*Plant Archives* Vol. 20, Supplement 1, 2020 pp. 593-596 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

# THE EFFECT OF IMPURITIES ON THE EFFICACY OF OZONE APPLICATION ON WHEAT (*TRITICUM AESTIVUM*) BEFORE AND DURING STORAGE

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## Abstract

In the present study the effect of ozone treatment on microbial stability of wheat (*Triticum aestivum*) before and during storage was evaluated. Two groups of wheat with 3.8% and 0.0% impurity were formed and treated with ozone gas at 3 ppm concentration for 1 hour. Groups were then placed in air-tight glass jars and stored for three months at varying temperatures which were 33.3°C, 29.6°C and 24.7°C respectively. The storage temperatures were chosen as average temperatures in Baghdad, Iraq from August to October. Ozone treated wheat samples were evaluated for their microbial (total bacteria, yeast and molds, total coliforms), moisture, ash and color properties during storage. Separation of impurities and ozone application caused a significant reduction in the numbers of microorganisms. The highest depreciation in the count of (bacteria, molds /yeast, coliform) was (0.48, 3.30, 0.91) log cfu/g respectively. There were no significant differences in moisture and ash contents of samples after ozone treatment. The statistical results of L*, a* and b* color values showed that the difference in the percentage of impurities had no effect on effectiveness of ozone.
***Keywords***: Ozone, separation, stored wheat, ozone application, microbial content, impurities.

## Introduction

The wheat is the main human food crop among the cereals in the world (Cooper, 2015). Wheat production in the World in 2019 production is around 757.4 million tons (Cephas *et al.,* 2014). The main parts of wheat grain are endosperm, bran and the germ. Endosperm contains mostly starch between 81-84% (Inamdar *et al.,* 2014). The germ part contains proteins, lipids, minerals and vitamins (Rajendrn, 2003). The bran which covers the endosperm forms 14 to 16% of the grain (Inamdar *et al.,* 2014). The bran consists of various layers such as pericarp, seed coat and nucellus. The external layer is rich in cellulose, hemicellulose and minerals. The aleurone is the innermost layer which is rich in protein, fats and minerals (Pomeranz, 1982). Before placing in silos, the wheat is separated of all foreign materials, stones, and other types of grains through the sorting processes. Most separation processes depend on the physical properties of grains such as size, weight, and color. In addition infected, damaged and broken grains were also removed. Chemicals agents like pesticides, antibacterials and fungicides are used during the storage of grains for the elimination of pests such as insects, rodents, bacteria, molds, yeasts and coliforms. Although these chemicals are quite effective, they have negative impacts on environment, grains and the health of people working in the sector. Therefore, it is necessary to develop alternative methods that does not affect the grain, environment and the humans, but effective as well. Ozone is one of the alternative methods due to its strong oxidizing nature, for this reason it is an effective antimicrobial that leads significant reduction in microbial content. Its destructive impacts on bacteria, yeasts, molds and other microorganisms and insects has been proven by many studies and. (Khadre *et al.,* 2001; Ibanoglu, 2002).

There are many factors which affect the application of ozone such as temperature, duration of application, concentration, method of ozone generation, and some others. (McDonough *et al.,* 2001; Hassan *et al.,* 2016). However there are some other factors related to materials on which the ozone will be applied, such as moisture content, surface properties of the substance, the type of the wheat and the primary microbial content before application (Ibanoglu, 2002; Raila *et al.,* 2006). The washing process is also proven to be highly effective in reducing microbial content, but increases the moisture content. This study was conducted for demonstration of the impact of the impurities and their separation on reducing the microbial content of wheat. Besides the effect of impurities on the efficiency of ozone that was applied for the extension of storage period of grains investigated.

## Material and Methods

### Wheat Samples

The wheat samples were obtained from Hediye Flour Company in Isparta, Turkey. The type of wheat was Bezostaja which is a hard-red winter wheat with an average of 1.9% impurities. The first group of wheat was totally separated from all physical impurities like chaff, stones and foreign seeds. The second group was prepared by increasing the impurities by two fold (3, 8%) in order to obtain more significant differences.

### Ozone Application

The 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 the system parameters were set, experimental groups were treated with 3 ppm ozone gas for 1 hour at 15$^{oC}$. Gaseous ozone was pumped to a glass air-tight chamber and the gas flow was controlled manually depending on the values generated by ozone sensor.

### Storage of the samples

After application of ozone 500 g wheat sample was placed in air-tight glass jars. Three parallel jars were prepared for each group (0.0% and 3.8 % impurities). All groups were stored for 3 months in a laboratory type incubator. The storage temperature was adjusted monthly, based on the average temperatures in Baghdad, Iraq beginning with August (August 33.3°C, September 29.6°C and October 24.7°C). The average temperatures were obtained from "The Global Atmospheric" site, Global current weather /Acc weather (Google, Global current weather, 2019).

### Microbial analysis

The total number of 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 (Maturin *et al.,* 1998). The total number of Yeasts and Molds (TYM) were determined by using the method of Bacteriological Analytical Manual, the plates were incubated for 48-72 h at 25°C (Tournas *et al.,* 2001). 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 (Feng *et al.,* 2002). All platings were carried out in triplicates for each dilution. The plates were counted as colony forming units (cfu) and their numbers were expressed as log cfu/g.

### Moisture Content

Moisture content of wheat samples was determined by using the method of AOAC 2000/ 925.10 (Horowitz *et al.,* 2000). Moisture content of the samples was calculated by using the formula below.

$$[(W_{initial}-W_{final})/W_{initial})] \text{ X } 100$$

### Color measurement.

The color of the wheat samples was determined by using CIE Lab system as L*a*b* values. The color values were measured with a Chroma meter 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.

### Ash Content

Ash content of wheat samples was determined by using the method of AOAC 2000/ 923.03 (Horowitz *et al.,* 2000). 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.

### Statistical analysis.

Statistical analysis was performed in order to make comparisons of the results. All data were analyzed by using the Minitab Statistical Software (Version 2019) program. The comparisons were made by using the average values at level of P<0, 01. Results represent mean averages of 3 measurements.

## Results and Discussion

### The Effect of ozone treatment on microorganisms

All impurities are removed before the storage for various reasons. These are reducing the time and cost of drying, protection of milling machinery and reduction of microbial load. The most common foreign materials are stones, other seeds, husks, insects, insect pieces, bacteria, yeasts and, molds.

**Table 1 :** Effect the ozone treatment on wheat samples with different impurities

| Impurity % | Initial | Month1 | Month 2 | Month 3 |
|---|---|---|---|---|
| 3.8 %<br>TAMB | 4.86± 0.05<br>A a | 4.72±0.06<br>AB a | 4.66±0.03<br>B a | 4.60±0.02<br>B a |
| 0.0%<br>TAMB | 4.38± 0.12<br>A b | 4.35±0.08<br>AB b | 4.28±0.07<br>AB b | 4.13±0.02<br>B b |
| 3.8 %<br>Yeast and Molds | 4.86 ±0.02<br>A a | 4.15±0.13<br>B a | 3.96±0.23<br>B a | 3.30±0.30<br>B a |
| 0.00%<br>Yeast and Molds | 3.77±2.10<br>A a | 3.60±0.30<br>A a | 2.00±1.73<br>A a | 0.00±0.00<br>A b |
| 3.8 %<br>Coliforms | 4.26±0.23<br>A a | 4.31±0.02<br>A a | 4.30±0.05<br>A a | 4.24±0.02<br>A a |
| 0.00%<br>Coliforms | 3.77± 0.30<br>A a | 3.43±0.51<br>A b | 3.50±0.34<br>A a | 3.33±0.35<br>A b |

(0.00% and 3.8%) represented the value of the impurities. Uppercase Letters represent months and lowercase letters represent application.

In order to determine the effect of impurities on microbial load and ozone treatment, natural wheat sample was screened and impurities were separated. After separation of all impurities, its impurity content became 0%. The amount of impurities in the other experimental group was increased to twice as 3.8%. Both samples were treated with ozone at a concentration of 3 ppm for one hour and placed in air-tight glass jars.

After treatment of both groups with ozone, the results showed that the differences between bacterial counts were decreased when compared to untreated samples. Statistical results showed there was significant a difference between values as the highest difference was determined in the third month ranging from 4.60 to 4.13 log cfu/g. The results were similar to that of Nur *et al.* (Nur *et al.,* 2015), (Khadre *et al.,* 2001) who reported that the ozone treatment affected bacterial counts and lead to inactivation or destruction of the bacteria.

The counts of bacteria in the two groups at the beginning of the experiment ranged between 4.86 to 4.60 log cfu/g, for the first group with 3.8% impurities and 4.38 to 4.13 log cfu/g for the second group with 0% impurity. The effect of grain purification and removal of impurities was observed to reduce bacterial counts and help to increase the effectiveness of ozone treatment (Rajendrn, 2003). Similar results were also determined for the numbers of yeast and molds. Through yeast and mold analyzes there were differences in the results of statistical analyzes, and the highest decrease was in the third month from 3.30 to 0.00 log cfu/g. These results are in accordance with the results obtained by Allen *et al.* (Allen *et al.,* 2003). There are many factors which may be contribute to this decline in addition to treatment with ozone and the process of separation of impurities such as temperature, moisture, storage material, oxygen. All factors may provide conditions not suitable for the growth of bacteria (McDonough *et al.,* 2001). The efficiency of ozone treatment depends on many factors related to ozone itself and other auxiliary factors that may negatively or positively affect the efficiency of ozone gas. In addition, differences in experimental conditions may affect the results as well (Zhu, 2018). It was clear that, the ozone application did not effectively eliminated the coliform group bacteria. The highest decrease was in the third month from 4.24 to 3.33 log cfu/g. These results are similar to the results presented by Khadre *et al.* (Khadre, *et al.,* 2001) who studied about the effect of ozone treatment on *E.coli* and some other bacterial species. The reason for this may be the Gram (-) structure of cell surface which contains higher amounts of lipids. A gram negative bacterium requires longer time of exposure and concentration of ozone.

**Effect the ozone treatment on the physical properties of wheat**

The results showed that the separation impurities of wheat affected the moisture content of the samples. The moisture content of samples were presented in Table. 2. The results extended in all the samples with highest increasing from 6.18±0.0% in (A) wheat samples to 6.40±0.0% in (B) wheat samples in the third month. The separation of impurities and reduced it from (3.8%) to (0.00%) resulted in an increase in moisture at a rate of 0.2%.

**Table 2 :** Effect the ozone treatment on moisture content with different impurities

| Month | (A) 3.8% impurities + ozone | (B) 0% impurities |
|---|---|---|
| Initial | 6.25±0.07 A b | 6.43±0.08 A a |
| 1 | 6.22±0.04 A b | 6.43±0.04 A a |
| 2 | 6.20±0.01 A b | 6.41±0.03 A a |
| 3 | 6.18±0.01 A b | 6.40±0.03 A a |

(0.00% and 3.8%) represented the value of the impurities. Uppercase letters

Represent months and lowercase letters represent application.

By comparing the statistical results, observed was that the effect of impurity separation on the moisture content causes a slight increase and there was no significant effect after application of ozone on the moisture content of wheat samples during the storage for three months. The results were similar to those reported by (Mendez *et al.,* 2003). who reported in their study that there was no significant effect on the hard wheat when 50 ppm ozone was applied for 30 days.

The results of ash analysis were presented in Table. 3. There was no change when ash amount in dry matter was considered. Statistical analysis also showed that there was no difference in ash content. (Nur *et al.,* 2015) also reported no significant differences in the analysis result of ash content in rice samples after ozone treatment. The samples were stored in the incubator for three months in air-tight glass jars. Studies demonstrated that, in infested wheat samples insect damage causes significant changes in ash content of grains during storage. In our study there was not any insect infestation, thus the ash content did not change in any of the groups. (Khadre *et al.,* 2001) reported that ash content did not change during storage unless they are not infested.

**Table 3 :** Effect the ozone treatment on the Ash content and color measurement with Different impurities.

| Physical Property | A month1 | B month1 | A month2 | B month2 | A month3 | B month 3 |
|---|---|---|---|---|---|---|
| ASH | 1.52±0.00 A a | 1.52±0.01 A a | 1.52±0.01 A a | 1.52±0.02 A a | 1.53±0.01 A a | 1.52±0.02 A a |
| COLOR | | | | | | |
| L* | 51.57±0.48 A a | 50.85±0.48 A a | 51.17±0.11 A a | 51.35±0.72 A a | 51.64±0.80 A a | 51.62±1.06 A a |
| a* | 7.77±0.14 A a | 8.08±0.24 A a | 7.72±0.07 A a | 7.84±0.07 A a | 7.60±0.64 A a | 7.41±0.13 A a |
| b* | 21.58±1.06 A a | 20.77±0.07 A a | 20.01±0.59 A a | 20.67±0.03 A a | 20.49±0.05 A a | 20.62±0.15 A a |

A represent the samples 3.8% impurities, B represent the samples 0.0% impurities

Uppercase letters represent months and lowercase letters represent application.

As it can be seen in Table. 3. And when statistical results were compared, there was no effect of impurity separation on the efficiency of ozone application regarding color values of the wheat. Statistical results also showed that there was no difference in the color values during the storage period after ozone application. These results are similar to the results which obtained by (Marston *et al.*, 2015). who reported that there was no difference in the results during storage after ozone treatment.

## Conclusion

Statistically significant reduction in the total bacteria, yeast/molds, coliform bacteria count in the wheat after using ozone application. Impurities separation process and reduced from (3.8 to 0.0) % caused to more ozone effectiveness. There was no significant effect for the separation of the impurities and the ozone application on the ash content and color properties. The separation of impurities and reduced it from (3.8%) to (0.00%) resulted in an increase in moisture at a rate of (0.2). While no insects were observed in wheat samples stored in air-tight containers under temperatures ranged from 24.7 to 33.3 $^{o}$C, but their large numbers were determined in the samples stored polyethylene sacks stored at room temperature. This shows that the storage of grain in airtight containers is effective against insect infestation may be due to the temperatures applied, oxygen consumption and accumulation of carbon dioxide.

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