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A Comparison between Chlorinated Water and Ozonated Water as an Antimicrobial Treatment during Tempering of Wheat

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Tempering of wheat is done to improve its physical state for milling. Chlorinated water is being used in industry to decrease the microbial load of tempered wheat but chlorine leaves a residue which limits its application in food industry. Ozone gas is highly reactive. Compared to chlorine, ozone gas is a stronger and more rapid antimicrobial agent. Ozone gas can be dissolved in water. It is expected that ozone treatment may inhibit or minimize bacterial, yeast and mold count which has been a dominant safety and quality concern for wheat grown in the Northern Plains. Hence, the objective of this study was to compare the efficacy of ozonated water with the efficacy of chlorinated water during tempering of durum wheat. Tempering was done using water containing 700 ppm chlorine and water containing 10 and 16 ppm ozone. Wheat grains were tempered at 17ºC. Wheat grains were tempered in two steps with resting time of 6 hours after each step to raise the initial moisture content (MC) from initial m.c. to a final of 17%(db), with an intermediate MC of 12.5% after first step. The wheat grains were also washed using 16 ppm of ozonated water. These tempered and washed grains along with their control samples were tested for total bacterial, yeast and mold count and also for color change and germination capacity.

Keywords: Bacterial count, germination capacity, Mold, Ozonated water, Tempering, Washing, Wheat grain, yeast

Introduction

Cereal grain contains a wide assortment of micro flora including bacteria, actinomycetes, molds and yeast. Cereal grains under storage have a water activity well below the minimum needed for microbial growth; however they still contain large numbers of viable but dormant bacteria. These bacteria can be active again given favorable temperature and humidity conditions. Molds and yeasts in milled products can also contribute to spoilage in products such as fresh pasta and bread. For these reasons, microbiological standards for cereals and milled products have been deemed necessary by food processors (*Manthey et al.*, 2004).

Tempering is a process of adding water to dry grain and allowing the grain to rest for a period of time before it is milled. The purpose of tempering is 1) to toughen the bran thus making it resistant to being broken into small particles during milling, toughening the bran reduces specks in semolina or flour and 2) to soften the endosperm which makes it easier to grind and requires less energy to milling (*Boyacioglu and Sunter*, 2004). Durum wheat (*Triticum turgidum* L.) and hard red spring wheat (*Triticum aestivum* L.) are tempered prior to milling into semolina (for pasta making) and into flour (for bread making), respectively. Chlorinated water at concentrations of 600-700 ppm is widely used for tempering in the pasta industry, as a disinfectant to reduce the microbial load of the grain. The moisture content of durum wheat must be raised from an initial 10-11% dry basis (db) to a final 17% (db).

Although chlorine is the most commonly used disinfectant for decontamination for food products including cereal grains, there are increasing concerns about the presence and safety of concomitant disinfectant by-products. Chlorine reacts with organic residues to form potentially mutagenic or carcinogenic reaction products, such as trihalomethanes and their impact on human and environmental safety have been raised in recent years (*Beltran et al.*, 2005). For this reason use of chlorine in certain fields like washing of fresh cut products is banned in several European countries including Germany, The Netherlands, Switzerland and Belgium. In particular, consumers of organic food reject the use of chlorinated water (*Baur et al.*, 2004). Chlorine may be present on wheat grain after tempering with chlorinated water. These chlorine residues are assumed to be removed with bran during de-braning, prior to milling. Ozone does not leave hazardous residues on food (*Khadre et al.*, 2001); therefore, ozonated water may serve as a potential alternative to chlorinated water in tempering of wheat.

Ozone gas has been included into the GRAS (generally regarded as safe) category by US regulations in 1997 (*Liangji*, 1999). This regulation brought ozone gas and ozonated water many applications in the food industry. A significant amount of research has been done to study the effect of ozonated water on the microbial load and shelf life of fresh-cut fruits and vegetables (*Sapers*, 2003). But limited research has been conducted using ozone or ozonated water on cereal and cereal based products. Influence of tempering and washing with ozonated water on the selected properties (milling, rheological, chemical, color and microbiological) of wheat flour has been studied. Ozonated water was prepared by dissolving ozone gas in circulating water. Ozone concentration was determined spectrophotometrically at 500 nm (*Ibanoglu*, 2001, 2002).

Research is needed to determine whether ozonated water may be used successfully for tempering. The critical differences between the ozonated water and chlorinated water need to be recognized. One of the major differences is that ozone has approximately 1.5 times more oxidizing power than chlorine (*Ibanoglu*, 2002) but it has very short half-life of several minutes in water. Ozone decomposes into O_2 as soon as it comes in contact with the first layer of organic matter. Tempering of wheat should be done in a way so as to get uniform grain treatment before ozone is depleted. Moreover maximum concentration of ozonated water achieved and its half life depends on the quality and temperature of water used.

Considering the aforesaid discussion the present study was carried out with the following objectives:

1) Develop a continuous system for monitoring and logging ozone concentration in water; 2) Characterize the kinetics of ozone generation and depletion and its dependence on quality and temperature of water; 3) Develop a system for tempering and washing durum and hard red spring wheat grain with ozonated water; and 4) Evaluate and compare the effect of ozonated water and chlorinated water on grain color, germination and microbial load.

Safety Emphasis

Gaseous ozone has been used in the study to make ozonated water. The action of ozone on the human organisms has already been dealt with in many studies and exposure limit values have been standardized in most user countries. In the USA and in most European countries the threshold limit value (TVL), i.e. the maximum concentration of ozone for a work time of 8 h a day, has been set at 0.1 ppm (*Damez and Vigouret*, 1981). Ozone Discovery Badges supplied by Ozone Solutions (Sioux Center, IA) are used as a measure to detect ozone level in the work area. These badges have a response time of 8 h for 0-0.080 ppm ozone concentration but responds within 5 minutes at high (> 8 PPM) ozone levels. Ozone badges usually indicated low to moderate concentration (<0.06ppm) of ozone in our work area. Disposable gloves (Microflex®) and mask (Dura-Mesh®) are used while handling samples and working with ozone.

Materials and Methods

System Development

A system was developed as shown in Figure 1. Ozone gas was produced using an OS-8C Ozone Generator (Ozone Solutions, Inc., Sioux Center, IA, USA). The generator has a capacity of 8 g/h ozone output. Ozonation of water was carried out in a Borosil® heavy-walled Erlenmeyer flask of 1.5 l capacity with tubulation for exhaust of excess ozone. The exhaust ozone went into a 450M Ozone Gas Monitor (Advanced Pollution Instrumentation, Inc., San Diego, CA, USA). Ozonated water was prepared by bubbling ozone gas into 1 l of water (distilled or tap) with the help of a Gas Dispersion Tube (Sparger) (Thermo Fisher Scientific, Barrington IL 60010 USA) having pore size 100 µm,12 O.D. and 20 mm long. The dissolved ozone concentration and water temperature was monitored continuously with a Q45H/64 Dissolved Ozone Analyzer (Analytical Technology, Inc., Collegeville, PA, USA). The water at a continuous flow of 0.2-0.5 l/min was circulated through the flow cell and returned to the flask by a Peristaltic Pump (Manostat, New York, NY. USA) and ¼'' tubing (Tygon® 3603). A CR10X Datalogger (Campbell Scientific, Inc., North Logan, UT, USA) and a Computer monitored and logged dissolved ozone concentration and water temperature at 0.2 sec intervals.

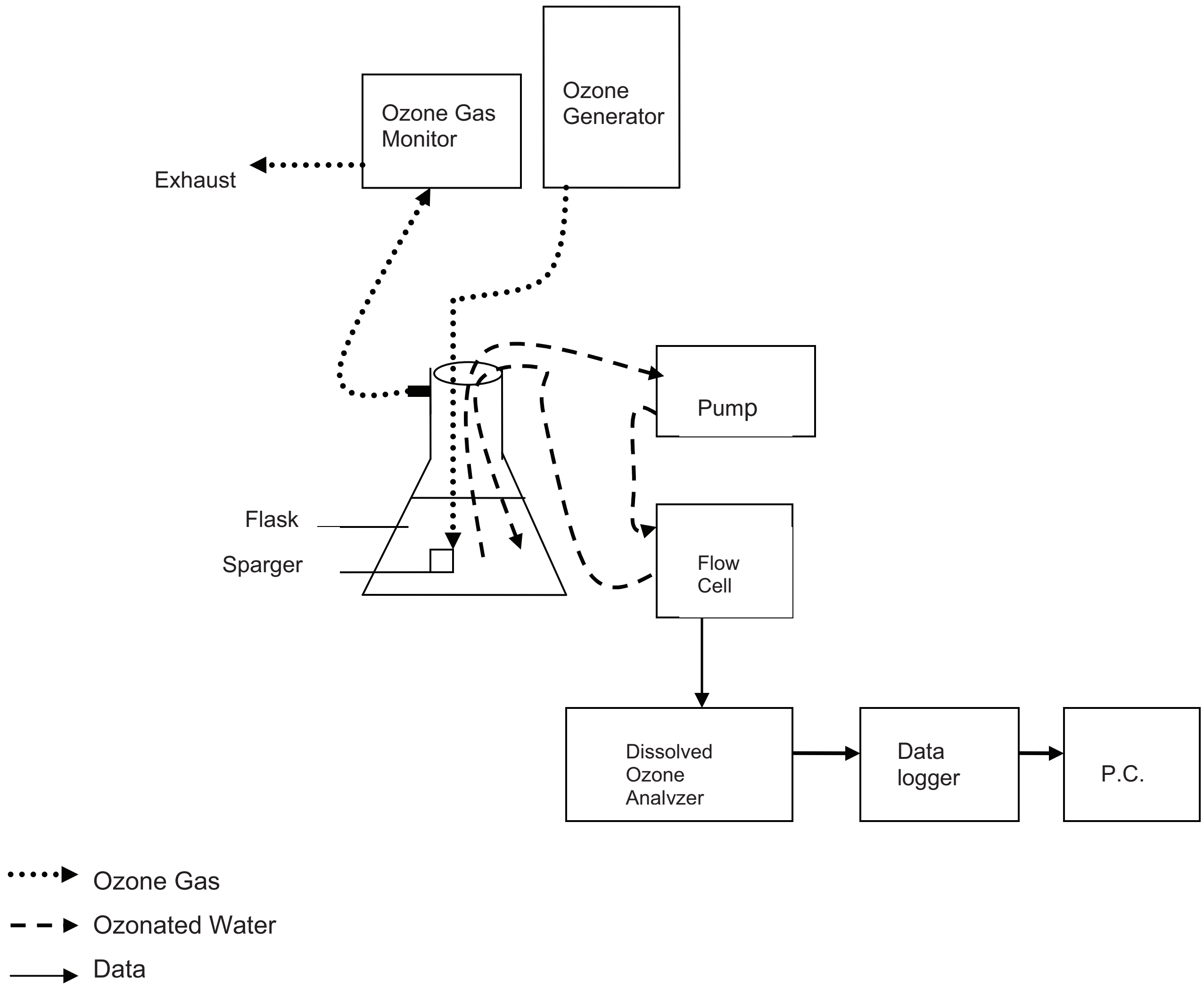

Figure 1: Diagram showing system for making ozonated water and its continuous monitoring and data recording.

Ozone Decay Analysis

Experiments were done to compare the kinetics of ozone generation and decay in tap and distilled water at 17 ± 1°C and pH 5 using 30,000 ppm of ozone gas at a flow rate of 1.5 cfm. One liter of tap or distilled water was placed in an Erlenmeyer flask and ozonated. The maximum ozone concentration reached and time taken to reach that concentration was monitored. Then the ozone generator was stopped and decay of ozone in ozonated water was recorded. Ozone decay curves were plotted and ozone decay constants were calculated fitting data to the formula:

$$y = y_o e^{-kt} \qquad \text{- eq. 1}$$

Where,

y = concentration of ozone at any time, t

y_o = initial concentration of ozone

k = ozone decay constant

t = time

And half-life of ozone in water was calculated as:

$$t_{1/2} = \ln(2)/k \qquad \text{- eq. 2}$$

Tempering of Wheat

Durum wheat samples were procured from Langdon, North Dakota in 2004 and stored in a cool, dry place until used. Moisture analysis on the wheat samples was done according to approved method 44-15A (AACC 2000); a forced-air oven was used to determine the moisture content of wheat samples. The initial moisture content of durum wheat samples was 8.7% (db). Durum wheat (150 g) was tempered using a two-stage tempering scheme in which the first stage raised the moisture content of wheat grain to 12.5% (db) and the second stage raised the moisture content to 16% (db). The amount of water to be added to each sample was calculated according to AACC method 26-95. For the first tempering, the grains were spread on a glass tray in a thin layer and 6.5 ml of ozonated water was uniformly sprayed on them. These grains were then collected in a pint glass jar and shook vigorously for 15 min for moisture absorption. The second tempering was done 24 h later in a similar way by spraying 4.35 ml ozonated water on 100 g of tempered samples. The concentrations of ozonated water used were 10 and 16 ppm. The tempering of wheat was also done by distilled water (control) and by chlorinated water of 700 ppm.

Survey samples (2006) of hard red spring wheat from North Dakota were also tempered. These samples had an initial moisture content of 12.1% (db); thus the first tempering was not needed. These samples were tempered in one stage to a final moisture content of 16% (db). Hard red spring wheat samples (100g) were treated with 4.64 ml of ozonated water (16 ppm). Similarly, control samples were prepared by tempering 100 g of grain with 4.64 ml of distilled water.

Wheat Washing

Durum and hard red spring wheat samples were washed with ozonated water at 10 and 16 ppm and also with distilled water (control). Wheat (100 g) was taken in a Borosil® graduated beaker and volume was made up to 250 ml with water. The samples were kept as such for 1 min and then excess water was removed by decanting grain and water over a stainless steel strainer. Washed grains were shifted to a pint glass jar and were shaken vigorously for 15 min to get uniform absorption of water. The final moisture content of grain was noted after 3-4 h.

Color Analysis

Color analysis of tempered wheat samples was done by Minolta (Colorimeter) Chroma Meter CR-310. The instrument was calibrated against a standard white tile (L = 91.9, a = -1.4 and b = 2.0). The grain sample was poured into the attachment provided with the instrument and color was measured in terms of 'L', '*a*', '*b*' values. The color was described by a Hunter Lab value where 'L' indicates intensity of color i.e. lightness which varies from L = 100 for perfect white to L = 0 for black. Letters '*a*' and '*b*' denote chromaticity dimensions which give understandable designations of color i.e. the value of '*a*' measures redness when positive, grey when zero and greenness when negative and the value of '*b*' measured yellowness when positive, grey when zero and blueness when negative. All measurements were replicated twice.

Germination Test

Standard germination tests followed Association of Official Seed Analysts (AOSA) procedures (AOSA, 1993). Tempered wheat (100 kernels) was placed on the wetted germination paper. The germination paper was rolled by placing another wetted germination paper over it. Three replications were done for all samples. These grains were incubated at 20° C for 5 days. The number of germinated kernels was recorded.

Microbiology

Microbiological analysis was initiated by aseptically mixing 11 g of tempered wheat with 99 ml of sterile Butterfield's phosphate-buffered dilution water (pH 7.0). Serial dilutions from 10^{-1} to 10^{-4} were made. Aerobic Plate Count (APC) and Yeast and mold Count (YMC) were done according to AOAC Official Method of Analysis. A pour plate technique was used for total bacterial count (plate count agar) and the surface spread method was used for YMC (malt extract agar). APC plates were incubated for 48 ± 3 h at 35 ± 1°C and YMC plates were incubated in dark for 5 days at 25ºC. The plates having less than 300 CFU (Colony Forming Units) were counted. APC plates were replicated twice and YMC plates were replicated thrice.

Statistical Analysis

The results obtained were analyzed statistically; trend lines and exponential equations were used for ozone decay analysis. Color data was analyzed using bar graphs and error bars. Mean and standard deviation was shown to interpret germination data and microbial results of treated samples were compared to their controls by *t*-test at 95% level of confidence.

Results and Discussion

Kinetics of ozone decay (Figure 2) in tap and distilled water was studied while keeping all other factors constant (like temperature, pH of water and ozone gas concentration).In tap water highest concentration reached was 15 ppm in 39 min whereas in distilled water the highest concentration reached was 18 ppm in 12 min. The ozone decay constant and half life of ozone in tap and distilled water was calculated using eqs. 1 and 2, respectively. The ozone decay constant was calculated to be 0.1034 min^{-1} (R^2 = 0.9952) for tap water and 0.0534 min^{-1} (R^2 = 0.9968) for distilled water. Half life of ozone in tap water was 6.7 min and for distilled water was 13 min. It was observed that when comparing distilled water to tap water, the highest concentration reached was greater; the time taken to reach that concentration was shorter and the half-life was almost doubled. The greater half-life in distilled water accounts for the higher steady state concentration. These observations strongly support the fact that less pure water results in reduced half life.

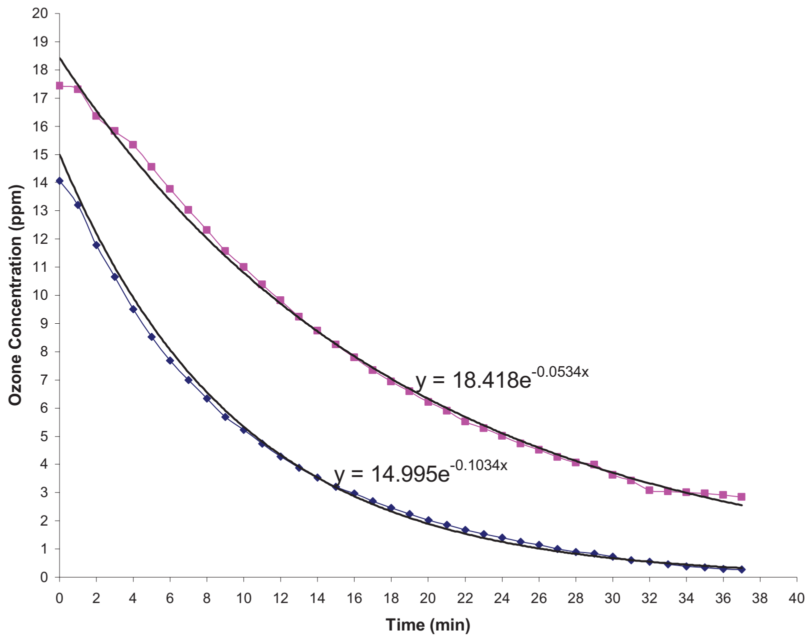

Figure 2: Ozone decay curves for ozonated tap water (diamonds) and distilled water (squares) at 17 ± 1°C Ozonated water was prepared in 1 l water in a glass flask and continuously monitored with a dissolved ozone analyzer by circulation through tygon tubing.

Color of grain samples was recorded in terms of 'L', '*a', 'b*' values, where 'L' value describes the lightness and 'b' value correlates with the pigmentation (yellowness) of the grain (*Ibanoglu*, 2001). Figure 3a summarizes the results of color comparison of treated durum wheat samples with their respective controls. Similarly, Figure 4 shows the results of color comparisons of hard red spring wheat samples. The error bars (±5%) shown on the graphs of treated samples overlaps with their controls in all cases of 'L', 'a', 'b' , by this it can be concluded that ozonated water applied during tempering or washing did not have any significant effect on the color of durum or hard red spring wheat grains. The results of this study showed that the ozone treatment applied during tempering and washing of grain samples was not sufficient in terms of ozone concentration and contact time to oxidize the pigments in the wheat.

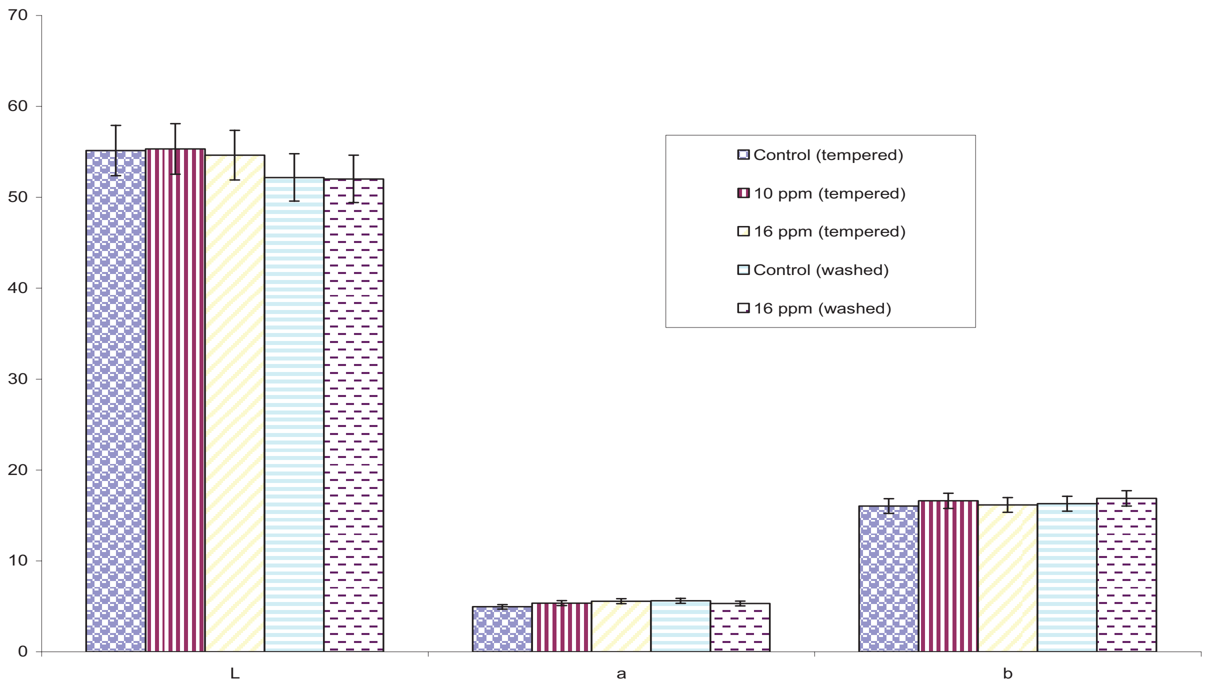

Figure 3a: A comparison of color in terms of 'L a b' values for ozonated water tempered and washed durum wheat samples with their controls. The ozonated water of 10 and 16 ppm concentration was prepared by circulating gaseous ozone in 1 l distilled water at 17 ± 1°C. The wheat grains were tempered using 10 and 16 ppm and washed using 16 ppm ozonated water. Control samples were prepared by tempering or washing with distilled water.

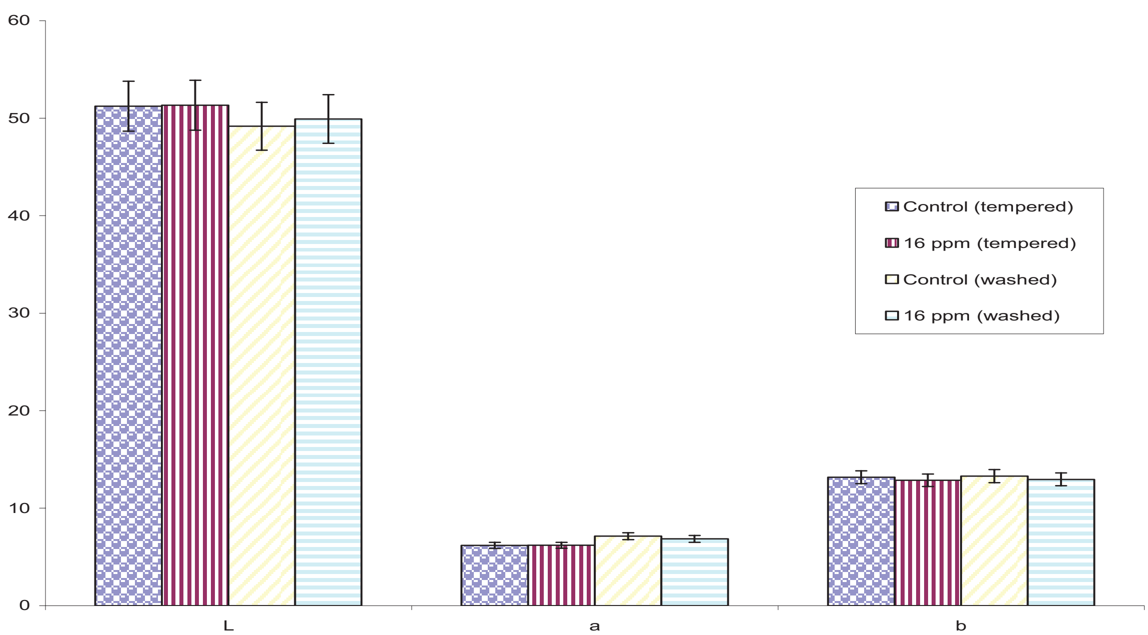

Figure 3b: A comparison of color in terms of 'L a b' values for tempered and washed hard red spring wheat samples with their controls. The ozonated water of 16 ppm concentration was prepared by circulating gaseous ozone in 1 l distilled water at 17 ± 1°C. The wheat grains were tempered or washed

using this16 ppm ozonated water. Control samples were prepared by tempering or washing with distilled water.

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There was no significant difference in the germination capacity of ozonated water tempered, washed grain as compared to distilled water tempered, washed grain (Table 1). One possible explanation for this could be ozone is a surface treatment and ozone in water gets depleted by the time water reaches the germ of wheat kernel. However, the germination capacity of hard red spring wheat grain was higher than that of durum wheat for both washed and tempered grain. This could be due to the fact that durum wheat samples were older, procured in 2004 and may be they have lost some of their germination capacity with time.

Table 1: Percent germination for different treatments and controls of durum and hard red spring wheat.

Treatment	**Germinated Grains (%)** [a]
Durum Tempering	
Distilled Water (control)	80 ± 5
Ozonated Water (10 ppm)	76 ± 5
Ozonated Water (16 ppm)	78 ± 5
Durum Washing	
Distilled Water (control)	76 ± 4
Ozonated Water (16 ppm)	77 ± 4
HRSW [b] **Tempering**	
Distilled Water (control)	96 ± 1
Ozonated Water (16 ppm)	96 ± 2
HRSW [b] **Washing**	
Distilled Water (control)	96 ± 2
Ozonated Water (16 ppm)	97 ± 1

[a] Average of three values ± standard deviation

[b] Hard red spring wheat

Analysis of microbiological results by *t*-test (α = 0.05) showed that both APC and YMC were significantly different from their controls for all treatments (Table 2). The results of YMC showed significant decrease in both durum and hard red spring wheat treated samples as compared to their controls. There was not a specific pattern observed for the results of APC of durum wheat samples. These unexpected results could be because of old samples used in analysis as fresh samples were not available when the experiments were started. The durum wheat samples were 3 years old, having 8.7% m.c. and stored under cool temperature. It is possible that the bacteria were in their dormant state under these conditions of storage and did not responded to ozonated water treatment; they became active only when they got sufficient water activity after tempering/washing and favorable temperature during incubation. However, there was decrease in APC of hard red spring wheat sample when ozonated water was used in tempering or washing.

Table 2: Microbial load (log CFU/g of dry weight) in terms of aerobic plate count (APC) and yeast and mold count (YMC) for treated and control samples of durum and hard red spring wheat.

Treatments	APC	YMC
Durum Tempering		
Distilled Water (control)	3.46	1.84
Ozonated Water (10 ppm)	3.59	1.24
Ozonated Water (16 ppm)	3.20	1.12
Durum Washing		
Distilled Water (control)	3.82	1.92
Ozonated Water (16 ppm)	3.95	1.87
HRSW [C] Tempering		
Distilled Water (control)	3.92	2.41
Ozonated Water (16 ppm)	3.36	2.37
HRSW [C] Washing		
Distilled Water (control)	3.78	2.81
Ozonated Water (16 ppm)	3.44	2.39

[C] Hard red spring wheat

Conclusion

A continuous system for monitoring and data logging ozone concentration in water was developed and evaluated for analyzing ozone generation and depletion behavior. Ozonated water of varying concentrations was used in tempering and washing of wheat grains. It was found that ozonated water treatment did not have any effect on the color and germination capacity of wheat grains. Ozonated water has significantly lowered the YMC in tempered and washed samples of both durum and hard red spring wheat. The APC results obtained were doubtful in case of durum wheat samples which can be attributed to old samples used and need further analysis with fresh samples. It is probable that if ozonated water had reduced the APC in hard red spring wheat samples it will give the same results with freshly harvested durum wheat. Comparison of the results with those obtained using chlorinated water is due and is expected to be accomplished soon.

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