

# Development and Evaluation of Ozonated Water System for Antimicrobial Treatment of Durum Wheat

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**Short version of title:** Ozonated water system for wheat treatment...

**Choice of journal section:** Food Engineering and Physical Properties

## ABSTRACT

Ozonated water is reported to be effective in reducing the microbial load in foods such as fruits, vegetables, and grains. Ozonated water may be an effective alternative to chlorinated water in treating durum wheat before milling. Therefore, durum wheat was washed with ozonated water, and analyzed for yeast and mold count (YMC) and aerobic plate count (APC). A system for producing and monitoring ozonated water was developed. The effect of water quality (tap, distilled, and ultra-pure), temperature (7, 15, and 25 °C), and pH (2, 4, and 6.5) was evaluated on the following: steady-state dissolved ozone concentration, ozone decay constant, half-life, mass transfer coefficient, equilibrium ozone concentration, and solubility ratio. The study of these parameters was important to attain a stable, high dissolved ozone concentration at the outset of washing, and to have information for system improvement and scale-up. A 1% acetic acid solution (pH 2) at 15 °C resulted in high dissolved ozone concentration (21.8 mg/L) and long half-life (9.2 min). Next, wheat was washed with 5 wash water types: distilled water, ozonated water (16.5 mg/L), chlorinated water (700 mg/L), acetic acid solution (1%), and acetic acid + ozonated water (1%, 20.5 mg/L), and then analyzed for YMC and APC. The last of these treatments was the most effective, with a reduction of 4.1 and 3.2 log<sub>10</sub> colony forming units/g in

YMC and APC, respectively. Though ozonated water was not very effective alone, it was useful in combination with acetic acid.

Keywords: acetic acid, bacterial count, ozonated water, pH, wheat washing

## Introduction

Grain contains a wide assortment of micro-flora including bacteria, actinomycetes, molds, and yeast. Durum wheat under storage conditions of 55% RH, 12-15 °C, and 9-10% (wb) moisture content, has a water activity well below the minimum required for microbial growth. However, the grain still contains a large population of viable but dormant micro-flora. This micro-flora can be active again given favorable conditions during processing. Controlling growth of microorganisms in the field, and during processing is a major challenge. For these reasons, millers and producers have set stringent microbial limits on the grain they accept. Microbiological standards for cereals and milled products have been deemed necessary by food processors (Manthey and others 2004).

Several methods have been investigated to reduce the microbial load in grain and its products, including physical methods such as milling or sieving, washing or soaking in water, use of chemicals such as chlorine, irradiation and/or thermal treatment with hot air. These methods have provided varying levels of success, but none was truly satisfactory (Young and others 1984; Trenholm and others 1992; Wolf and Bullerman 1998; Yumbe-Guevara 2003; Kottapalli and others 2006). Chlorinated water is used to control bacteria and mold in grain milling (EPRI 2000). Chlorinated water (600-700 mg/L) is commonly used to disinfect the wheat grain during tempering in the pasta industry (A. Friedt, personal communication, 7 June 2007). However, there are growing safety concerns about the use of chlorine since it reacts with organic residues to form potentially carcinogenic reaction products (Beltran and others 2005).

Ozone is one of the oxidizing and sterilizing agents that has potential applications in the food industry. Ozone has GRAS (generally regarded as safe) status in the U.S., and can also be used in organic foods, as it leaves no residues (Liangji 1999). Ozone can be applied as a gas or as an aqueous solution.

Ozone gas has been widely studied as a treatment of cereal grains such as wheat, barley, and corn, still the information on the successful antimicrobial use of gaseous ozone is very limited (Wu and others 2006). Very little research has been reported using ozonated water on cereal and cereal based products. Ibanoglu (2002) found a reduction of 0.8 log<sub>10</sub> colony forming units (CFU)/g in the aerobic plate count (APC) and 0.4 log<sub>10</sub> CFU/g in the yeast and mold count (YMC) in flour prepared from wheat grain washed with ozonated water (11.5 mg/L).

Ozone decomposes rapidly to diatomic oxygen and has a short half-life of 1-10 min in water (Wu and others 2006). Therefore, ozonated water should be prepared on site and applied immediately. High dissolved ozone concentration and long half-life are desirable for better

disinfection (Kim and others 1998). The ozone decay constant, mass transfer coefficient, and solubility ratio are other important parameters that reflect system performance; thus, are measured to help improve and scale up the system. These values are rarely reported in food applications but are commonly determined in waste water treatment and ozone solubility studies (Wu and Wang 2001; Zhou and Smith 2000; Bin 2006).

Effectiveness of ozonated water also depends upon how it is applied. The rapid washing of the grain with ozonated water is one possible approach. Washing ensures full, uniform treatment of the grain. Washing of grain before milling is commercially practiced and is mandatory in India (BIS, 1965).

Acetic acid is another effective agent against bacteria, yeast and mold; it also lowers the pH to increase dissolved ozone concentration and half-life of ozone in water. Therefore, it may enhance the effectiveness of ozonated water, and, when used in combination, may have a synergistic effect against microbes. An ozonated acetic acid solution was more effective in carrot washing compared to ozonated water or acetic acid alone; the combined treatment resulted in complete elimination of yeast and molds (Bang and others 2007).

Thus, the objectives of this study were to develop a system for producing ozonated water for cereal grain washing; characterize the kinetics of ozonation and ozone decay in this system; and evaluate the effectiveness of the ozonated water, with and without acetic acid, for durum wheat washing.

## **Materials and Methods**

### **Sample Preparation**

Durum wheat samples were procured from Minot, North Dakota in 2007. The samples were stored in a cool (15 °C), dry place until cleaned. The wheat samples were cleaned using a dockage tester (Carter-Day, Minneapolis, MN, U.S.A.), and moisture content was determined to be 8.8% (wb) using a forced-air oven method 44-15A (AACC International 2000). The samples were combined into a single lot and mixed thoroughly using a drum mixer. The wheat samples were then packed in air and moisture-tight polyethylene bags and stored in a deep freezer (-18 °C) until used. The required wheat sample was warmed to 18 °C before use.

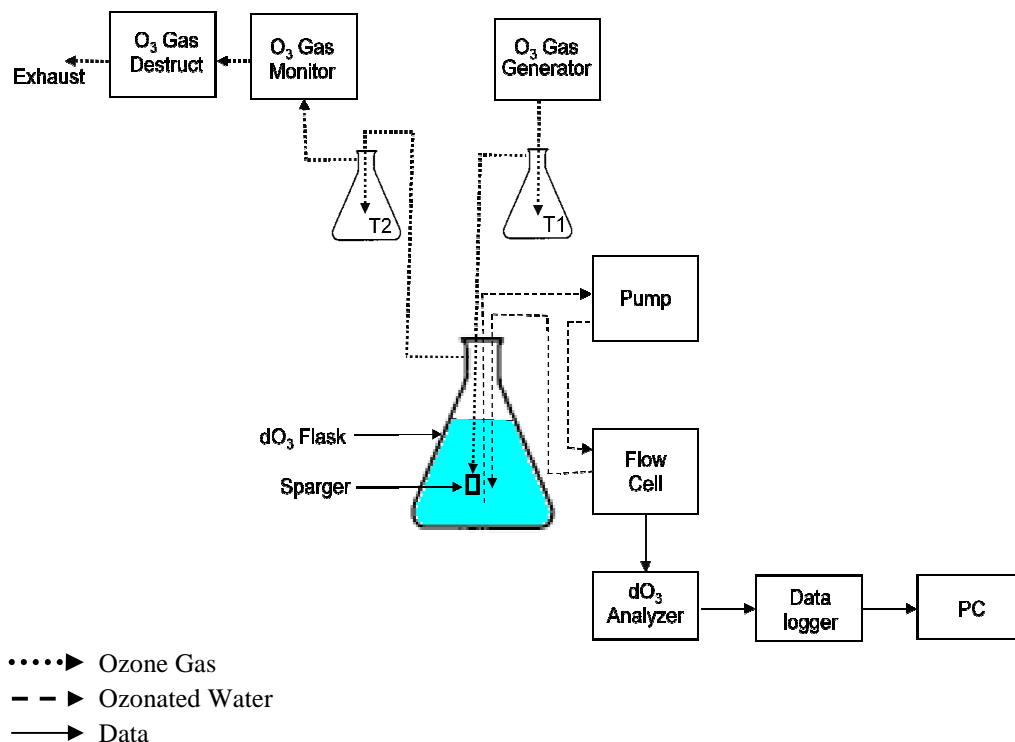
Tap and distilled water were procured from the outlets in the laboratory. Ultra-pure water was prepared by a Milli-Q<sup>®</sup> ultra-pure water purification system (Millipore, U.S.A.). Acetic acid solution was prepared by adding glacial acetic acid (EMD Chemicals Inc., Gibbstown, NJ, U.S.A.) and chlorinated water was prepared by adding AZONE hypochlorite solution (Lynde Co., St. Paul, MN, U.S.A.), to distilled water.

### **System Development**

A system was developed to produce and monitor ozonated water (Figure 1). Ozone-rich gas was produced from ambient air by an OS-8C ozone generator (Ozone Solutions, Inc., Sioux Center, IA, U.S.A.). The ozone generator has a capacity of 8 g/h ozone output. Inside the ozone generator, the incoming ambient air passes through a dehydrator that removes moisture and dust particles; the air then passes through a molecular sieve that produces a stream of pure oxygen. The ozone gas is produced from oxygen via corona discharge. The ozone gas stream was

manually controlled to have 3.5% [w/w] of ozone in the oxygen, and the flow rate was maintained at 0.75 L/min. This ozone gas stream was used for ozonation of water, and the exhaust gas stream was monitored by a 450 M ozone gas monitor (Advanced Pollution Instrumentation, Inc., San Diego, CA, U.S.A.). A permeation dryer inside the ozone gas monitor completely dries the incoming gas stream, thereby protecting moisture-sensitive internal components, which is especially useful in the case of ozonated water applications. An ozone gas destruct unit inside the monitor converts most of the ozone into oxygen before it is exhausted from the instrument. An additional ozone gas destruct was constructed using a CARULITE<sup>®</sup> 200 granular catalyst (Carus Chemical Company, LaSalle, IL, U.S.A.) to ensure complete ozone destruction from the final exhaust stream.

Ozonation of water was carried out in a Borosil<sup>®</sup> heavy-walled Erlenmeyer flask of 1.5 L capacity with tubulation for exhaust gas. Ozonated water was prepared by bubbling the ozone-rich gas into 1 L of water using a sparger (Thermo Fisher Scientific, Barrington, IL, U.S.A.) having 100  $\mu\text{m}$  pore size, 12 mm OD, and 20 mm length. The sparger was positioned in the center of the flask and 25 mm above the bottom level to achieve maximum mixing. The dissolved ozone concentration was monitored continuously with a flow cell and a Q45H/64 dissolved ozone analyzer (Analytical Technology, Inc., Collegeville, PA, U.S.A.). The water was circulated through the flow cell at a flow rate of 0.5 L/min and returned to the flask by a peristaltic pump (Manostat, New York, NY, U.S.A.) via 6.35 mm tubing (Tygon<sup>®</sup> 3603). A CR10X-datalogger (Campbell Scientific, Inc., North Logan, UT, U.S.A.), and a PC monitored and logged the dissolved ozone concentration and water temperature at 0.5 s intervals. The datalogger was programmed with a quadratic equation developed to directly give the dissolved ozone concentration (mg/L). The equation is based on voltage change associated with the change in ozone concentration as detected by the dissolved ozone analyzer. The PC displayed data in near-real time. Two traps (T1 and T2) were made of vacuum flasks to protect the ozone gas generator and monitor, respectively, from moisture and impurities. T1 is helpful in case of back pressure (returned gas or water) into the ozone gas generator.



**Figure 1** - System for producing and monitoring ozonated water.  $dO_3$  is dissolved ozone, T1 and T2 are moisture traps for ozone gas generator and monitor streams, respectively.

The performance of the ozone gas generator and monitor was checked once per month by connecting the generator output directly to the monitor input; the system was checked for consistency of ozone output at each setting of ozone gas concentration level (on the generator) and ozone gas flow rate level (on the monitor). The performance of the dissolved ozone analyzer was cross checked by a visual detection method using Dissolved Ozone Vacuivials (K-7402, Ozone Solutions, Inc., Sioux Center, IA, U.S.A.). The flow rate, ozone gas concentration, and ozonation time were selected to maximize dissolved ozone concentration without pressure build-up in the flask. The peristaltic pump speed was optimized and kept constant to achieve precision in determining ozone half-lives and rate constants.

## Evaluation of System Performance

Experiments were carried out to observe and evaluate the system performance parameters as dependent on the following factors: water quality (tap, distilled, and ultra-pure), temperature (7, 15, and 25 °C), and pH (2, 4, and 6.5). Water (1L) was ozonated for 12-15 min, until the ozone concentration remained constant for 1 min; the water was then assumed to be at steady-state dissolved ozone concentration ( ). The ozonation was stopped at that point, and the decay of the ozone in water was monitored until the concentration fell below 1 mg/L. Ozonated water was circulated to the flow cell for 1 min of each 5 min interval to minimize loss of ozone from a small manufactured gap in the flow cell covering.

Ozone kinetics experiments were replicated thrice, and data obtained was used to calculate, ozone decay constant, half-life, mass transfer coefficient, equilibrium ozone concentration, and solubility ratio.

### Ozone Decay Constant and Half-life

The rate of ozone decay followed a first order reaction (Eq. 1), which was in agreement with Alder and Hill (1950); and Roth and Sullivan (1981).

$$\frac{d[O_3]}{dt} = -k[O_3] \quad (1)$$

Here,  $\frac{d[O_3]}{dt}$  is the rate of ozone decay (mg/L min),  $k$  is the ozone decay constant ( $\text{min}^{-1}$ ), and  $[O_3]$  is ozone concentration (mg/L). The integrated form of Eq. 1 was used to find  $k$  (Eq. 2).

$$[O_3]_t = [O_3]_o e^{-kt} \quad (2)$$

Here,  $[O_3]_t$  and  $[O_3]_o$  are concentrations of ozone (mg/L) in water at any time ( $t$ ) and at the start of the decay analysis, respectively. An exponential trend line was fitted to  $[O_3]_t$  versus  $t$  decay data to find  $k$ .

Half-life (min) of ozone in water ( $t_{1/2}$ ) was calculated from  $k$  (Eq. 3).

$$t_{1/2} = \ln(2)/k \quad (3)$$

### Mass Transfer Coefficient

The mass transfer coefficient was calculated from the change in ozone concentration over time during ozonation (Eq. 4) as given by Roth and Sullivan (1981). This rate equation takes into account simultaneous ozone absorption and decay (Eq. 1).

$$\frac{d[O_3]}{dt} = k_L a ([O_3]^* - [O_3]) - k[O_3] \quad (4)$$

Here,  $\frac{d[O_3]}{dt}$  is the rate of ozonation (mg/L min),  $k_L a$  is mass transfer coefficient ( $\text{min}^{-1}$ ), and  $[O_3]^*$  is the equilibrium ozone concentration (mg/L) in water.

At  $[O_3]_{ss}$  there is no further change in ozone concentration over time. Thus, the rate of ozone absorption is equal to the rate of ozone decomposition (Eq. 5).

$$k_L a ([O_3]^* - [O_3]_{ss}) = k[O_3]_{ss} \quad (5)$$

Equation 5 shows that  $[O_3]_{ss}$  is less than  $[O_3]^*$ , because a portion of the ozone decomposes during the ozonation process. Using Eq. 4 and 5 the rate of ozonation can be calculated (Eq. 6).

$$\frac{d[O_3]}{dt} = (k + k_L a) ([O_3]_{ss} - [O_3]) \quad (6)$$

Equation 7 was obtained by integrating Eq. 6.

$$([O_3]_{ss} - [O_3]_t) = [O_3]_{ss} e^{-(k+k_L a)t} \quad (7)$$

The value of  $(k + k_L a)$  was calculated by fitting an exponential trend line to  $([O_3]_{ss} - [O_3]_t)$  versus  $t$  data (Eq. 7).

### Equilibrium Ozone Concentration and Solubility Ratio

As noted above,  $[O_3]^*$  is greater than  $[O_3]_{ss}$  due to simultaneous decomposition occurring during the absorption process.  $[O_3]^*$  can be calculated from  $[O_3]_{ss}$ ,  $k$ , and  $k_L a$  by rearranging Eq. 5 to the form used in Eq. 8 (Roth and Sullivan 1981).

$$[O_3]^* = [O_3]_{ss} \left(1 + \frac{k}{k_L a}\right) \quad (8)$$

The solubility ratio ( $s$ ) is defined as the ratio of ozone concentration in the aqueous phase ( $[O_3]^*$ ; mg O<sub>3</sub>/L water) to ozone concentration in the gaseous phase ( $[O_3]_g$ ; mg O<sub>3</sub>/L O<sub>2</sub>).  $[O_3]_g$  was measured as % [w/w] of oxygen and then converted to mg O<sub>3</sub>/L O<sub>2</sub> by using the density of oxygen as calculated from ideal gas law. The value of  $s$  was calculated experimentally and also predicted from an empirical relation. The experimental  $s$  was calculated from its definition (Eq. 9).

$$s \text{ (experimental)} = \frac{[O_3]^*}{[O_3]_g} \quad (9)$$

The value of  $s$  was predicted from Henry's law constant developed by multilinear regression (Eq. 10; Roth and Sullivan 1981).

$$H = 3.84 \times 10^7 [OH^-]^{0.035} e^{\left[-\frac{2428}{T}\right]} \quad (10)$$

Here,  $H$  is Henry's law constant (atm/mol fraction),  $[OH^-]$  is hydroxide ion concentration (g-mol/L), and  $T$  is the absolute temperature (K).

The value of  $H$  from Eq. 10 was substituted in Eq. 11.

$$s \text{ (predicted)} = T / (0.218 \times H) \quad (11)$$

Predicted values of  $H$  and  $s$  were based on the pH after ozonation.

### Preparation of Wash Water

Tap water, distilled water, ultra-pure water, and acetic acid solutions were used to determine ozone decay constants and to evaluate system performance (Table 1). The acetic acid solution was prepared to pH 4 (0.6%) and pH 2 (1%). The wash water temperature was controlled by immersing the flask in a water bath maintained at 3 °C lower than the desired temperature in the flask. The water temperature was manually recorded before, during (inside the flow cell), and after ozonation, using an alcohol thermometer. The pH and electrical conductivity were recorded before and after ozonation. There was a decrease in pH of 0-0.2 as a result of ozonation.

**Table 1 - Characteristics of water ozonated for kinetic study experiments.**

Water type	Temperature (°C)	pH <sup>a</sup>	Electrical conductivity (μS/cm)	
Tap	25	7.5	217.0	
Distilled	25	6.5	10.3	
	15	6.5	10.3	
	7	6.5	10.3	
Ultra-pure	25	5.8	0.8	
Acetic acid solution				
	(0.6%)	15	4	-
	(1.0%)	15	2	-
	(1.0%)	7	2	-

<sup>a</sup> The pH values shown are before ozonation.

Electrical conductivities of the acetic acid solutions were above the limits of detection (> 300 μS/cm).

## **Durum Wheat Treatment**

Distilled water, ozonated water (16.5 mg/L), chlorinated water (700 mg/L) at pH 10.8, acetic acid solution (1%), and acetic acid + ozonated water (1%, 20.5 mg/L) were prepared at 7 °C and used for wheat washing experiments.

Prepared water (300 mL) was poured into the flask containing the durum wheat sample (150 g). Ozonated water was used within 30 s of the cessation of ozonation. The flask was immediately stoppered to avoid loss of ozone into the atmosphere. The flask was fixed on a vibratory shaker set at 100 RPM for 3 min. The grain was strained out by placing a stainless steel mesh over the flask mouth, inverting the flask, and then tapping it 15 times in 10 s. The washed grain was stored in zip lock bags and placed on ice for up to 30 min until analyzed. The wheat washing experiments were replicated 9 times.

Microbiological analysis was initiated by aseptically weighing 11 g of washed wheat grain. A  $10^{-1}$  dilution was prepared by adding weighed grain to 99 mL of sterile Butterfield's phosphate-buffered solution (pH 7.0). All decimal dilutions were prepared with 90 mL sterile diluents plus 10 mL previous dilution. Dilutions were shaken 25 times in a 30 cm arc to suspend the material, in order to facilitate preparation of subsequent dilutions and inoculations. APC and YMC were performed according to AOAC Official Methods 990.12 and 997.02, respectively (AOAC International 2005). For APC, 1 mL of dilution was transferred onto an APC Petrifilm (3M Microbiology, St. Paul, Minnesota). Samples were plated in duplicate and were incubated for  $48 \pm 3$  h at  $35 \pm 1$  °C. For YMC, 1 mL of dilution was transferred onto an YMC Petrifilm (3M Microbiology, St. Paul, Minnesota). Samples were plated in triplicate and were incubated for 5 d at room temperature (20-25 °C). APC Petrifilms having less than 300 colonies were counted, and YMC Petrifilms having less than 150 colonies were counted.

## **Statistical Analysis**

The results of rate constants, half-lives, equilibrium ozone concentration, solubility ratio, and microbial experiments were subjected to analysis of variance (ANOVA) at 5% level of confidence, and the difference between means was determined by Duncan's Multiple Range Test. Statistical software SAS version 9.1 (SAS Institute, Cary, NC, U.S.A.) was used to analyze data.

## **Safety Emphasis**

Gaseous ozone was used in the study to make ozonated water. The action of ozone on humans has already been discussed in many studies, and exposure limits have been standardized in most user countries. In the U.S.A. and in most of the European countries, the threshold limit value, that is, the maximum ozone concentration for a work time of 8 h/d, has been set at 0.1 mg/L (Damez and Vigouret 1981). Ozone Discovery Badges (Ozone Solutions, Sioux Center, IA, U.S.A.) were used in the work area as a measure to detect ozone levels. These badges have a response time of 8 h for an ozone concentration of 0 to 0.08 mg/L, but they respond within 5 min at high (> 8 mg/L) concentrations. Ozone badges usually indicated low to moderate (<0.06

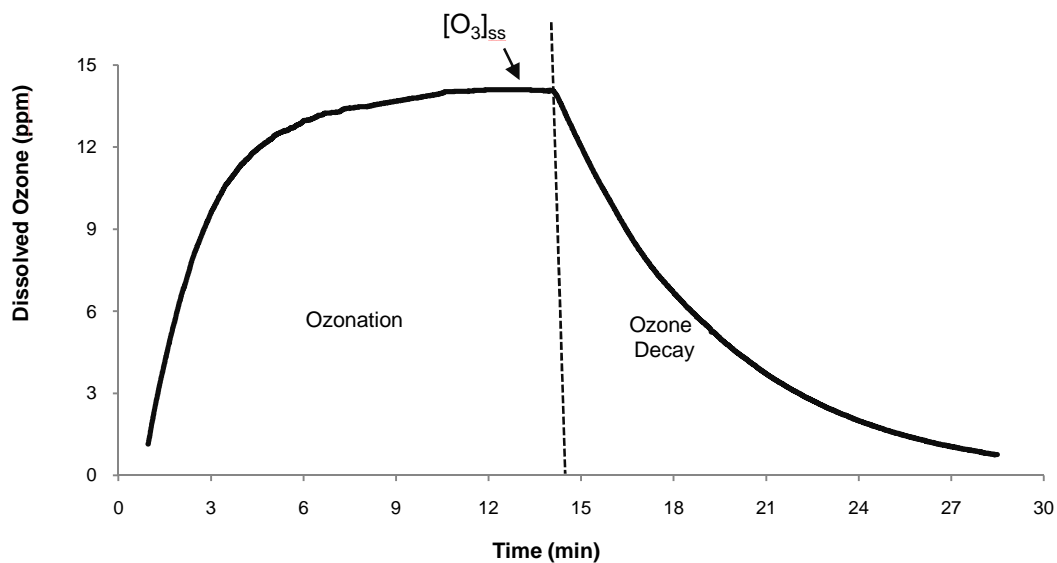
mg/L) concentrations of ozone in our work area. Disposable gloves (Microflex<sup>®</sup>) and masks (Dura-Mesh<sup>®</sup>) were used while handling samples, and when working with ozone and ozonated water.

## Results and Discussion

The system developed to produce water with controlled ozone concentration (Figure 1) worked as intended. The system was constructed of leakage-proof and ozone-safe material to minimize degradation reactions between ozone and the system. Some advantages of this system were continuous, automated, and accurate monitoring and datalogging of the dissolved ozone concentration. The sensing element in the dissolved ozone analyzer is a polarographic membrane that measures ozone directly, and is readily interfaced with a datalogger. The commonly reported methods for measuring dissolved ozone concentration are based on the indigo method (Bader and Hoigne 1981), which is colorimetric and requires periodic manual sampling, analysis and recording. In some instances, these methods are slow and subjective, and the sampling step may result in some ozone loss before analysis.

### Steady-state Dissolved Ozone Concentration

The antimicrobial effect of ozone depends on its concentration in the solution. Ozone is unstable in an aqueous solution; thus, its effectiveness as a disinfectant depends on the rate at which it decomposes, or its half-life (Gurol and Singer 1982). Ozone kinetic experiments were carried out to obtain a combination of water quality, temperature, and pH at which a high steady-state dissolved ozone concentration with a long half-life could be achieved. Ozone kinetic experiments were divided into two parts: attainment of steady-state dissolved ozone concentration (during ozonation) and ozone decay as an indicator of half-life (Figure 2).

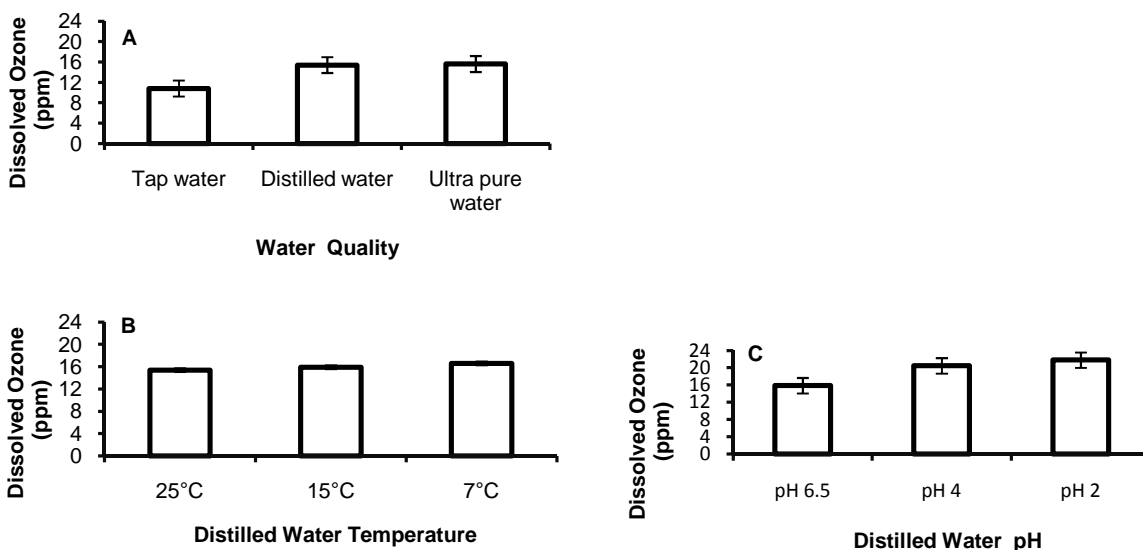


**Figure 2** - Ozonation to attain steady-state aqueous ozone concentration ( $[O_3]_{ss}$ ), followed by ozone decay (after ozonation was stopped), using distilled water (pH 6.5) at 7 °C.

Tap, distilled, and ultra-pure water were evaluated at 25 °C and had pH values of 7.5, 6.5, and 5.8, respectively (Figure 3A). In distilled water,  $[O_3]_{SS}$  achieved was 43% higher than in tap water. Use of ultra-pure water showed no significant ( $P < 0.05$ ) advantage over distilled water.

The effect of temperature (25, 15, and 7 °C) on  $[O_3]_{SS}$  was evaluated using distilled water (Figure 3B). The  $[O_3]_{SS}$  obtained at 15 °C was 3.2% greater than that obtained at 25 °C, decreasing the temperature to 7 °C further increased  $[O_3]_{SS}$  by 4.4%. The  $[O_3]_{SS}$  obtained at 7 °C, was significantly ( $P < 0.05$ ) higher than that obtained at 25 °C. The increase in  $[O_3]_{SS}$  based on temperature decrease was smaller than that found through decrease in pH or increase in water quality. This difference may be because ozone decay mechanism is affected less by variation in temperature than by variations in water quality and pH. A water temperature much below 7 °C was not feasible; the dissolved ozone analyzer was not designed to operate at temperatures lower than 5 °C.

A temperature of 15 °C was easily reached with this system and offered a significant benefit described below. Thus, the effect of pH (6.5, 4, and 2) on  $[O_3]_{SS}$  was evaluated using distilled water at 15 °C (Figure 3C). Glacial acetic acid was used to achieve the lower two pH values. A significant increase ( $P < 0.05$ ) of  $[O_3]_{SS}$  with a decrease in pH was observed. The  $[O_3]_{SS}$  value for distilled water at pH 2, 15 °C (21.8 mg/L) was twice that of tap water pH 7.5, 25 °C (10.8 mg/L).



**Figure 3** - A comparison of steady-state dissolved ozone concentration ( $[O_3]_{SS}$ ) with change in water quality (A), water temperature (B), and water pH (C). Error bars show standard error. The readings are based on an average of 3 replicates.

### Ozone Decay Constant, Half-life and Mass Transfer Coefficient

Ozone decay in water was monitored in conjunction with the study of  $[O_3]_{SS}$  to observe the effect of water quality, temperature, and pH on the decay constant and the half-life of dissolved

ozone in water (Table 2). The decay constant is inversely related to the half-life, as shown in Eq. 3; therefore, the trends observed with the decay constant will be the inverse of the half-life trends. The half-life was more than doubled by using distilled water over tap water, and use of ultra-pure water provided an additional 35% increase over distilled water. As noted above, a long ozone half-life is desirable for disinfection. The longer half-life in distilled and ultra-pure water partly accounts for their higher  $[O_3]_{SS}$ . The observed lower value of  $[O_3]_{SS}$  and half-life in tap water could be due to organic carbon and other minerals (barium, bromate, copper, chloramines, fluoride, nitrate/nitrite) present in tap water, but not in distilled or ultra-pure water.

The effect of temperature on decay constants and half-life was evaluated using distilled water ( $\text{pH } 6.5 \pm 0.2$ ). The decay constant decreased while the half-life increased with decrease in temperature (Table 2). The use of 15 °C temperature increased the half-life by 30% over 25 °C and use of 7 °C temperature further increased half-life by 15%.

The effect of pH on decay constants and half-lives was observed by lowering pH with glacial acetic acid. The half-life increased significantly ( $P < 0.05$ ) with a decrease in water pH (Table 2). The pH of 4 increased the half-life by 15% over pH 6.5, and use of pH 2 further increased the half-life by 5%. Acetic acid is one of the ion scavengers reported by Tomiyasu and others (1985). Hence, acetic acid may have acted as a scavenger for hydroxide ions responsible for ozone decay in water, and might have slowed ozone decomposition and extended the half-life. A similar effect was not observed when HCl was used to lower water pH (Smedt and others 2001); in the same study, the half-life of ozone was increased from 5 to 225 min when pH was decreased from 7.5 to 2 using acetic acid. In another study, Kong and others (2003) observed that half-life of ozone in ground water increased from 14.1 to 17.6 min upon reducing pH from 10.8 to 3.5, and in the case of deionized water, half-life increased from 31.9 to 38.6 min upon reducing pH from 10.2 to 3.9. Unfortunately, the system setups and other experimental conditions such as temperature,  $[O_3]_{SS}$ , and  $[O_3]_g$  used in these studies were not described.

**Table 2 - Ozone decay constant ( $k$ ), half-life, and mass transfer coefficient ( $k_L a$ ) as affected by water quality, temperature and pH.**

Water type	$k$ ( $\text{min}^{-1}$ )	Half-life (min)	$k_L a$ ( $\text{min}^{-1}$ )
Water Quality (25 °C)			
Tap	0.30A	2.27A	0.54
Distilled	0.12B	5.81B	0.63
Ultra-pure	0.08CD	7.82D	0.50
Temperature (pH 6.50 ± 0.2)			
25 °C	0.12B	5.81B	0.63
15 °C	0.09C	7.56C	0.55
7 °C	0.08CD	8.68E	0.44
pH (15 °C)			
6.5	0.09C	7.56C	0.55
4.0	0.08CD	8.72F	0.51
2.0	0.07D	9.19G	0.49

Readings within a column followed by same letter are not significantly ( $P < 0.05$ ) different from each other, based on ANOVA and Duncan's Multiple Range Test. Significant ( $P < 0.05$ ) differences were not found for  $k_L a$ . The results are based on 3 replicates.

The values of the mass transfer coefficient ( $k_L a$ ) ranged from 0.44 - 0.63  $\text{min}^{-1}$  (Table 2). A significant ( $P < 0.05$ ) difference in  $k_L a$  values was not observed with changes in quality, temperature, and pH of water within the ranges in this study; the standard deviations among replicates were very high, 40% in some cases. The value of  $k_L a$  is expected to increase significantly with an increase in the gas flow rate and decrease in gas sparger pore size (Zhou and Smith 2000); these two parameters were constant during our experiments. Gurol and Singer (1982) calculated  $k_L a$  to be 1  $\text{min}^{-1}$  for their system; they reported gas concentration of 5% [w/w] of ozone in the oxygen, and the gas flow rate was 0.75 L/min, but did not state sparger pore size.

### Equilibrium Ozone Concentration and Solubility Ratio

The equilibrium ozone concentration ( $[O_3]^*$ ) was calculated from  $[O_3]_{SS}$ ,  $k$ , and  $k_L a$  using Eq. 8. The solubility ratio ( $s$ ) is the ratio of aqueous to gaseous ozone concentration, thus commonly reported without units. The value of  $s$  is expected to be independent of the gaseous ozone concentration used; however, it is expected to depend upon factors such as temperature and pH. The value of  $s$  was calculated experimentally from  $[O_3]^*$  (Eq. 9) and empirically from temperature and pH (Eq. 11). The value of  $s$  increased significantly ( $P < 0.05$ ) when distilled or ultra-pure water was used as compared to tap water and also increased with decreasing pH (Table 3).

**Table 3 - Comparison and trend of equilibrium ozone concentration (  $[O_3]^*$  ) and solubility ratio ( $s$ ).**

Water Type	$[O_3]^*$ (mg/L)	$s$ (experimental) [(mg $O_3$ / L water) / (mg $O_3$ / L $O_2$ )]	$s^a$ (predicted) [(mg $O_3$ / L water) / (mg $O_3$ / L $O_2$ )]
Water Quality (25 °C)			
Tap	16.8A	0.33A	0.21
Distilled	18.3B	0.36B	0.23
Ultra-pure	18.1C	0.36B	0.24
Temperature (pH 6.5)			
25 °C	18.3B	0.36B	0.23
15 °C	18.5D	0.37B	0.29
7 °C	19.6E	0.39C	0.36
pH (15 °C)			
6.5	18.5D	0.37B	0.29
4.0	23.7F	0.47D	0.36
2.0	24.9G	0.49E	0.42

<sup>a</sup>The  $s$  (predicted) is calculated from Eq. 11 and is based on pH after ozonation.

Readings within a column followed by same letter are not significantly ( $P < 0.05$ ) different from each other, based on ANOVA and Duncan's Multiple Range Test. The results are based on 3 replicates.

The experimental values of  $s$  ranged from 0.33 – 0.49 and the predicted values of  $s$  ranged from 0.21 – 0.42, over a temperature range of 7 - 25 °C. In a literature review, the values of  $s$  for dissolved ozone were found to lie between 0 and 0.64 for a temperature range of 0 - 60 °C (Roth and Sullivan 1981). Gurol and Singer (1982) compiled literature values of  $s$  for dissolved ozone at 19 - 20 °C and found them to range from 0.23- 0.41. In our study, the difference between experimental and predicted values of  $s$  varied between 8 and 30% at 7 and 15 °C; at 25 °C this difference increased and was between 50 and 57%.

### Variation in Data and Challenges

A wide variation in values of reaction order, half-life, steady-state dissolved ozone concentration, and solubility ratio can be seen in the literature. In a literature review by Gurol and Singer (1982), it was noted that researchers disagree on whether the order of the ozone decay reaction is first, one-and-a-half, or second, or a combination of any of these two (depending on phase of the decomposition reaction). In another review, Roth and Sullivan (1981) listed significant variation in the solubility ratios as calculated by different researchers, even when accounting for temperature. Gurol and Singer (1982) attributed these differences to the use of different, sometimes questionable techniques to measure dissolved ozone concentration; the effect of solution composition, for example, the ionic medium on decomposition; the possible presence of impurities in the reagents; and uncertainty in the data analysis and interpretation. Tomiyasu and others (1985) observed that ozone studies reported in the literature were generally

not under comparable conditions because of different ozone concentrations, pH, and ionic media used, presence or absence of buffers, and possible scavengers and promoters used. Many investigators did not recognize the impact of a simultaneous decay reaction occurring during ozonation; and, therefore, assumed steady-state ozone concentration to be equilibrium concentration. Some researchers also assumed the ozone decay constant to be negligible in mass transfer coefficient calculations. These two closely related assumptions might have caused a significant bias error in the solubility ratio and mass transfer coefficient values (Roth and Sullivan 1981), as indicated by Eq. 7 and 8.

Other factors that might have contributed to a disagreement in the reported results are failure to report all the conditions during the experiment, for example, gaseous ozone concentration, flow rate, ozonation time, temperatures, water properties, apparatus used, and interval between dissolved ozone readings. The ozone parameters are also system-specific. Even if investigators attempt to closely document and control such factors, some factors are difficult to control precisely in laboratory-scale systems. Despite the variability in values reported in literature, quantities such as half-life, mass transfer coefficient, and solubility ratio are of great importance in the analysis, and scale-up of ozone-based disinfection processes.

### Durum Wheat Treatment

The developed ozonated water system was used for preparing treatment waters. The results of the microbial analysis of the washed samples are summarized in Table 4. Treated grains ( $18 \pm 1\%$  wb after washing) were compared to dry grain (8.8% wb). A  $1 \log_{10}$  CFU/g reduction is equivalent to a 90% reduction in colony forming units per g of wheat grain.

**Table 4 - The effect of different washing treatments on yeast and mold count (YMC) and aerobic plate count (APC) of durum wheat grain.**

Treatment	YMC ( $\log_{10}$ CFU/g)	APC ( $\log_{10}$ CFU/g)
Dry grain (Control)	4.3 A	4.9 A
Distilled water wash	4.2 A	4.9 A
Ozonated water (16.5 mg/L) wash	3.8 B	4.7 A
Chlorinated water (700 mg/L) wash	2.4 C	4.5 A
Acetic acid (1%) wash	0.5 D	1.9 B
Acetic acid + ozonated water (1%, 20.5 mg/L) wash	0.2 E	1.7 B

Readings within a column followed by the same letter are not significantly ( $P < 0.05$ ) different from each other, based on ANOVA and Duncan's Multiple Range Test. The results are based on 9 replicates.

Inhibition of micro-organisms by ozone takes place by breaching the cell wall, and the resulting changes in numerous cellular constituents, including proteins, unsaturated lipids and respiratory enzymes in cell membranes, peptidoglycans in cell envelopes, and nucleic acids in the cytoplasm (Khadre and others 2001). Our study showed a  $0.5 \log_{10}$  CFU/g reduction in YMC and a  $0.2 \log_{10}$  CFU/g reduction in APC with ozonated water (16.5 mg/L) washing. Similar results were shown by Ibanoglu (2002), who found that ozonated water (11.5 mg/L) washing of

wheat reduced YMC by 0.4 and APC by 0.8 log<sub>10</sub> CFU/g in the flour produced from washed wheat. Ibanoglu (2001) also reported that tempering of wheat with ozonated water (11.5 mg/L) resulted in 1.0 and 1.5 log<sub>10</sub> CFU/g reduction in YMC and APC, respectively.

Chlorine acts on microbial membranes, altering their permeability, inactivating enzymes, inhibiting membrane transport and causing lethal DNA damage (Venkobacher and others 1997). Our study showed a 1.9 log<sub>10</sub> CFU/g reduction in YMC and a 0.4 log<sub>10</sub> CFU/g reduction in APC with chlorinated water (700 mg/L) washing. Baur and others (2004) found a 0.7-1.5 log<sub>10</sub> CFU/g reduction in aerobic mesophilic, pseudomonades, and *Enterobacteriaceae* with chlorinated water (200 mg/L) washing of shredded lettuce.

The antimicrobial action of organic acids, such as acetic acid, depends upon several factors, including reduction in pH, dissociation of the acid, chain length, cell physiology, and metabolism (Akbas and Olmez 2007). Acetic acid is a weak acid thus only partially dissociated. Less dissociation results in greater antimicrobial activity, as the undissociated acid can penetrate the cell membrane and decrease the internal pH of the cell (Brudzinski and Harrison 1998). The acetic acid solution (1%) was more effective than ozonated water (16.5 mg/L) for eliminating micro-organisms. Unlike ozone, acetic acid is chemically stable, therefore, it is better able to penetrate crevices on the wheat grain surface to cause disinfection. Acetic acid + ozonated water (1%, 20.5 mg/L) was found to be the most effective treatment with YMC and APC reductions of 4.1 and 3.2 log<sub>10</sub> CFU/g, respectively. Our results are similar to the findings of Akbas and Olmez (2007) that dipping of fresh-cut iceberg lettuce in 5 g/L of organic acid (citric and lactic acid) for 2 min resulted in a 1.5-2.0 log<sub>10</sub> CFU/g reduction in the aerobic mesophilic and psychrotropic bacteria, and *Enterobacteriaceae*. Organic acid (5 g/L) was more effective than ozonated water (4 mg/L) and chlorine (100 mg/L) dippings for fresh-cut lettuce. Bang and others (2007) found that the combined treatment of 1% organic acid (citric, lactic, and acetic) and ozonated water (4 mg/L) washing of carrots resulted in a complete elimination (3.8 log<sub>10</sub> CFU/g reduction) of YMC. The combined treatment was more effective than ozonated water washing or organic acid washing alone, both in the case of carrots and cabbage.

Table 4 results show an additive effect of acetic acid + ozonated water; that is, the log reduction of these two agents together was similar to the sum of the log reductions for these two agents when used separately.

## Conclusion

A system for producing ozonated water, which includes integrated dissolved ozone monitoring, was developed. The highest ozone concentration of 16.6 mg/L was obtained using distilled water (pH 6.5) at 7 °C, and 21.8 mg/L was obtained in an acetic acid solution (pH 2) at 15 °C. The system performance parameters; decay constant (0.07 to 0.30 min<sup>-1</sup>), mass transfer coefficient (0.44 to 0.63 min<sup>-1</sup>), and solubility ratio (0.21 to 0.49) were similar to the literature values under similar conditions. Microbial analysis of washed wheat samples showed that ozonated water (16.5 mg/L) moderately reduced the microbial count, but was less effective than chlorinated water (700 mg/L) and acetic acid (1%). Acetic acid + ozonated water (1%, 20.5 mg/L) was the most effective treatment.

This research has highlighted two areas for further study. First, the ozonated water showed a pronounced antimicrobial effect only when used in combination with 1% acetic acid. Although acetic acid does not have residue issues as does chlorine, future research should be conducted to strive to eliminate microbes using ozonated water alone. Ozonated water alone can have a significant antimicrobial effect if used at higher dissolved ozone concentration. Knowing current system performance and how it relates to system design, it is possible to achieve even higher dissolved ozone concentration, for example, by increasing gaseous ozone concentration. Another component of this research could be to find the ozonated water additives that can be used in least amount (mg/L) to increase dissolved ozone concentration, have antimicrobial effect, and are also acceptable by cereal industry standards. Second, industry may find washing treatment expensive since washing results in the addition of more moisture to grain than required (before milling), hence the process requires a drying step. For this concern, a washing system and process can be designed to accomplish rapid application of ozonated water, thorough moisture removal from surface, and rapid, mechanical removal of free water; hence, accomplishing washing without undue drying costs and undue risks of grain spoilage.

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## **Acknowledgements**

This research was funded through the USDA National Research Initiative and the North Dakota Agricultural Experiment Station (Grant No.: USDA-CSREES-NRI-Food Safety #2005-35201-16667). We are thankful to Darrin Haagenson, Research Specialist, NDSU Agricultural and Biosystems Engineering Department, for his help in setting up the system, and providing technical assistance whenever needed. We are also thankful to Anuradha Boddeda, Research Specialist, NDSU Veterinary and Microbiological Sciences Department, for her help in performing the microbiology work. Special thanks are due to Kenneth Hellevang, Professor, NDSU Agricultural and Biosystems Engineering Department for providing valuable suggestions and guidance in research.

Disclaimer: This is the version of manuscript that was submitted to Journal of Food Science (JFS). The manuscript is published by JFS as:

Dhillon, B., D. Wiesenborn, C. Wolf-Hall, and F. Manthey. 2009. Development and evaluation of ozonated water system for antimicrobial treatment of durum wheat. *J. Food Science* 74(7): E396-E403.