonated water is $10^7$ CFU/mL or 7 log CFU/g. From Fig. 1, it can be seen, the initial character of the tofu with a bacterial count of $1.0 \times 10^7$ CFU/g and as a
comparison of 3 variations of contact time. Blanko has the lowest elimination rate of TBMA bacteria up to 0 log (CFU/gram) at day 7. While the highest TBMA bacteria elimination rate that occurs in tofu with contact time 120 minutes, eliminated 6,65 log (CFU/gram) at day 7. From the Graph it can be seen that after tofu was treated with water of ozonation at certain contact duration, there was a decrease in the number of TBMA.

![Graph](image1)

**Figure 1.** Influence of duration contact in ozonated water to TBMA.

As at 40 minutes’ contact time, there was a reduction in the number of TBMA up to 6,34 log (CFU/gram) then at 80 minutes of contact, the reduction occurred to 6,60 log (CFU/gram). Furthermore, at 120 minutes’ contact time, significant TBMA reduction occurred up to 6,65 log (CFU/gram). This happens because there is an ozone contact with a tofu sample containing TBMA. Ozone will disinfect the bacteria contained in the tofu.

3.3.2. Effect of Contact Duration of the Ozonated Water on pH. Fig. 2 shows that the longer it stored, the pH of tofu will also be higher. This is because the longer will increase will increase the number of microorganisms that can lead to the occurrence of degradation of proteins that produce NH₃ which is alkaline so that the pH in the tofu increases. According to Asgar [2] that the amount of protein degradation will lead to the increasing number of NH₃ compounds produced, it will cause the increase in pH tofu. The effect of ozone concentration interaction with storage time to pH tofu can be seen in Fig. 2 below.

![Graph](image2)

**Figure 2.** Influence of duration contact in ozonated water to pH.
The pH parameter not only affects the process of ozone decomposition; the pH can also affect the content of microorganisms in water. In the pH range the initial character of the tofu sample of 5.07 still allows the presence of *E. coli* bacteria as listed in the above table, wherein *E. coli* can live in the pH range of 4.4-9.0. After submersion, the water will be allowed to contact for 40 minutes, 80 minutes and 120 minutes before the residual ozone check. Once contacted, it appears that high water turbidity can protect *E. coli* and prevent contact between disinfectants and *E. coli* in the water.

### 3.3.3. Effect of Contact Duration of Ozonated Water to Protein Content

In this study, observed the effect of contact duration of the water on the proteins in the tofu, this is because proteins in the tofu can be reduced over time due to decomposition by microbial contaminants and other oxidizing compounds such as O$_3$. Protein value at each treatment of contact time variation is shown in Table 3. In the storage period, the bacteria continuously growing. Bacteria will break down proteins into polypeptides, amino acids, and amines and then some other species can also break down fats into glycerol and fatty acids. Changes that can be seen from the outside when it has been damaged, which is the smell of acid to the rotten, the surface of tofu mucus, the texture becomes soft, reduced compactness, color and appearance is not bright, sometimes mouldy on the surface.

#### Table 3. Protein value at each treatment of contact time variation.

| Variation       | Protein Content (%) |
|-----------------|---------------------|
|                 | 0 Hour  | 168 Hours |
| Blank           | 12.27   | 9.85      |
| Duration Contact 40' | 11.35   | 9.20      |
| Duration Contact 80' | 10.51   | 10.46     |
| Duration Contact 120' | 9.92    | 9.85      |

### 3.4. Effect of Contact Temperature Ozonated Water to Quality of Tofu

In this study, the second variation is the variation of contact temperature of the water is zoned with tofu. It is aimed to know the effect of temperature in contacting the water with the tofu. On its contact, it is made into 3 variations of temperature. Temperature Incubator (37°C), Room Temperature (25°C) and Temperature Fridge (5°C). Then, after contact is stored in the fridge chiller with temperature (8°C) for 7 days. The sample control of tofu (blanko), is not contacted with ozone, also observed in this study. Then the tofu sample will be observed the quality change every 2 days’ interval.

#### 3.4.1. Effect of Contact Temperature Ozonated Water on Total Aerobic Mesophilic Bacteria

In Fig. 3 we can see the result of contact temperature variation on the contact of the aerated water. The amount of TBMA contained in the tofu at the initial character is 1.0x10$^7$ CFU/mL. Then after contact (Clock 0), there is a decrease in the number of TBMA at room temperature up to 5.5x10$^6$ CFU/mL. However, at the incubator temperature, there was a growth of 1.0x10$^6$ CFU/mL. While at refrigerator temperature, the decrease is more effective up to 3.0x10$^6$ CFU/mL. During storage, up to day 7 the temperature of the refrigerator has the least growth rate. During storage period, TBMA in refrigerator temperature variation experienced growth up to 5.9x10$^8$ CFU/mL. Meanwhile, the most rapid growth rate experienced by the variation of incubator temperature, which on the 7th day contained TBMA of 7.6x10$^8$ CFU/mL. This is also experienced by Blanko containing TBMA of 7.5x10$^8$ CFU/mL. Similarly, according to Zhou *et al.* [6], that exposure to ozone is getting longer, will disinfect bacteria, especially *E. coli* getting bigger. In addition, ozone is able to withstand bacterial growth becomes slower in its cell division. The ozonation technology has been used as a disinfection method for improving water quality. On the one hand, ozone acts as a powerful oxidizer and excellent disinfectant so that it can deactivate the enzyme work on the cell membrane of the bacteria in such a way that it dies entirely [7].
3.4.2. Effect of Temperature Contact Water Temperature on pH Tofu. The influence of the contact temperature of the ozonated water to the change in pH of the tofu can be seen in Fig. 4. The results show that the lower the contact temperature of the ozonated water with tofu, the slower the pH increase rate. At 168 hours the lowest pH is found in the sample with the contact temperature at the refrigerator temperature of 5.92. During storage, microbial activity occurs which causes the pH value to rise. Some microorganisms, especially mesophilic bacteria and E. coli, can break down acids naturally present in foodstuffs. Therefore, it can lead to a sufficient increase in pH to allow the growth of decaying species previously inhibited by their growth.

3.4.3. Effect of Contact Temperature Water Temperature on Protein Content. In this study, protein content was tested by the Kjeldahl method, in which the benchmark is the number of Nitrogen atoms present in the sample. Decreased protein levels can mean that ozone can damage proteins either physically or chemically. According to Young and Setlow [8], secondary structures of proteins linked to hydrogen bonding characteristics between C=O and N-H groups on polypeptides. This C=O bond is sensitive to ozone attacks. Cysteine protein may be the type of amino acid that was first oxidized by radical oxygen.
Protein content contained in tofu before being treated is 12.27%. In Fig. 5 it is seen that, the protein content decreases at each contact temperature variation. At the time after contact (0 Hours), the decrease in protein content occurs in 3 variations of contact temperature. Significant decrease occurred at incubator temperature from 12.27% to 9.70%. Then followed by room temperature, the decrease occurs from 12.27% to 10.20%. While at refrigerator temperature, protein content decreased, but not very significant, only from 12.27% to 11.50%.

3.5. Effect of Ozone Dosage in Ozonated Water to Quality of Tofu

In this study, also conducted an analysis of the effect of ozone dosage on the quality of tofu. Variation of ozone dose used in this research that is equal to 0.28 mg/L and 0.33 mg/L. The treatment of the samples was soaking by tofu water with each dose for 120 minutes. Then, after 120 minutes, the ozonated water already used to soak the tofu is discarded. Samples are stored in the refrigerator for 7 days. Sampling to be tested is shortly after reboot (hour 0), then storage clock 1, hour 72, hour 120 and hour 168.

3.5.1. Effect of Contact Duration of Ozonated Water to Total Mesophilic Aerobic Bacteria. In Fig. 6 compared to the treatment of tofu with different doses of ozone in decreasing the total number of aerobic mesophyll bacteria, the graph shows that, with higher ozone doses, the higher the ozone-carrying power of the total bacteria of aerobic mesophyll. There are 2 doses compared to this time. Dose of ozone 1 is 0.28 mg/L, this dose is a dose that researchers use to retrieve research data. While the dose of ozone 2, is the result of research [9], which is equal to 0.33 mg/L.
3.5.2. Effect of Ozone Dosage on Protein Content. Fig. 7 shows the influence of ozone dosage to protein content in tofu. It can be observed that there is a substantial decrease in protein content after contact with an aqueous water. The initial character of protein content in the dose of ozone 1 and the dose of ozone 2 is 12.27% and 14.54%. Then, after being contacted with water, it decreases. At the dose of ozone 1, there was a decrease of up to 11.5% and at the dose of ozone 2 to 14.01%. An increasing amount of ozone will increase the amount of degraded protein, as Cataldo [5] has noted, which observes a decrease in the number of invertase proteins as ozone levels are added.

Figure 7. Influence of ozone dosage to protein content in tofu.

With higher ozone doses (0.33 mg/L), quality guarding of the tofu will be more effective because the denatured protein is fewer, only 0.53% while the 0.28 mg/L ozone dose is 0.77% denatured protein content. This is because the number of TBMA bacteria successfully eliminated at 0.33 mg/L ozone dose is higher than when the ozone dose is 0.28 mg/L.

4. Conclusion
Conclusion that can we obtained from the objective is the longer the contact duration between the tofu and the water is zoned, the number of TBMA in the know is less. Among 40, 80, and 120 minutes, the most optimum contact duration in disinfecting TBMA was 120 minutes with disinfection capability 51%. The lower the contact temperature of the water is zoned, the number of TBMA to know the less. Among the refrigerator temperatures, the incubator temperature and room temperature, the most optimized ozonated water in the disinfection of TBMA is the temperature of the refrigerator with 96% disinfection capability. The longer the contact duration between the tofu and the ozonated water, the lower the rate of increase in pH, moisture content, and decreased protein levels during storage. The lower the contact duration of the ozonated water, the lower the rate of increase in pH, moisture content, and decreased protein levels during storage. The higher the ozone dose increase, The greater the number of deactivated TBMA, The greater the decrease in protein content after immersion, The greater the decrease in pH after immersion, The lower the increase in moisture during storage.

Acknowledgements
This work was supported by PDUPT 2018 with Contract Number 404/UN2.R3.1/HKP05.00/2018. The authors would like to thank Directorate of Research and Community Service UI for facilitating this grant.

References
[1] Andarwulan N, Kusnandar F and Herawati D 2014 Pengelolaan Dara Analisis Pangan, (Jakarta: Universitas Terbuka) pp 1-41
[2] Asgar A 2014 Teknologi Ozonasi Untuk Mencuci Sayuran. In: Iptek Hortikultura: Balai Penelitian Tanaman Sayuran) pp 10-3
[3] Sotelo J L, Beltran F J, Benitez F J and Beltran-Heredia J 1987 Ozone decomposition in water: kinetic study Industrial & Engineering Chemistry Research 26 1 39-43
[4] Nasional B S 2017 Tahu SNI 01-3142-1998 Badan Standardisasi Nasional Jakarta
[5] Cataldo F 2006 Ozone degradation of biological macromolecules: proteins, hemoglobin, RNA, and DNA Ozone Sci. Eng. 28 5 317-28
[6] Zhou G, Xu X and Liu Y 2010 Preservation technologies for fresh meat–A review Meat Sci. 86 1 119-28
[7] Mailia R, Yudhistira B, Pranoto Y, Rochdyanto S and Rahayu E S 2015 Heat Resistance of Escherichia coli, Staphylococcus aureus, Bacillus cereus and Spore Forming Bacteria Contamination Isolated from Tofu Production at Sudagaran Yogyakarta Agritech-Jurnal Teknologi Pertanian 35 3 300-8
[8] Young S and Setlow P 2004 Mechanisms of Bacillus subtilis spore resistance to and killing by aqueous ozone J. Appl. Microbiol. 96 5 1133-42
[9] Ma’ruf A, Dewi S S and Wardoyo F A 2017 Waktu Paparan Gas Ozon Terhadap Pertumbuhan Bakteri Escherichia coli Prosiding Seminar Nasional & Internasional