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Effect of Ozone Treatment on Microbiological Properties of Stored Wheat

Hussein Al-Sahho, Hakan Kuleaşan

Department of Food Engineering, Suleyman Demirel University, Isparta, Türkiye
Email: hussienalsa327@gmail.com

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Abstract

In this study, ozone gas was applied to samples of durum wheat stored in four experimental groups (durum wheat without any treatment for comparison, durum wheat treated with ozone, purified durum wheat, and purified durum wheat treated with ozone). Two groups were treated with ozone gas at 3 ppm concentration for 1 hour. Groups were then placed in air-tight glass jars and stored for 6 months at variable temperatures between 24.7˚C to 34.8˚C. Microbiological (total count bacteria, yeast/molds and coliform) and physical properties (moisture, color and ash) evaluated. Ozone application statistically caused a significant reduction in the numbers of bacteria, yeast, molds and coliforms. Ozone application, washing process and storage temperature are the major factors affecting the microbial counts. No significant differences were determined in moisture and ash contents of samples after ozone treatment. The color measurement results showed that color values of wheat samples were affected by ozone treatment, storage and washing.

Keywords

Wheat Storage, Ozone Application, Wheat Impurities, Microbial Enumeration

1. Introduction

Wheat is one of the stable foods in the world and belongs to the genus triticum in Gramineae poaceae (grass) family [1]. Harvest is the first step to crop production and to transfer grains from the field to the warehouse [2]. Various physical tests are applied before taking wheat to the silos. Cleaning before storage is crucial to protect the grain from microorganisms and insects. Most pests originate from the field [3]. The main objectives of the cleaning process are to remove any materials other than wheat, such as metals, foreign materials and stones. Removal

of other types of grain seeds, damaged wheat, and infected wheat [4]. Grain washing is one of the most effective ways to clean grain from all foreign materials and organisms [5]. Before storing grain it is necessary to dry the wheat sometimes even with the use of simple sunlight, to prevent the growth of microorganisms, the destruction of wheat and extending the stored life of the grain [2]. One of the traditional methods for fumigating stored wheat is the use of chemical pesticides. Scientists and researchers have worked to find alternative methods because of the effects that these materials have on the environment, human health, and treated grains as well. Ozone treatment is a new alternative method for fumigation of grains [6]. Ozone is a powerful oxidizing agent discovered by Christian Friedrich Schönbein in 1839 [7]. The use of ozone as a sterilization agent began in the 1900s [8]. Ozone can be produced through four processes (corona discharge, electrochemical method, UV light, and radioactive chemicals [9].

All methods used to generate ozone rely on application of large energy to break the bond between two oxygen atoms and allow them to separate and then re-formation [10]. Ozone has no effect on grain components like proteins, lipids, starch and density [11]. Ozone application affects the fat and starch content by oxidation, which modifies the physical chemical content of the grains. Reduce yellowness and promote whiteness [12]. The aim of this study is to study the effect of ozone on the microbiological and physical properties of grains during the storage period.

2. Materials and Methods

2.1. Wheat Samples

Two different types of wheat samples were obtained (1.9%) impurities. The first sample was taken directly from loading platform without any process (natural). The second sample was taken from the same batch after all processes (sieving, cleaning, washing etc.) were applied (cleaned). The wheat samples were purchased from Hediye Flour Company in Isparta, Turkey. The type of wheat was Bezostaja which is a hard-red winter wheat.

2.2. Ozone Application

Ozone application was carried out in the laboratory of the Department of Horticulture, Faculty of Agriculture, Isparta Uygulamalı Bilimler University, Isparta, Turkey. Ozone gas was generated from ozone generator by corona discharge method (Ozonoks-Ozone Systems, Narlıbahce, Antalya, Turkey) using pure oxygen. After preparation of the system test groups were treated with 3 ppm ozone gas for 1 hour at 15˚C. The gas flow was controlled manually depending on the sensor's reading. The ozone gas pumped through a glass air-tight cabinet

2.3. Sample Storage

After ozone application the wheat samples were placed in air-tight glass jars (500 g per jar). Six jars were prepared for each group (Natural, Natural Ozone, Cleaned,

and Cleaned Ozone). All groups were stored for 6 months in a laboratory incubator. The storage temperature was adjusted monthly based on the average weather temperatures in Baghdad, Iraq beginning with May (May, June, July, August, September, October). The average temperatures were obtained from "The Global Atmospheric" site, Global current weather/Acc weather [13] as shown in Table 1.

2.4. Microbial Analysis

The number of Total Aerobic Mesophilic Bacteria (TAMB) in wheat samples was determined by the method of Bacteriological Analytical Manual, the plates were incubated for 24 - 48 h at 30˚C [14]. The number of total Yeasts and Molds (TYM) in wheat samples were determined by the method of Bacteriological Analytical Manual, the plates were incubated for 48 - 72 h at 25˚C [15]. Enumeration of total coliform bacteria was done by using the procedures of Bacteriological and Analytical Manual, the plates were incubated for 24 h at 37˚C [16]. Both pour and spread plates were carried out in triplicate for each dilution. The plates were counted as colony forming units (cfu) and numbers were expressed as log cfu/g.

2.5. Moisture Content

Moisture content of wheat samples was determined by using the method of AOAC 2000/925.10 [17]. Moisture content of the samples was calculated by using the formula below.

$$[(\text{Winitial-Wfinal})/\text{Winitial})] \times 100$$

2.6. Color Measurement

The color of the wheat samples was evaluated by using CIE Lab system as L*a*b* values. The color values were measured with a chromameter carrying D65 light source (MINOLTA CR-400, Japan). L* values represents the lightness from black (0) to white (100), a* value represents the redness from green (−) to red (+), and b* represents the yellowness from blue (−) to yellow (+) of the samples. All measurements were in triplicates.

2.7. Ash Content

Ash content of wheat samples was determined by using the method of AOAC 2000/923.03 [17]. This process was continued until the samples reached a constant weight. Ash content was calculated depending on the initial and final weight of the samples.

Table 1. The average of the temperature in Baghdad, Iraq.

Months	May	June	July	Aug.	Sep.	Oct.
Temperature ˚C	28.4	33.4	34.8	33.3	29.6	24.7

2.8. Statistical Analysis

Statistical analysis was performed to compare the experimental results, All results were analyzed and compared using the (minintab statistical analysis) (version 2019) program to determine the average values during the comparison at level of $P < 0.01$. Results represent mean averages of 3 data.

3. Results and Discussion

3.1. The Effect of Ozone Treatment on the Microorganisms at the Natural Samples Wheat

Bacterial enumeration results of experimental groups were presented in Table 2. The total number of bacteria significantly decreased after ozone treatment. Statistical results showed that the decline of bacterial numbers also continued during all months. According to results the number of bacteria decreased from 4,93 to 3.87 log cfu/g in natural sample and from 4.51 to 3.10 log cfu/g for ozonated natural sample after six months of storage. The decrease may be due to several reasons, including the lack of adaptation of bacteria, sudden changes in the environment in which they live [18]. Another factor that causes the decrease in the number of bacteria may be the high storage temperatures in this study which were 24.7˚C to 34.8˚C. Incubation temperatures applied in our study were higher than appropriate growth tempertatures for microorganisms in general (25˚C to 32˚C) [3]. The high temperatures in general lead to an increase in the impact of ozone treatment and to increase rate of destruction [19]. In addition to these reasons, it is also necessary to mention that wheat samples in our study were in an air-tight glass jars and with high temperatures which leads to increase respiration rate and therefore consume oxygen and accumulation of carbon dioxide could help to increase the effect of ozone. The results showed that the bacterial numbers in gradually decreased during six months to 3.87, 3.10, log cfu/g for the samples natural and natural ozone respectively.

Table 2. Effect of the ozone treatment on the microorganisms (total count bacteria, yeast and molds, coliform bacteria) in the natural wheat samples during six months of storage.

SAMPLES	MON. 0	MON. 1	MON.2	MON.3	MON.4	MON.5	MON.6
Pca natural	4.93 ± 0.06 A a	4.66 ± 0.05 A ab	4.51 ± 0.04 A ab	4.43 ± 0.08 A ab	4.39 ± 0.07 A ab	4.30 ± 0.06 A ab	3.87 ± 0.44 A b
Pca natural oz.	4.53 ± 0.09 B a	4.43 ± 0.01 A a	3.85 ± 0.09 B b	3.63 ± 0.03 A bc	3.40 ± 0.20 B bcd	3.20 ± 0.10 B cd	3.10 ± 0.10 A d
Pda natural	4.72 ± 0.06A a	4.02 ± 0.05A a	3.99 ± 0.06A a	3.96 ± 0.07A a	3.89 ± 0.06A a	3.76 ± 0.0A a	3.54 ± 0.07A b
Pda natural oz.	4.63 ± 0.03A a	3.46 ± 0.32A ab	3.50 ± 0.27A ab	3.62 ± 0.09A ab	3.43 ± 0.13A ab	2.10 ± 1.05B b	0.00 ± 0.00B c
Coliform natural	4.15 ± 0.06A a	3.33 ± 0.20A a	3.32 ± 0.16A a	3.10 ± 0.10A a	2.10 ± 1.05A ab	2.00 ± 1.00A ab	0.00 ± 0.00A b
Coliform natural oz.	3.53 ± 0.12B a	3.30 ± 0.17A ab	3.10 ± 0.10A ab	2.10 ± 1.05A ab	2.10 ± 1.05A ab	2.00 ± 1.00A ab	0.00 ± 0.00A b

When we compared the results obtained in this study between the values of natural wheat samples before and after ozone treatment, which are shown in Table 2. We noted a slight decrease in yeasts and molds. Decreasing of values was consistent during 6 month storage period which were 3.54 at the beginning and 0.00 log cfu/g at the end. The results were parallel with the results obtained by Nur *et al.* [18] who reported the effect of ozone treatment on fungi counts leads a serious reduction. Allen *et al.* and Wu *et al.* also reported in their study that the gaseous ozone was very effective in the inactivation of fungi in the wheat [20] [21].

There are some factors that may help or accelerate the decrease in the number of yeasts and molds such as temperature which increases the effectiveness of ozone [22]. In addition, increased temperatures leads to increased respiratory rate and therefore consumes oxygen inside the airtight jars and cause drastic decreasing in the numbers of yeast and molds [3] [23]. The elimination of molds requires the elimination of oxygen because of their obligate aerobic nature. Anaerobic conditions together with ozone application increases the inhibiton rate of yeasts and molds [24].

The effect of ozone application on coliform bacteria was presented in Table 2. Above were observed. There were not significant differences in the counts of coliform bacteria, except the first month between applications. The fact that coliforms are not affected by ozone treatment may be due to their resistance factors like being gram negative [25]. The highest difference occurred in the first month as 4.15 to 3.53 log cfu/g. Results of enumerations were similar to the values reported by Tiwari *et al.*, [9] They reported in their study that, the ability of ozone gas in reduction of coliform bacteria is limited. In addition to the storage of samples in sealed glass jars prolonged ozone exposure also has inhibitory effect. Perhaps increased carbon dioxide levels may also increase the effectiveness of ozone treatment [24]. When the storage results inspected there was a decrease in total count of coliform bacteria during a period of six months. Coliform bacteria were gradually eliminated during six months.

3.2. The Effect of Ozone Treatment on the Microorganisms at the Cleaned Samples Wheat

The cleaning of the wheat alone leads to a reduction in the number of bacteria the effect was much higher when combined with ozone treatment. The application of ozone to cleaned wheat helped to reduce bacterial content more effectively and this is evident through the results presented in Table 3. Throughout the experiment (six months) and statistical results showed that there is a significant difference in the results after treatment with ozone. Besides the highest difference was in the 4th month where the number of bacteria decreased from 3.62 to 0.00 log cfu/g. The results were in accordance with the results determined İbanoglu [26] who reported the values from 5.5 to 5.1 and 4.7 log cfu/g.

Ozone treatment was more effevtiveness with the cleaned sample (separation and washing) which has high moisture content [27]. Results were also comparable

Table 3. Effect of the ozone treatment on the microorganisms (total count bacteria, yeast and molds, coliform bacteria) in the cleaned wheat samples during six months of storage.

SAMPLES	Mon.0	Mon.1	Mon.2	Mon.3	Mon.4	Mon.5	Mon.6
Cleaned pca	4.95 ± 0.05 A a	4.54 ± 0.01 A b	3.94 ± 0.08 B c	3.78 ± 0.09 A cd	3.62 ± 0.09 B cd	3.49 ± 0.12 B d	3.52 ± 0.04 A d
Cleaned oz. pca	4.04 ± 0.14 C a	3.69 ± 0.12 B a	3.33 ± 0.20 C a	1.10 ± 1.10 B b	0.00 ± 0.00 C b	0.00 ± 0.00 C b	0.00 ± 0.00 B b
Cleaned pda	4.74 ± 0.12A a	3.58 ± 0.29A ab	3.38 ± 0.25A ab	3.20 ± 0.20B ab	3.23 ± 0.23A ab	2.26 ± 1.13A ab	1.00 ± 1.00A b
Cleaned oz. pda	4.27 ± 0.12B a	3.56 ± 0.04A ab	3.26 ± 0.14A ab	3.16 ± 0.16B ab	2.10 ± 1.05A abc	1.00 ± 1.00A bc	0.00 ± 0.00A c
Cleaned coliform	4.51 ± 0.08A a	3.81 ± 0.08A b	3.26 ± 0.14A c	3.10 ± 0.10A c	3.10 ± 0.10A c	2.50 ± 0.08A d	0.00 ± 0.00A d
Cleaned oz. coliform	3.81 ± 0.08B a	2.10 ± 1.05A ab	1.10 ± 1.10A ab	1.00 ± 1.00A ab	0.00 ± 0.00A b	0.00 ± 0.00A b	0.00 ± 0.00A b

with the data reported by İbanoglu [28] who reported the values between 4.2 to 3.5 log cfu/g which represented the reduction of the total number of bacteria after treated the wheat with ozonated water.

The high temperatures in the third and fourth months between 34.8˚C to 33.3˚C respectively also help to increase the efficiency of ozone by affecting the concentration stability and decomposition of ozone. This increases the rate of ozone interaction with microorganisms and increase the rate of destruction with increased temperature [19] [29]. The results showed that the bacterial numbers in gradually decreased during six months to, 3.52, 0.00 log cfu/g for the, cleaned and cleaned ozoned respectively [21]. We concluded that all 4 factors as increased storage temperature, ozone application, oxygen depletion and cleaning effects had a combined effect on the reduction of bacteria.

The results were relatively similar to values ranged from 3.1 to 2.4 log cfu/g reported by İbanoglu, [28] and Allen *et al.* [20] reported that the wheat which was high in moisture content led to more effectiveness for ozone treatment and increase the rate of reduction and inactivation the fungal spores [9].

There are some factors that may help or accelerate the decrease in the number of yeasts and molds such as temperature which increases the effectiveness of ozone [22]. The results obtained in this study showed that the process of cleaning had a clear impact on the total number of yeasts and molds and caused a decrease in their numbers. It was also shown that ozone gas is very effective especially in airtight conditions during the storage [3] [23] [24].

From the results obtained in the table above we note that there is no significant difference in the results before and after the process of cleaning. The statistical analysis results showed that there is no difference between the results after washing. There was even a slight increase in the numbers of coliform bacteria from 4.15 to 4.51 log cfu/g. The limited differences in the numbers of coliform bacteria may because of the equipment with bacterial contaminations which may increase the grain contamination during washing or may not cause a reduction [30]. When the storage results inspected there was a decrease in total count of

coliform bacteria during a period of six months. Coliform bacteria were gradually eliminated during six months. Based on the information mentioned above, ozone treatment alone is not effective for elimination of coliforms. We think that some factors such as temperature, pH, water purity, ozone concentration and time of ozone exposure are also very effective [19] [31].

3.3. The Effect of Ozone Treatment, Cleaning and Storage on the Physical Properties

The results showed that the cleaning process of wheat affected the moisture content of the samples. The moisture content of samples was presented in **Table 4**. The results extended from 8.26% ± 0.05% in natural wheat samples to 11.06% ± 0.15% in cleaned samples. Cleaning process including washing step increased the moisture of the samples about 2.8%. These results were similar to those reported by İbanoglu [28] who reported the moisture content of wheat increases about 3% through the cleaning process. In **Table 4**, the moisture content of the natural and ozone applied natural wheat samples varied between 8.26% ± 0.05% to 8.46% ± 0.00%. The moisture content obtained in this study was relatively similar to the values obtained by Mudawi *et al.* [32] who reported that the moisture content extended from 12.361 to 13.701%, but there was no significant effect on the moisture content of the wheat. As it can be seen in **Table 4**.

Table 4. The effect of ozone treatment, cleaning and storage on the physical properties.

period	Natural	Natural ozone	Cleaned	Cleaned O
Moisture 1	8.26 ± 0.05A a	8.46 ± 0.00A a	11.06 ± 0.15A b	11.08 ± 0.27A b
Moisture 2	8.19 ± 0.04A a	8.36 ± 0.06A a	10.60 ± 0.06AB b	10.66 ± 0.05AB b
Moisture 3	8.16 ± 0.10A a	8.24 ± 0.10A a	10.16 ± 0.03B b	10.25 ± 0.13B b
L* value 1	41.58 ± 1.13B b	49.83 ± 1.47A a	43.49 ± 0.47B b	48.34 ± 1.71B a
L*value 2	49.16 ± 0.81A b	50.36 ± 0.86A b	56.65 ± 2.30A a	55.54 ± 0.45Aa
L *value 3	51.71 ± 1.29A b	50.65 ± 1.50A b	57.45 ± 1.31A a	56.55 ± 0.74A a
a* value 1	6.34 ± 0.15C a	5.98 ± 0.07B b	6.01 ± 0.02B b	6.03 ± 0.06B b
a* value 2	7.84 ± 0.29A a	7.99 ± 0.68A a	7.06 ± 0.06A a	7.31 ± 0.45AB a
a* value 3	7.22 ± 0.05B a	7.18 ± 0.54AB a	6.55 ± 0.51AB a	6.82 ± 0.09A a
b* value 1	11.96 ± 0.46B b	15.76 ± 0.80B a	12.99 ± 0.07B b	15.05 ± 0.39B a
b* value 2	20.04 ± 0.02A c	20.99 ± 0.39A b	24.46 ± 0.10A a	24.58 ± 0.11A a
b* value 3	20.26 ± 1.34A b	20.44 ± 0.78A b	24.54 ± 0.88A a	23.90 ± 0.95A a
% Ash (in dry material) 1	1.51 ± 0.00A a	1.52 ± 0.00A a	1.62 ± 0.00A a	1.61 ± 0.00A a
ASH 2	1.51 ± 0.01A a	1.52 ± 0.00A a	1.63 ± 0.00A a	1.60 ± 0.02A a
ASH 3	1.50 ± 0.03A a	1.52 ± 0.00A a	1.64 ± 0.01A a	1.62 ± 0.01A a

Results represent the averages of triplicates. Uppercase Letters represents months, Lowecase letters represents application.

Application of ozone treatment does not significantly change the moisture content of wheat samples [33]. **Table 4** Shows that there is no significant clear effect on the moisture content of grains after treatment with ozone gas where it was noted that the moisture content of the clean wheat sample was 11.06% and after treatment it was 11.08% at the beginning of the application. The moisture content for the clean wheat sample changed from 10.16% to 10.25% after six months [26] [32].

The results confirm that grain moisture loss depends on several factors such as temperature, grain moisture content, and relative humidity as well. The losses were increased by increasing the rate of moisture content and increasing the temperature. There is a close and interrelated relationship between temperature and grain moisture content and relative humidity [34].

The color is one of the major criterias which determines the quality of grains [7]. L* (lightness) values of wheat samples measured before and after ozone treatment were shown in **Table 4**. There were significant differences in L* values at the first month and following months. When statistical analyzes were inspected the highest difference at first month were between natural sample which was 41.58 and ozone treated natural sample as 49.83. Results were in accordance with the results presented by Marston *et al.* [35] who reported that ozone has the ability to oxidize pigments such as carotenoids found in some food ingredients. Zhu [7] also supported the idea that increasing L* may be the result of oxidation of carotenoids and polyphenols found in grains. But there may be some other factors like the characteristics of food (the type), and application conditions such as temperature and humidity. Ozone treated foods, *i.e.*, rice, wheat or nuts, may show different behaviors due to their physical structures and chemistry [7] [12] [36] [37].

The a* value indicates the degree of redness of the surface color of the grain from red to green. The numbers of a* value was not constant with storage due to oxidation of some pigments and other chemical compounds. The results were similar to results of Wang *et al.* [12] reported also decrease in a* values after ozone treatment. In general there was no difference in the results of months that followed. Although there were changes in a* values of the samples the change was quite minor. Marston *et al.* [35] also reported that there was no difference in the results during storage after ozone treatment.

The b * value in measurement of color indicates the degree of yellowness of the surface. When the results were inspected, ozone treatment caused an increase in b* values in all samples. Increasing was continued gradually during 6 months of storage. Our results did not correspond to the results obtained by Zhu [7] and İbanoglu [28]. We thought that the difference may be due to the different conditions of the storage and the impact of some other factors such as temperature and moisture content. Besides, the concentration of ozone, duration of exposure, oxygen consumption occurred in air-tight jars and the type of wheat might cause the difference in results [7].

The results of ash analysis were presented in Table 4. Although the ash content of samples was changed regarding total weight, there was no change when ash amount in dry matter was considered. Statistical analysis also showed that there were no differences in ash content. Nur *et al.* [18], İbanoglu, [28] Mudawi [32] also reported that there was no significant effect on the ash content after treated the wheat by ozonated water.

4. Conclusion

Treatment of hard wheat samples with ozone gas reduced the total numbers of bacteria, yeast, molds and coliforms. Cleaning process along with ozone application was more effective compared to the untreated samples. The treatment of wheat samples with ozone caused minor changes in the color values of wheat. Cleaning process, storage temperature and storage conditions were also found to be effective on color measurements. There was no significant effect of either storage or ozone application on the ash content of wheat. The moisture content of wheat samples increased after washing process. In addition to reducing the contamination of grains washing step increased the efficiency of ozone treatment. It was also noted that the effect of ozone in grains with higher moisture content was higher. While no insects were observed in wheat samples stored in air-tight containers under temperatures ranged from 24.7˚C to 34.8˚C, their large numbers were determined in the samples stored polyethylene sacks stored at room temperature.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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