Effects of ozonation time on the internal quality of milk: A meta-analysis

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Abstract. The aim of that study was to evaluate the effectiveness and suitability of its use according to local conditions and ensure compliance with applicable food safety standards. A raw database had been constructed based on peer-reviewed and published research articles that reported the use of ozonation time on the internal quality of milk. The results of the observations were data collected using Microsoft Excel, followed by using Microsoft Word for formula processing before processing the data using the SAS application. That research used meta-analysis techniques to integrate 29 pieces of data from six pieces of literature that observed and researched the effects of the length of time of the ozonation process on milk quality. The variables observed were the effect of the long ozonation process for dairy products on the number of bacteria, protein levels, aflatoxin levels, and pH. The results of observing the effect of long ozonation on milk to determine the number of bacteria had an insignificant effect (p > 0.05). The protein results had a significant effect (p > 0.05) on the duration of ozonation; the results of aflatoxin levels found in milk had a significant effect (p > 0.05) on the duration of ozonation in milk; and the pH of milk also had a significant effect (p > 0.05) on the duration of ozonation in milk. In summary, using ozonation time was safe for the internal quality of milk.

1 Introduction

Milk is a product that is easily damaged or perishable because of its high nutritional content and its nature, which is easily affected by external factors. One process of handling milk that can cause damage is heating it at high temperatures. Heating milk at high temperatures can cause denaturation or changes in the protein structure of milk [1]. Milk proteins, especially whey proteins such as whey protein and casein, have complex structures and are susceptible to high temperatures. When milk is heated to too high a temperature, protein bonds can be disrupted and the protein structure can change. This can result in a loss of biological and functional activity of the protein as well as changes in the texture and taste of the milk. Apart from that, bacterial activity can also cause damage to milk. Bacteria in milk can produce enzymes that damage the nutrients in milk.

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Good milk quality is very important to maintain the cleanliness, safety, and nutritional value of the milk produced. In an effort to preserve milk, the preservation method commonly used is sterilisation. Sterilisation aims to destroy or inactivate pathogenic and spoilage microorganisms that can cause damage to milk. Commonly used sterilisation methods include thermal (heating) and non-thermal methods [2]. One technology that can be used to improve milk quality is ozonation. Ozonation is the process of using ozone to clean, disinfect, and remove unwanted odours from water, air, or surfaces [3]. In the context of milk, ozonation can be used to address various problems that can affect milk quality, such as bacteria, viruses, pathogenic microorganisms, unpleasant odours, and other contaminants.

The application of ozonation in milk processing has several significant benefits. Several aspects relate to ozonation and milk quality: killing of microorganisms, elimination of unpleasant odours, increasing shelf life, and reducing the use of chemicals. The application of ozonation in milk processing is beneficial not only for milk producers but also for consumers who consume dairy products. By improving the quality of milk, ozonation can help maintain the cleanliness and safety of dairy products, as well as the taste and nutritional value presented to consumers. It should be noted that ozonation must be carried out carefully and in accordance with appropriate guidelines. Therefore, it is important to follow the recommended instructions and guidelines and exercise strict supervision in the application of ozonation in milk processing.

In the context of the dairy industry in Indonesia, ozonation can be a technology that has the potential to help improve the quality of milk and, consequently, the quality of the milk products produced. However, the implementation of ozonation in the Indonesian dairy industry requires further study and research to evaluate the effectiveness and suitability of its use according to local conditions and ensure compliance with applicable food safety standards.

2 Materials and Methods

2.1 Literature search and Dataset development

A raw database was constructed based on peer-reviewed and published research articles which reported use of ozonation time on the internal quality of milk. Articles were selected based on the [4] method. An algorithm literature was constructed with a search in Scopus, Web of Science, PubMed, and Medline using the MESH terms “bacteria”, “ozonation”, “milk”, “aflatoxin”, and “pH”. A single search from Google Scholar was also undertaken to identify additional studies that may have been relevant to our objectives.

2.2 Data analysis and coding

Prior to statistical meta-analysis, data analysis and coding were performed using SAS ODA Academics. The modelling used by following [5].

\[ Y_{ijk} = \mu + S_i + \tau_j + S\tau_{ij} + \beta_1 X_{ij} + b_i X_{ij} + \beta_2 X^2_{ij} + b_i X^2_{ij} + e_{ijk} \]  

Where: \( Y_{ijk} \) = dependent variable, \( \mu \) = overall mean value, \( S_i \) = random effect of the \( i \)th study, assumed to be \( \sim N_{iid} (0, \sigma_S^2) \), \( \tau_j \) = fixed effect of the \( j \)th of \( \tau \) factor, \( S\tau_{ij} \) = random interaction between the \( i \)th and \( j \)th level of \( \tau \) factor, also assumed to be \( \sim N_{iid} (0, \sigma^2_{S\tau}) \), \( \beta_1 \) = overall value of the linear regression coefficient of \( Y \) to \( X \) (a fixed effect), \( \beta_2 \) = overall coefficient value of the quadratic regression of \( Y \) to \( X \) (a fixed effect), \( X_{ij} \) dan \( X^2_{ij} \) =
continuous values of the predictor variable (in linear and quadratic form, respectively), \( b_i = \) random effect of the study on the regression coefficient of \( Y \) to \( X \), assumed to be \( \sim N_{iid}(0, \sigma^2_b) \), and \( e_{ijk} = \) residual value from unpredictable error. \( S_{\tau_{ij}} \) dan \( S_i \) are taken to be independent variables that are chosen at random. A validation and significance test were conducted on the model. The significance of the values was determined using a one-way analysis of variance. It is significant if the P-value (P or P-val) < 0.05 and tends to be significant if the P-value between 0.05 and 0.1. As a result, \( P_l \) represents the P-value for the linear constant \( (\beta_1) \) and \( P_q \) represents the P-value of quadratic constant \( (\beta_2) \). Therefore, the validation test was conducted using the root mean square error (RMSE) and Nakagawa determination coefficient (R2) or \( R_{GLMM}(c)^2 \)

\[
RMSE = \sqrt{\frac{\sum (O - P)^2}{NDP}}
\]

\[
R_{GLMM}(c)^2 = \frac{\sigma_f^2 + \sum (\sigma_i^2)}{\sigma_f^2 + \sum (\sigma_i^2) + \sigma_e^2 + \sigma_d^2}
\]

Note: \( O = \) actual value, \( P = \) estimated value, \( NDP = \) number of data point, \( \sigma_f^2 \) is the variant of a fixed factor, \( \sum (\sigma_i^2) \) is the sum of all variants of the component, \( \sigma_e^2 \) is the variant due to the predictor dispersion and \( \sigma_d^2 \) is the specific distribution of the variant.

### 3 Results and discussion

The results obtained using the SAS application to determine the number of bacteria showed that the length of time of ozonation of milk had insignificant effect (P > 0.05) on the across whole parameters. Before the ozonation process was carried out, milk had a very high bacterial content, according to the treatment results from previous research. The results of observations of total bacteria before and after ozone treatment are presented in Table 1.

#### Table 1. Studies included in the effects of ozonation time on the internal quality of milk

<table>
<thead>
<tr>
<th>No</th>
<th>References</th>
<th>Level</th>
<th>Countries</th>
<th>Kind of Milk</th>
<th>Form</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>[6]</td>
<td>0-10</td>
<td>Turkey</td>
<td>Milk concentrate</td>
<td>Liquid</td>
<td>5-10</td>
</tr>
<tr>
<td>2</td>
<td>[7]</td>
<td>0.10</td>
<td>Iran</td>
<td>Whey concentrate</td>
<td>Liquid</td>
<td>5-10</td>
</tr>
<tr>
<td>3</td>
<td>[8]</td>
<td>3-12</td>
<td>Indonesia</td>
<td>Fresh milk</td>
<td>Liquid</td>
<td>5-10</td>
</tr>
<tr>
<td>4</td>
<td>[9]</td>
<td>3-12</td>
<td>Indonesia</td>
<td>Dairy milk</td>
<td>Liquid</td>
<td>3-12</td>
</tr>
<tr>
<td>5</td>
<td>[10]</td>
<td>0-9</td>
<td>Indonesia</td>
<td>Goat milk</td>
<td>Liquid</td>
<td>3-9</td>
</tr>
</tbody>
</table>

#### Table 2. Descriptive table of the effects of ozonation time on the internal quality of milk

<table>
<thead>
<tr>
<th>No</th>
<th>Parameters</th>
<th>Unit</th>
<th>NDP</th>
<th>Mean</th>
<th>SD</th>
<th>MAX</th>
<th>MIN</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AFM</td>
<td>µg/kg</td>
<td>19</td>
<td>0.36</td>
<td>0.13</td>
<td>0.62</td>
<td>0.20</td>
<td>0.03</td>
</tr>
<tr>
<td>2</td>
<td>pH</td>
<td>No unit</td>
<td>19</td>
<td>6.64</td>
<td>0.05</td>
<td>0.013</td>
<td>6.75</td>
<td>6.54</td>
</tr>
<tr>
<td>3</td>
<td>Bacteria</td>
<td>Log/CFU</td>
<td>19</td>
<td>51390</td>
<td>71149</td>
<td>202500</td>
<td>938</td>
<td>0.19</td>
</tr>
<tr>
<td>4</td>
<td>Protein</td>
<td>No unit</td>
<td>19</td>
<td>3.54</td>
<td>0.52</td>
<td>4.27</td>
<td>2.99</td>
<td>0.14</td>
</tr>
</tbody>
</table>

AFM – Aflatoxin; CFU – colony forming unit; Kg – kilogram; Log- logarithm; NDP – Number data point; Max – Maximum; Min – Minimum; pH- potential hydrogen; SD – standard deviation; SEM- standard error mean, µg – microgram
The number of milk bacteria in the group that received ozonation treatment was much lower than the control group (without ozone treatment). From the literature results, it was found that before the ozonation process was carried out, the number of bacteria exceeded the number of SNI applied, based on SNI 3141:2011, with a maximum figure of $1 \times 10^6$ CFU/ml. The results after administering ozonation with an average time span of 3–12 minutes, reviewed from the three literatures, showed a process of reducing the number of bacteria to $0.098 \times 10^6$ CFU/ml. The amount of bacterial contamination decreased ($P > 0.05$), and increasing the time of the ozonation process would reduce the number of bacteria found in milk even though the thermal sterilisation process was not used. It is known that the longer the ozone exposure, the lower the number and activity of bacteria found in milk. The increasing exposure time to ozone in the process of sterilising consumption milk will influence the chemical and organoleptic properties as well as the activity of spoilage microbes found in pure milk, so it can be seen that ozone can indicate and provide significant effects [4].

The results of observations and research using the meta-analysis concept prove that the administration of ozone with the effect of a long exposure time can eliminate and sterilise the microbial content or number of bacteria in consumed milk. States that non-thermal
sterilisation is an alternative process to replace thermal sterilisation, where the non-thermal sterilisation process will maintain product nutrition even though there has been a decrease in the number of bacteria found in milk [3].

Aflatoxin levels found in milk are the result of the process of breaking down the energy contained in milk nutrients by spoilage microbes. Aflatoxin itself is a type of mycotoxin, namely a group of natural poisons that can be produced by fungi such as Aspergillus sp. and have adverse effects on humans, animals, and plants that can result in disease and economic losses. The higher the level of aflatoxin contained in milk, the higher the level of bacteria contained in the milk processing process before consumption. In general, it is known that there are two types of fungi that produce aflatoxins found in milk, namely Aspergillus flavus, which produces aflatoxin B1 and Aspergillus parasiticus, which produces four types of aflatoxins. Some of the types of aflatoxin listed above are toxins that can damage agricultural products, especially livestock products. Aflatoxin itself is obtained from feed contaminated by the fungus Aspergillus sp., which becomes a residue in the livestock's body, thus becoming AF contamination. The presence of AF contamination, especially AFB1, in animal feed, apart from endangering animal health, also causes residues of aflatoxin and its metabolites such as aflatoxin M1, aflatoxicol (Ro), aflatoxin Q1, and aflatoxin P1, which are deposited in meat, milk, and eggs [6]. The results of observations and analysis showed that for the aflatoxin content contained in the table above, there had been a decrease in aflatoxin residue during the ozonation process in milk.

According to the provisions of BSN 7385-2009 and Regulation of the Head of the Republic of Indonesia Food and Drug Supervisory Agency No. HK.00.06.1.52.4011 sets the maximum threshold for AFM content in milk and processed milk by-products at 500 ng/L in consumed feed and converted into residue left in milk to be 0.5 µg/L (ppb). Thus, the use of ozone can be ensured as a strong antimicrobial agent in meeting the needs of the food processing industry, which can maintain consumer health [12]. The most common way to stop the development of microorganisms in milk is to use a pasteurisation process or heat it to a temperature of 700–850°C, but it is found that the nutritional content will drop drastically or damage will occur. Therefore, it is necessary to have an effective process to reduce the development of milk-destroying microbes without reducing the nutritional content of the product too much. One of these methods is the ozonation method, where ozone technology is able to have a minimal impact on the nutritional quality of milk but can also kill bacteria found in milk [4].

Based on the results listed in table 4, it shows that the decrease in milk protein levels in terms of the length of time exposure to ozone had on milk protein levels had a significant influence, where after the ozonation process, the protein levels experienced a decrease that was not too sharp. The decrease in protein levels can be concluded that ozone given over a long period of time will prevent damage or loss of too much protein levels [4]. Based on table 4 above, it is known that the protein levels before being exposed to ozone correspond to a time range between 0 and 12 minutes, the protein levels in cow's milk have relatively the same amount. It is known that the ozonation process can result in protein denaturation but not too high, in contrast to the pasteurisation process, where during the production process it will have a very high protein denaturation effect due to the continuous thermal process given to the milk, but reviewed again the ozonation process described is appropriate. with different lengths of time, the level of protein denaturation is very low, so statistically the reduction in protein has no real effect on the nutritional content of milk products.

The results of other research strengthen the results of observations regarding the effect of ozonation as a substitute for developing non-thermal food processing technology in the field of dairy products, which stated that milk protein levels experienced a decrease that was not too significant by 0.10% for each length of time given and remaining above the established standard protein content, where the protein content is in accordance with SNI 3141.1:2011,
processed cow's milk must contain a minimum of 2.8% protein from the total product [13]. This means that the process of using ozonation as a sterilisation for milk processing can minimise damage to protein levels in milk processing, being able to maintain it optimally and not experiencing a drastic decrease in the percentage of protein levels. The results of observations from the research cited showed that milk that received ozone exposure for a period of 3 to 12 minutes in accordance with the respective literature had relatively the same pH value, namely an approach to normal pH (8). These results show that the longer the treatment time, the milk tends to increase the pH value, although the increase is not very significant, and statistically, these results have no effect on milk nutrition. The cause of damage to milk is an increase in lactic acid production due to the breakdown of milk lactose, resulting in an increase in lactic acid production, which can cause the acidity of the milk to increase, and a decrease in the pH value, which causes the milk to become damaged and unfit for consumption. The acidity level of milk is an important indicator of the overall quality of milk [3].

4 Conclusion

To sum up, ozone treatment and exposure have a specific impact on the number of bacteria, aflatoxin levels, protein, and pH in milk and other dairy products. The use of ozone can be proposed as a powerful antimicrobial agent that can meet the needs of the food industry and achieve food regulatory agency approval and consumer acceptance. The long processing time of using ozonation as a non-thermal sterilisation technology can be used to improve the microbiological and nutritional quality of fresh milk and processed milk by-products.

References

3. F. Listiana, R. S. S. Santos, Y. Subagyo. Prosiding Seminar Nasional Teknologi Agribisnis Peternakan (STAP), 9 (2022)
7. R. Widiastuti, WARATOZA. 24 (2014)
9. BPOM RI, Badan Pengawasan Obat dan Makanan Republik Indonesia 2012 (Jakarta, 2012)
10. BSN, Badan Standarisasi Nasional. 2009. (Jakarta Indonesia, 2009)
11. D. Sert, E. Mercan. LWT. 144 (2021)