Effect of ozone treatment on microbiological and physicochemical properties of soymilk beverage

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Abstract. Ozone is a strong oxidant and potent desinfecting agent. An ozonation process for microbial inactivation in some beverages has been done. Accordingly, the ozonation process was performed in soymilk. Soymilk was ozonated under different exposure time: 1, 3, 5, 10, and 15 minutes and evaluated for the changes of microbiological and physicochemical properties. The assays were carried out by using an experimental design fully randomized and results were statistically evaluated by Duncan’s Multiple Range test (p < 0.05). Following the ozone treatment, a significant reduction in pH occurred at 1 minute treatment, whereas a significant increasement was recorded after 3 minutes treatment. A significant increasement in total dissolve solid was observed at 5, 10, and 15 minutes. Microbial reduction was recorded 0.19 log cycles at 1 min., 0.35 log cycles at 3 min, and 0.57 log cycles at 5 minutes ozone treatment. In contrast, an increase of microbial population occurred at 10 and 15 minutes. The findings presented here could be a prelude for the potential application of ozone treatment of soymilk in the food industry.

Keywords: Ozone treatment, Soymilk, Microbial inactivation, Physicochemical properties

1. Introduction
Soybean (*Glycine max.*) is a food material that known contain highly nutrition. Soybean contains 35–50% protein and essential amino acids [1]. Quality of protein that contained in soybean is similar to protein in milk. Besides its protein content, soybean is a good essential fatty acids and isoflavonoids source. Due to its high nutrition, soybean can reduce risk of coronary disease, breast and prostate cancer, also improve skin health [2]. These high nutrition brings popularity to soybean, so there are many food product that was derived, such as tofu and soymilk.

Soymilk is a liquid based food product, made from extraction of soybean. This beverage has consumed for centuries in Asia. This beverage contain essential fatty acids and high quality protein...
with no cholesterol, gluten, and lactose. Soymilk is suitable as substitution of cow’s milk for people with lactose-intolerant [3]. Soymilk was also used as base for other product, such as soy yoghurt and cheese [4]. Due to its short shelf-life, consumption of fresh soymilk has limit to areas close to the production site [4]. This characteristic leads to research on attempt to extend shelf-life of fresh soymilk.

Thermal treatment has been used on preservation of soymilk. This method was used due to the effectivity on microbial inactivation [5]. However this method has its shortcoming. Thermal treatment could affect nutrition in food product and cause several change in organoleptic characteristics such as color and flavor. This changes could drop consumer’s interest [6]. In attempt to preserved quality of soymilk, non-thermal treatment was used. There are several non-thermal treatment to preserved food product, such as UV-treatment, ultrasound, and ozone.

Ozone (O₃) is a strong oxidant and antimicrobial agent that has been applied in several fields, such as food industries, water treatment, and medicine. Its high reactivity, penetrability, and spontaneous decomposition to non-toxic product cause ozone was used to ensuring microbiological safety in food products. In food industries, oxidizing potential of ozone was applied for food preservation, extension of shelf-life, sterilization of used equipment [7]. Application of ozone has been reported in various beverages including apple cider [8], orange juice [9], blackberry juice [10] and raw milk [11].

Ozone is a powerful antimicrobial agent due to its oxidizing capacity, has numerous potential to be applied in food industry [12]. Its effectivity to reduce microorganism and free of chemical residues is the benefits of ozone. Mechanism of ozone as antimicrobial agent involve oxidation and destruction of cell walls and cytoplasmic membranes. Initially, ozone directly oxidized and destroyed bacteria’s cell wall and cytoplasmic membranes. After that, ozone moves in the cell and work on its DNA so bacteria did not develop resistance over ozone. There are some different microbial sensitivity to ozone, depend on the structure of its cell walls [13].

Ozone can be generated in several ways, such as electrical discharge, electrochemical method, UV method, and radiochemical method [14]. Electrical discharge is a commonly used in application of ozone. This method was called corona discharge as well as dielectric barrier discharges (DBD). DBD method worked with plasma sourced from oxygen or air, caused electron energy transferred in dominant gas molecules such as N₂, O₂, H₂O through collision process. Primary radical (O*, N*, OH* and others), positive ions, negative ions, and excited molecules were produced by this ion collision. One of the primary radical formation, electron-ion, ion-ion reaction and release of electrons toproduce more secondary radical such as O₂* and H₂O [15].

The object of this paper was to determine the effect of ozone treatment on microbiological and physicochemical properties of soymilk, through quantitatively evaluating total plate count (TPC), pH value, and total dissolved solid (TDS).

2. Methodology

2.1. Material
The material used in this study is soymilk fresh obtained from Mr. Bowo’s small and medium enterprise located at 18 Bharata alley, Tembalang, Semarang, Central Java.

2.2. Sample preparation
The soymilk samples was stored in plastic packaging, maintained at ambient conditions at a temperature of 23 ± 2 °C before treatment.

2.3. Ozone treatment
The ozone generator is connected with a device that functions to enlarge the surface area of the soymilk to form one layer, so that ozone is only exposed to the surface of soymilk. Before ozonation, the device was cleaned using sterile alcohol, rinsed with sterile distilled water, and ozone flowed with a concentration of 16 ppm for 2 minutes. A total of 200 mL of the sample was flowed and treated with
ozonation with a controlled concentration up to 8 ppm. The device was operated at 2.7 kV then pure oxygen flowed at 0.6 L/min. The ozonation time is varied for 1, 3, and 5 minutes. Then, the samples was analyzed for microbial and psychocemical characterization.

2.4. Total plate count
The working principle of TPC analysis is the calculation of the number of bacterial colonies present in the sample with dilutions as needed and done duplo. All work is done aseptically to prevent undesirable contamination and duplicate observation can improve accuracy. The number of bacterial colonies that can be calculated is between 30–300 colonies. 1 ml each soymilk sample was serially diluted with 0.90% sterile saline solution (5 to 6 dilutions), and appropriate dilutions of 0.1 mL was piped to the sterilized petri dish then PCA media was poured evenly. The total microbia was counted after 24 hours incubated at 30 °C. The log reduction was calculated considering the initial count (control sample) and the count’s obtained after ozonation process.

2.5. Physicochemical properties
pH and total dissolve solid was measured using a digital pH/TDS meter Hanna HI 98130 to represent physichochemical properties of soymilk after ozonation. A beaker containing 10 mL soymilk sample was analyzed in triplicates and the pH meter was calibrated with standard solutions prior to carrying out each measurement [16].

2.6. Statistical analysis
The analysis were replicated three times. SPSS version 16 statistical software (SPSS, Inc., United States) was used to analyze the results. Means of the three replicates were compared using one-way analysis of variance (ANOVA). The differences between DBD plasma treatment means were evaluated using Duncan's test (p < 0.05).

3. Results and discussion
3.1. Total plate count
The stability of soymilk depends upon the extent to which the microflora alters its freshness. Various fruits and beverages have different levels of background microorganisms. The cold plasma inactivation efficacy of microorganisms varies and depends on many factors such as microbial species, types of reactive species generated, duration of exposure, pH and the surrounding environment of the microorganisms [17].

The microbial inactivation in soymilk using ozone was presented in table 1. The total plate count for the untreated soymilk was 5.97 log CFU/ml. After ozone treatment from 1 to 15 mins, the total plate count were reduced, but after 10 and 15 mins ozonation were increased again. The ozone treatment after each 1, 3, and 5 min resulted in 0.19 log, 0.35, 0.57 log reductions. In contrast, an increase of microbial population occurred at 10 and 15 minutes each 0.03 and 0.87 log. This result is not conform with Indonesian National Standard for soymilk (SNI 01–3830–1995), the total plate count ≤ 2.3 log CFU/ml. The ozonation treatment can reduce the microbial count, but has not reached the expected number. This may be overcome by increasing hygiene when processing, concentrating, or completing proper fulfillment, can also be combined with storage in cold temperatures. These results are consistent with Khuddhir's (2017) study, where ozonated milk samples can be returned microbes to 5.01 logs at room temperature storage (± 30 °C) and 5.89 log at cold temperature storage (± 4 °C) [18].
Table 1. Impact of various ozone treatment time on soymilk total plate count.

| Treatment time (min) | Total Plate Count (log CFU/ml) | Log reduction (log CFU/ml) |
|----------------------|-------------------------------|---------------------------|
| Control              | 5.97                          | -                         |
| 1                    | 5.78                          | 0.19                      |
| 3                    | 5.62                          | 0.35                      |
| 5                    | 5.40                          | 0.57                      |
| 10                   | 6.00                          | 0.03*                     |
| 15                   | 6.84                          | 0.87*                     |

*Increase of microflora

The efficacy of ozone treatment on soymilk sample can be determined by certain organics, inorganics, or suspended solids. Dissolved organic matter reduces the disinfection activity by consuming ozone to produce compounds with little or no microbiocidal activity, thereby reducing the concentration of active species available to react with microorganisms [19]. The inactivation of E. coli in orange juice, and found that the efficacy of ozonation was reduced in the presence of ascorbic acid and organic matter [20]. Meanwhile, our results showed an inactivation trend from 1 min to 5 min, and then log increase in 10 and 15 min of ozone treatment. This could be explained that ozone concentration present or available in the medium was a parameter determining ozone efficacy. Increased ozone concentration causes saturation and thus makes addition of further ozone to the reactor ineffective, resulting in longer times to achieve the same log-reduction values [19]. In addition, a longer ozonation time allowed the sample to come into contact with the air of many bacteria that precisely contaminated soymilk sample because the ozonation device is discontinuous.

3.2. pH

There are some factors that affect ozonation in food product, such as ozone concentration, temperature, and pH [21]. pH value in a solution is important to determining capability of decontamination in a system [22]. Besides, pH is one of the main quality parameters to determine product freshness [23].

pH value of treated sample was shown in Table 2. Following treatment with ozonation, mostly pH value of treated sample increased slightly except for sample with 1 min. treatment time. Reduction in pH value for sample with 1 min. treatment time may happen due to reaction of reactive species, such as ozone, with water on water-gas interface [24]. The different pH result can be attributed to buffering capacity of the liquids [25]. Silva (2015) researched the effect of combining ozone and heat treatment on sugarcane juice [26]. Use of ozone in this research did not change the pH of sugarcane juice.

Table 2. pH and total dissolve solid contents of various ozone treated soymilk samples.

| Treatment time (min) | pH        | Total Dissolve Solid (ppm) |
|----------------------|-----------|-----------------------------|
| Control              | 7.00 ± 0.006<sup>a</sup> | 312.00 ± 3.512<sup>ab</sup> |
| 1                    | 6.64 ± 0.010<sup>a</sup>  | 298.33 ± 9.905<sup>a</sup>  |
| 3                    | 7.22 ± 0.007<sup>c</sup>  | 324.33 ± 1.202<sup>b</sup>  |
| 5                    | 7.23 ± 0.006<sup>c</sup>  | 339.67 ± 4.410<sup>c</sup>  |
| 10                   | 7.33 ± 0.003<sup>d</sup>  | 353.33 ± 1.856<sup>cd</sup> |
| 15                   | 7.34 ± 0.003<sup>d</sup>  | 360.00 ± 2.082<sup>d</sup>  |

*Mean ± SE was for three replicates
Means with the same letter within the same column are not significantly different (\( \rho \geq 0.05 \))
3.3. Total dissolve solid
Total dissolve solid is substances as inorganic salt and other organic substances that dissolved in water. Total dissolve solid includes anion and cation of a sample [27]. Total dissolve solid can be used as emulsion stability indicator [28]. Total dissolve solid of treated sample shown in table 2. This result shows that there were increasing of total dissolve solid on treated sample, except sample with 1 min. treatment time. These increasement can be caused by reaction of ozone with organic components of soymilk. Abhilasha (2018) stated ozone reacts with organic components as an electrophilic or nucleophilic agent and the reaction was with unsaturated compounds [29].

4. Conclusion
After ozonation from 1 to 15 mins, the total plate count were reduced, but after 10 and 15 mins ozonation were increased. The microbial count was not conform with Indonesian National Standard for soymilk (SNI 01–3830–1995). The ozonation treatment can reduce the microbial count, but has not reached the expected number. The results show that most of pH value and total dissolve solid of treated sample increased slightly except for sample with 1 min. treatment time. Thus, the results highlight the ozonation as an alternative to reduce microbial load in soymilk. This may be overcome by increasing hygiene when ozonation processing, control the concentration, or treatment time, can also be combined with storage in cold temperatures. Further research on optimal ozonation conditions and the effects of ozonation on nutritional compounds are needed.

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