

EXPOSURE OF WHEAT TO FLAMELESS CATALYTIC INFRARED RADIATION ON  
TEMPERATURES ATTAINED, WHEAT PHYSICAL PROPERTIES, MICROBIAL LOADS,  
MILLING YIELD, AND FLOUR QUALITY

by

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## Abstract

Organic, hard red winter wheat of 11% moisture was tempered with distilled water to moisture levels of 16 and 18% and held for 8, 16, and 24 h. At each moisture and holding time wheat was unexposed (control) or exposed to infrared radiation for 1, 1.5, and 2 min using a bench-top flameless catalytic infrared emitter. The mean external grain temperatures for 16% moisture wheat measured with thermocouples during infrared exposure of 1, 1.5, and 2 min ranged from 77.4-83.1, 93.7-101.2, and 91.2-98.3°C, respectively; corresponding mean internal temperatures were 67.3-76.4, 80.0-85.6, and 81.3-93.2°C. Minor differences in kernel moisture, hardness, and weight were observed among treatments. Tempered wheat after infrared exposure among treatments lost 1.5-2% moisture. Infrared exposure of wheat reduced initial bacterial loads ( $6.7 \times 10^4$  CFU/g) by 98.7% and fungal loads ( $4.3 \times 10^3$  CFU/g) by 97.8% when compared with those on untreated wheat. Wheat tempered to 18% and exposed for 2 min to infrared radiation lost 2% moisture, and this wheat when milled had a yield of 73.5%. The color of flour from infrared-exposed wheat was slightly dark (color change,  $\Delta E = 0.31$ ) when compared with untreated flour. Differential scanning calorimetry showed that flours from infrared exposed wheat had lower enthalpy (3.0 J/g) than those unexposed to infrared (3.3 J/g). These flours were adversely affected because they had longer mixing times (7-15 min) at all infrared exposures due to the presence of insoluble polymeric proteins (up to 60%). Microbial loads in flour from wheat tempered to 18% and exposed for 1-2 min had 0.6-2.4 log reduction compared to flour from untreated wheat.

Wheat tempered to 18% moisture with electrolyzed-oxidizing (EO) water reduced bacterial and fungal loads up to 66%. EO water tempered wheat exposed for 1, 1.5, and 2 min to infrared radiation showed microbial reductions of 99.5% when compared with control wheat. Infrared treatment of tempered wheat cannot be recommended as it adversely affected flour functionality. The use of EO water for tempering as opposed to potable water that is generally used in mills slightly enhances microbial safety of hard red winter wheat.

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## **Dedication**

I dedicate this thesis to my parents, Mr. Deliephan G. Pillai and Mrs. Mohana Deliephan.

**Chapter 1 - Rationale for evaluating flameless catalytic infrared radiation on wheat microbial loads, milling yield and quality**

## **1.1. Rationale for research**

Wheat is the world's most important food crop providing about one-third of the global production of cereals, yielding over 600 million metric tons annually (<http://www.ers.usda.gov>). The United States ranks third in the world in wheat production and produces about 64 million metric tons annually. About 31 million metric tons are exported to 100 countries while 33 million metric tons are consumed domestically (<http://www.uswheat.org>). In 2000, Kansas stored 30 million bushels of hard red winter wheat on the farms and 245 million bushels was stored off the farm.

Wheat in storage is susceptible to spoilage by insects, fungi, and bacteria. Depending on the length of storage, grain moisture, and use and level of pest management, stored wheat is attacked by a variety of insect species (Storey et al., 1984; Reed and Pedersen, 1987; Kenkel et al., 1992; Martin et al., 1997), bacteria (Ottogalli and Galli, 1979; Eyles et al., 1989; Richter et al., 1993; Manthey et al., 2004), and fungi (Christensen, 1957; Cornell and Hoveling, 1998; Daisuke et al., 2001).

Bacterial and fungal growth on stored grain causes significant reductions in both the quantity and quality (Paster et al., 1995; McKenzie et al., 1997). For example, in the United States, the annual storage losses caused by fungi and insects are estimated at more than \$500 million (Kells et al., 2001). Many species of fungi produce mycotoxins which are highly toxic to animals and humans (Paster et al., 1995; McKenzie et al., 1997).

New and innovative technologies need to be constantly explored for effective disinfestation and disinfection of wheat, because of problems associated with pesticide residues, resistance development in pests, assure food safety, and to meet quality demands of domestic and foreign buyers. Very few pest management options are available for managing pests for organic stored wheat. One such alternative that appears to be the use of infrared radiation for disinfesting and disinfecting grains.

Infrared radiation is that part of the electromagnetic spectrum with wavelengths between those of ultra-violet and of microwave radiation. The wavelengths of infrared radiation range between 0.5  $\mu\text{m}$  and 1000  $\mu\text{m}$  and interact with food materials by reflection, absorption, transmission and scattering (Rosenthal, 1996). The penetration of infrared rays into the food material causes the water molecules to vibrate at a frequency of 60,000-150,000 MHz and this

causes rapid heating. The dissipation of radiative energy as heat results in particular surface temperatures and penetration depths specific to the food material treated, depending on the infrared wavelength, product composition, water activity and product thickness (Sakai and Hanzawa, 1994). Infrared radiation does not heat up the surrounding medium.

Infrared radiation can be divided into three categories namely near infrared (NIR; 0.75 – 1.4  $\mu\text{m}$ ; temperature less than 400°C), mid infrared (MIR; 1.4 – 3  $\mu\text{m}$ ; temperature in the range of 400°C to 1000°C), and far infrared (FIR; 3-1000  $\mu\text{m}$ ; temperature more than 1000°C) (Sakai and Hanzawa, 1994; Skjöldebrand, 2001). Shorter wavelength infrared radiation has greater penetration while producing lower temperature compared to longer wavelength radiation (Skjöldebrand, 2001). Infrared heating has several advantages such as (1) higher heat transfer capacity, (2) instant heating because of direct heat penetration, (3) high energy efficiency, (4) faster heat treatment, (5) fast regulation response, (6) better process control, (7) no heating of surrounding air, (9) uniform heating, (10) preservation of vitamins, and (11) less chance of flavor losses from burning of foods (Dagerskog and Österström, 1979; Afzal et al., 1999; Skjöldebrand, 2001).

The use of intense infrared radiation (FIR) was evaluated two decades ago in the United States for killing immature stages of stored- grain insects developing within kernels as well as adults (Tilton and Schroeder, 1963; Cogburn, 1967; Cogburn et al., 1971; Kirkpatrick and Tilton, 1972; Kirkpatrick et al., 1972, 1973; Tilton et al., 1983). Sakai and Hanzawa (1994) indicated that FIR energy penetrates very little; almost all the energy is converted to heat the surface of food. These findings were consistent with the study of Hashimoto et al. (1993) who evaluated FIR heating technique as a surface sterilization method to kill *Escherichia coli* and *Staphylococcus aureus* suspended in phosphate-buffered saline solution. Hamanaka et al. (2000) and Uchino et al. (2000) investigated sterilization of wheat surface using infrared radiation. Infrared radiation is transmitted through water at short wavelength whereas at longer wavelengths it is absorbed at the surface. Hamanaka et al. (2000) examined the disinfection effect of infrared radiation on wheat inoculated with *Aspergillus niger*, in which the wheat was treated with infrared heating at 2 kW for 30 sec, followed by cooling for 4 h, and again treated for 30 sec with infrared radiation to obtain a 1.6 log reduction in colony forming units (CFU)/g. Uchino et al. (2000) exposed wheat to infrared radiation for 28, 37, and 63 sec and evaluated microbial load reduction. However, sterilization effects of infrared treatment for extended exposure times have not been studied.

Moreover, in studies conducted on insects and microbes, temperature of substrates exposed to infrared radiation was measured immediately after exposure and not in real time. Therefore, in these studies, actual temperatures attained by the grain were under estimated. Infrared radiation sources used were lamps or tubes with low power (0.5 kW), or gas-fired emitters with high power ranges (14 kW) resulting in very high temperatures (900°C). In previous studies with insects natural gas or propane were combusted over ceramic panels in the presence of oxygen and had open flames.

Unlike gas-fired emitters, the flameless catalytic infrared emitter developed by Catalytic Industrial Technologies LLC, Independence, KS, USA (<http://www.catalyticdrying.com>), uses propane or natural gas combusted over a platinum catalyst in the presence of oxygen to emit infrared energy in the 3-7  $\mu\text{m}$  wavelength range resulting in temperatures below 500°C. The catalytic infrared heaters are environmentally friendly and do not produce any  $\text{NO}_x$  or CO. The co-products of catalytic oxidation-reduction reaction are carbon dioxide, water vapor, and infrared radiation.

Pan et al. (2008) used flameless catalytic infrared radiation to simultaneously dry and disinfest paddy rice. Eggs and adults of the lesser grain borer, *Rhyzopertha dominica* (F.) and Angoumois grain moth, *Sitotroga cerealella* (Olivier) were exposed to infrared radiation along with the paddy rice. A total of exposed 250 g paddy rice of 20.6 and 25.0% moisture (wet basis, wb), on a drying bed in a single layer for 15, 40, 60, and 90 sec. After 15, 40, 60, and 90 sec exposure the 20.6% moisture paddy rice attained temperatures of 42.8, 54.3, 61.2 and 69.4°C, respectively; corresponding temperatures of 25.0% moisture paddy rice were 42.8, 55.5, 59.1, and 68.0°C. The study recommended heating paddy rice to 60°C in 60 sec, and moisture loss after exposure of paddy rice at the two moistures was about 1.7%. This recommended treatment regimen resulted in 0.7-1.2% higher total rice yield when compared with conventionally dried paddy rice.

Khamis et al. (2010; 2011a,b) studied the effectiveness of flameless catalytic infrared radiation against all life stages of the rice weevil *Sitophilus oryzae* (L.), red flour beetle, *Tribolium castaneum* (Herbst), and *R. dominica* infesting hard red winter wheat of 12% moisture (wb). In these studies, temperatures were measured in real time during infrared exposure. They reported that exposure of 113.5 g of wheat in a single layer at a distance of 8 cm from the infrared emitter surface for 1 min killed all life stages of the three insect species. The

temperatures required for complete disinfestations of the three species ranged from 90-114°C. The temperatures attained by wheat did not adversely affect wheat milling yield and flour quality (Khamis et al., 2011c). Wheat quality was perhaps unaffected because Khamis et al. (2011c) wheat was tempered to 16% moisture (wb) after infrared exposure. It is unclear whether infrared exposure of tempered wheat has any adverse effects on milling yield and flour quality. This question formed the basis of my thesis research.

## 1.2. Infrared radiation basics

Temperature of the heating element determines the wavelength at which maximum radiation occurs as described by Planck's law, Wien's displacement law, and Stefan-Boltzmann's law (Dagerskog and Österström, 1979; Sakai and Hanzawa, 1994).

The amount of heat emitted from a black body (a theoretical object that absorbs 100% of the radiation that fall on it) can be determined using the Stefan-Boltzmann's equation:

$$Q = \sigma AT^4$$

where,  $Q$  is the rate of heat emission in J/s,  $\sigma$  is the Stefan-Boltzmann's constant ( $5.7 \times 10^{-8} \text{ W/m}^2\text{K}^4$ ),  $A$  is the surface area in  $\text{m}^2$ , and  $T$  is the absolute temperature in °K. This law clearly indicates that even a small increase in temperature can lead to higher rate of heat emission.

Infrared heating element emits radiation in range of wavelengths with varying intensities. The wavelength at which maximum radiation occurs depends on the lamp temperature according to Planck's equation:

$$E_{b\lambda}(T, \lambda) = \frac{2\pi^5 h c_o^2}{15 n^2 \lambda^5 \left[ e^{\left(\frac{hc_o}{n\lambda kT}\right)} - 1 \right]}$$

where,  $E$  is the emissive power of a black body,  $h$  is the Planck's constant ( $6.626 \times 10^{-34} \text{ Js}$ ),  $c_o$  is the speed of light in km/s,  $n$  is the refractive index of the medium,  $\lambda$  is the wavelength in  $\mu\text{m}$ ,  $k$  is the Boltzmann's constant ( $1.3806 \times 10^{-23} \text{ J/K}$ ) and  $T$  is the absolute temperature in °K. As the temperature of the heating element increases, the wavelength at which maximum radiation occurs decreases and the total energy emitted increases.

Wien's law states that the wavelength corresponding to maximum emission is inversely proportional to the black body temperature according to Wien's law:

$$\lambda_{max} = \frac{2900}{T}$$

where,  $\lambda_{max}$  is the wavelength corresponding to maximum emission in  $\mu\text{m}$  and  $T$  is the absolute temperature of the black body in  $^{\circ}\text{K}$ .

### **1.3. Interaction of infrared radiation with food materials**

As food is exposed to infrared radiation, it is either absorbed, reflected, or scattered. Absorption intensities at different wavelengths vary with food components. As the infrared radiation is absorbed by food components, the vibration and rotation of the molecules change because of a decrease or increase in the distance between atoms, movement of atoms, or vibration of molecules which leads to radiative heating (Skjöldebrand, 2001). A significant portion of the infrared radiation is reflected depending upon the wavelength. Less than 10% of the radiation is reflected back in the FIR wavelength region (Skjöldebrand, 2001).

The rate of heat transfer to food material depends on several factors such as the composition, depth, temperature of the infrared source, moisture content of the food material, shape and surface characteristics of the food material (Skjöldebrand, 2001). Water and organic compounds such as proteins and starches which are the main components of food absorb infrared energy at wavelengths greater than  $2.5 \mu\text{m}$  (Sakai and Hanzawa, 1994).

### **1.4. Pathogen inactivation mechanism of infrared heat treatment**

Infrared heating can be used for pathogen inactivation. There are limited studies performed on the application of infrared radiation for pathogen inactivation in food systems (Uchino et al., 2000; Daisuke et al., 2001; Jun and Irudayaraj, 2003). Several researchers have suggested numerous targets for inactivation such as DNA, RNA, ribosome, cell envelope, and proteins in the cell. Mid to far-infrared radiation is used in the pasteurization of food products, because the absorption of infrared energy by water and organic materials is high in this wavelength region (Sawai et al., 1995). The sensitivity of *E. coli* to the antibiotics like rifampicin and chloramphenicol increased following heat treatment with far infrared irradiation and thermal conduction, suggesting that the damage to RNA polymerase and ribosome occurred leading to cell death (Sawai et al., 1995).

### **1.5. Role of infrared radiation in wheat conditioning**

Wheat is conditioned before milling primarily to improve its physical state. Tempering involves adding water to wheat before milling to toughen the bran and soften the endosperm, and thus improve the efficiency of flour extraction. Wheat conditioning methods using heat, practiced in European countries, reportedly shortened the time required for conditioning as well as improved the baking quality of soft wheat (Bradbury et al., 1960). Studies on warm conditioning (at temperatures less than 46.1°C), hot conditioning (at temperatures over 46.1°C) and steam conditioning of wheat have been reported by Bradbury et al. (1960).

Early studies on Kansas hard wheat were undertaken to determine whether the improvements in milling/baking quality accompanying the ‘aging’ of wheat immediately after harvest could be achieved by moistening and heating the wheat (Bradbury et al., 1960). Swanson et al. (1916) used temperatures ranging from 45 to 98°C and quantities of water ranging from 25 to 100 cc/kg of wheat at different holding times for each treatment, and concluded that heating of moistened wheat improved the milling quality of wheat and baking quality of flour to some extent. The chief advantage claimed for hot conditioning is that the use of heat reduces the standing time required for penetration of moisture into the grain. Mellowing of hard wheat kernels is largely dependent on the entrance of moisture. From the data given by Polewka (1929) it appears that heat of conditioning facilitates mellowing process. He determined texture of kernels by examining sections with a diaphanoscope. Schafer (1954) attributed the easier separation of bran from endosperm to the hardening of protein contents of the cells of aleurone layer. This hardening was considered to have made it mechanically easier for the flutes of the rolls to scrape the outermost endosperm cells from the bran.

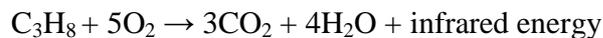
Bradbury et al. (1960) indicated that infrared radiation had been used to some extent in Germany to dry wheat grain for milling and that the cost was compensated for by the increased yield and quality of flour. Rumpf (1955) stated that infrared radiation of 2.5 to 3 kW can be used to condition 100 kg of washed wheat grains to 40-80°C for improved flour yield and quality. He also observed that the over extensible gluten in sprout damaged/soft wheat were strengthened due to radiative heat. Pan et al. (2008) stated that heating by conventional methods has disadvantages like slow heating and non-uniform heat distribution, which results in moisture gradients within paddy grain. The moisture gradient could induce tensile and compressive stresses resulting in fissures after cooling. Pan et al. (2008) observed these effects on infrared

heating of paddy grain. I hypothesize that similar effect of stresses and fissuring could be caused in wheat due to non-uniform conventional heating. My premise is that infrared radiation can provide a solution for achieving fast and relatively uniform heating resulting in reduced moisture gradient within the grain, which in turn may induce cleaner separation of bran resulting in improved milling yield.

### **1.6. Flameless catalytic infrared heating**

Catalytic Drying Technologies LLC, Independence, KS, has developed a proprietary flameless infrared technology for diverse drying applications. The company received an award from the United States Environmental Protection Agency's Pollution Prevention Program for developing the flameless catalytic infrared radiation source, under the "Environmentally Preferable Products" category. Unlike flame infrared emitters, in flameless infrared emitters, propane or natural gas chemically react at the surface of a platinum catalyst below gas ignition temperatures, delivering peak radiant energy in the 3-7  $\mu\text{m}$  range, and the resulting temperatures are below 500°C (Khamis et al., 2010).

The bench-top flameless catalytic infrared emitter has a circular heating surface of 613.4  $\text{cm}^2$  and is fueled by a propane cylinder (Ozark Trail Propane Fuel, Bentonville, AR) at 27.9 cm (0.40 psi) of water column pressure. To initiate the reaction, the coil is heated with a 110 volts electrical supply for 15 min. The propane reacts with oxygen in the presence of a platinum catalyst and emits infrared radiation. The co-products of this reaction are carbon dioxide and water vapor. The total heat energy output of this unit is 1.5 kW/h (Khamis et al., 2010). The reaction producing infrared energy is shown below:



### **1.7. Research Hypotheses**

Wheat tempered to different moisture contents at different holding times, and subsequently treated with infrared radiation for different exposure times could result in a substantial reduction of microbial loads (bacteria and fungi), and an increase in milling yield without adversely affecting flour quality.

## 1.8. Research objectives

The research reported in this thesis focuses on exploring infrared radiation as a tool for reducing bacterial and fungal loads in wheat and increase milling yield. This study also examined wheat physical properties, flour functionality and end-use qualities. Furthermore, this study explored the use of electrolyzed-oxidizing (EO) water as a tempering agent for wheat to reduce microbial loads. Specific objectives of this work included: (1) measuring external and internal temperature profiles of wheat grain during infrared heating, (2) evaluating effects of infrared heating on microbial loads of whole wheat and flour, (3) determining effects of infrared heating on wheat physical properties, germination, milling yield, and flour quality and functionality, and (4) evaluating effects of EO water used for tempering wheat plus infrared radiation on microbial loads of whole wheat and flour.

This thesis has five chapters including this introductory chapter. The first chapter describes infrared radiation principles and basics and provides a rationale for the work reported here. In chapters two through four, we exposed wheat (16 and 18% moisture [mc], wet basis [wb]) tempered for 8, 16 or 24 h to flameless catalytic infrared radiation for 1, 1.5 and 2 min. In the second chapter, the internal and external temperatures of wheat kernel during infrared heating were measured using thermocouples and compared statistically to determine differences in temperatures attained. Thermal conductivity and diffusivity were calculated for wheat tempered to 18% mc for 24 h and exposed to infrared radiation for 2 min. In the third chapter, reduction in bacterial and fungal loads on tempered wheat during infrared radiation exposure was evaluated, and the average infrared radiation exposure time required for one-log reduction of microbial loads (*D*-value) was determined. In the fourth chapter, the changes in wheat physical properties namely moisture, weight and hardness, and germination were evaluated. Wheat tempered to 18% mc and IR treated for 1.0, 1.5 or 2 min was milled and the milling yield was calculated. The flour was evaluated for changes in color, falling number, enthalpy, baking quality and amount of insoluble polymeric protein present. In the fifth chapter, wheat was tempered to 18% mc for 24 h with distilled water and EO water. The reduction in bacterial and fungal loads on wheat unexposed and exposed to infrared radiation for 1, 1.5 and 2 min were evaluated and the corresponding *D*-values were calculated.

Described below are standard microbial plating procedures that are not mentioned in detail within each chapter:

*(a) Preparation of media*

Bacterial counts were estimated using Tryptic Soy Broth Agar (TSBA) base (Beckton, Dickinson and Company, Sparks, MD, USA). To 1.0 L of distilled water, 23.0 g of TSB and 12.0 g of nutrient agar were added and placed on a heater and shaker (Thermolyne, Dubuque, IA, USA) with a magnetic stirrer placed inside the medium for homogenous mixing. After mixing for 15 min, the medium was autoclaved in a sterilizer set to 121°C at a pressure of 2038.9 cm of water column (29 psi). The medium is then poured into 100 × 15 mm Petri plates and allowed to solidify. After solidification the plates were inverted.

Fungal counts were estimated using Potato Dextrose Agar (PDA) base (Oxoid Inc., Hampshire, England). To 1.0 L of distilled water, 30.0 g of PDA was added and the medium was prepared following the same procedure as indicated for bacterial counts. Chloramphenicol (75 mg; Genlantis, San Diego, CA, USA) dissolved in 0.5 ml of ethanol was added to the medium before pouring into petri plates as mentioned above.

*(b) Preparation of inoculum*

Blanks consisting of 100 ml distilled water each, for all samples were prepared by dissolving 1 g of peptone (Beckton, Dickinson and Company, Sparks, MD, USA) in 1 L distilled water and autoclaved at 121°C at 2038.9 cm of water column (29 psi). Test tubes containing 9 ml distilled water were also autoclaved along with this. After cooling down, 10 g of sample was dissolved in the 100 ml blank. From this sample, 1 ml of inoculum was transferred to the 9 ml distilled water in test tube which makes the inoculum concentration to  $10^{-1}$ . Similarly inoculums of  $10^{-2}$ ,  $10^{-3}$  and  $10^{-4}$  concentrations were prepared.

*(c) Inoculating media plates and enumeration*

Inoculums (100 µL) were poured onto the media and spread uniformly using sterilized glass rod. The inoculated media plates were placed in an incubator under controlled conditions (25°C and 65% RH). The bacterial and fungal colonies were counted after 48 h of incubation.

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**Chapter 2 - Thermal diffusivity in wheat kernels exposed to  
flameless catalytic infrared radiation**

## Abstract

Organic hard red winter wheat of 11% moisture (wb) (control) was tempered to 16 and 18%, and wheat at each moisture content was held for 8, 16, and 24 h in sterile plastic bags. Wheat (113.5 g) in a circular stainless steel pan at each moisture and holding time was subjected to infrared radiation for 1, 1.5, and 2 min at a distance of 8 cm from the emitter surface. During infrared exposure, temperature on the surface of the wheat kernel and at a depth of 2 mm within the kernel were measured continuously with J-type thermocouples. These measurements were made for a kernel placed at the center of the pan. Simultaneously, temperatures of the wheat in the pan center during infrared exposure were measured continuously with a non-contact infrared thermometer placed at a distance of 85 cm. In all treatment combinations, mean external temperature of grain measured by thermocouple during 1, 1.5, and 2 min exposures ranged from 74.0-83.1°C, 93.3-101.4°C, and 91.2-99.3°C respectively. Mean internal temperature of grain measured by thermocouple during 1, 1.5, and 2 min exposures ranged from 67.3-76.4°C, 80.0-85.6°C, and 81.3-94.3°C respectively. Mean surface temperature of grain measured by infrared thermometer during 1, 1.5, and 2 min exposures ranged from 81.3-99.8°C, 90.9-104.2°C, and 101.5-110.4°C respectively. The thermal conductivity and diffusivity were determined for 11% mc (control), 16% and 18% mc wheat at 1, 1.5 and 2 min exposure, and the values were not significantly different among treatments ( $P>0.05$ ). Linear regression model ( $y = a + bx$ ) were fit to the grain surface temperatures vs. internal temperatures. The corresponding parameter estimates showed the relationship between the two. This helps in predicting the internal grain temperature when the external surface temperature is known and vice versa during infrared heating.

## 2.1. Introduction

Infrared radiation is used for grain drying (Bradbury et al., 1960; Schroeder and Rosberg, 1960; Faulkner and Wratten, 1969; Pan et al., 2008), for controlling various life stages of internal and external stored-product insects (Tilton and Schroeder 1963; Cogburn et al., 1971; Kirkpatrick and Tilton, 1972; Tilton et al., 1983) and for pathogen inactivation on cereals (Hamanaka et al., 2000; Uchino et al., 2000; Jun and Irudayaraj, 2003). In all these studies, grain temperatures were measured after infrared exposure and not in real time, which may have resulted in under-reporting actual temperatures attained by the grain because of natural cooling after exposure at ambient conditions. Infrared radiation sources used were lamps or tubes with low power (0.5 kW), or gas-fired emitters with high power ranges (14 kW) resulting in very high temperatures (900°C). They used natural gas or propane combusted over ceramic panels in the presence of oxygen and had open flames.

Unlike gas-fired emitters, the flameless catalytic infrared emitter developed by Catalytic Industrial Technologies LLC, Independence, KS, USA (<http://www.catalyticdrying.com>), uses propane combusted over a platinum catalyst in the presence of oxygen to emit infrared energy in the 3-7  $\mu\text{m}$  wavelength range resulting in temperatures below 500°C. The catalytic infrared heaters are environmentally friendly and since they do not use any flame, these heaters do not produce any  $\text{NO}_x$  or CO. The co-products of catalytic oxidation-reduction reaction are carbon dioxide, water vapor, and infrared radiation.

Pan et al. (2008) used flameless catalytic infrared radiation to simultaneously dry and disinfest paddy rice. They exposed 250 g paddy rice with moisture contents 20.6% and 25.0%, on drying bed in a single layer, for 15, 40, 60 or 90 sec. The average infrared intensity at the paddy rice surface was 5348  $\text{W}/\text{m}^2$ . After 15, 40, 60, and 90 sec exposure the 20.6% moisture paddy rice attained temperatures of 42.8, 54.3, 61.2 and 69.4°C, respectively; corresponding temperatures of 25.0% moisture paddy rice were 42.8, 55.5, 59.1, and 68.0°C. The study recommended heating rice for 1 min to attain a temperature of 60°C to completely disinfest eggs and adults of the Angoumois grain moth, *Sitotroga cerealella* (Olivier) and lesser grain borer, *Rhyzopertha dominica* (F.). This recommended treatment regimen resulted in 0.7-1.2% higher total rice yield when compared with conventionally dried paddy rice.

Pan et al. (2008) also measured temperatures of grain bed after infrared exposure using a T-type thermocouple (Omega Engineering Inc., Stamford, CT, USA).

Khamis et al. (2010; 2011a,b) studied the effectiveness of flameless catalytic infrared radiation against all life stages of the rice weevil *Sitophilus oryzae* (L.), red flour beetle, *Tribolium castaneum* (Herbst), and *R. dominica* infesting hard red winter wheat of 12% moisture (wb). In these studies, temperatures were measured in real time during infrared exposure. They reported that exposure of 113.5 g of wheat for in a single layer at a distance of 8 cm from the infrared emitter surface for 1 min killed all life stages of the three insect species. The temperatures required for complete disinfestations of the three species ranged from 90-114°C. The temperatures attained by wheat did not adversely affect wheat milling yield and flour quality (Khamis et al., 2011c). Wheat quality was perhaps unaffected because Khamis et al. (2011c) wheat was tempered to 16% moisture (wb) after infrared exposure. The exposure of tempered wheat to infrared radiation on temperatures attained, wheat physical properties, flour quality, and microbial loads are unknown.

In the present investigation, experiments were designed to determine ‘real-time’ temperatures attained by wheat tempered to 16 and 18% moisture (wb) and held for 8, 16, and 24 h prior to milling exposed to infrared radiation for 1, 1.5, and 2 min. The latter two exposures were greater than those used by Khamis et al. (2010; 2011a,b,c). The effects on wheat physical properties, microbial loads, germination, milling yield and flour quality are reported in chapters three to five. Like Khamis et al. (2010; 2011a,b,c) temperature of wheat in all treatment combinations were measured continuously using a non-contact infrared thermometer. In addition, thermocouples placed on a wheat kernel surface and at a depth of 2 mm from the surface were also measured. Specific research objectives were to (1) compare the internal and external temperature profiles of wheat during infrared exposures with those measured by infrared thermometer and (2) calculate thermal conductivity and diffusivity of tempered wheat subjected to infrared radiation.

## **2.2. Materials and Methods**

### ***2.2.1. Wheat samples***

Uninfested, organic hard red winter wheat (var. Jagger) was procured from Heartland Mills, Marienthal, KS, USA. The initial mean  $\pm$  SE moisture content of wheat was determined

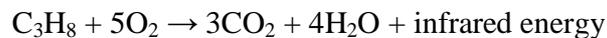
using the Perten 4100 Single Kernel Characterization System (SKCS, Perten Instruments North America Inc., Springfield, IL, USA), and was found to be  $10.8 \pm 0.1\%$  ( $n = 300$ ). About 200 g of wheat was placed in sterile polythene Ziploc bag (Fisher Scientific, Pittsburg, PA, USA) and distilled water was added to increase the grain moisture to 16 or 18%. The amount of distilled water added was calculated using the following formula (Harris and Lindblad, 1978):

$$\text{Amount of water added (in ml)} = \left[ \frac{100 - \text{Initial moisture}}{100 - \text{Final moisture}} - 1 \right] \times \text{Amount of wheat (in g)}$$

The bag with wheat after adding water was shaken vigorously for 10 mins to provide form coverage of the added water on all kernels. The mean  $\pm$  SE actual moisture contents verified by SKCS at 16 and 18% wheat were  $15.9 \pm 0.0\%$  and  $17.7 \pm 0.1\%$ , respectively. The wheat at each moisture content was allowed to temper for 8, 16, and 24 h in an environmental growth chamber (Percival, Perry, IA, USA) at  $28^\circ\text{C}$  and 65% RH.

### ***2.2.2. Infrared heating***

A bench-top flameless catalytic infrared emitter (Catalytic Drying Technologies LLC, Independence, KS, USA) was used for heating wheat. It has a circular heating surface of  $613.4 \text{ cm}^2$  and is fuelled by a propane cylinder (Ozark Trail Propane Fuel, Bentonville, AR) at 27.9 cm (0.40 psi) of water column pressure. To initiate the reaction, a heating element within the emitter casing is heated for 15 min. The propane reacts with oxygen in the presence of a platinum catalyst and emits infrared radiation in the 3-7  $\mu\text{m}$  range. The co-products of this reaction are carbon dioxide and water vapor. The total heat energy output of this unit is 1.5 kW/h. The reaction producing infrared energy is shown below:



A steel pan (28.0 cm in diameter and 3.8 cm in depth) with a handle was used to expose 113.5 g of wheat in a single layer to infrared radiation at a distance of 8.0 cm from the emitter surface.

### ***2.2.3. Temperature measurements***

Two iron-constantan J-type thermocouples ( $T_1$  and  $T_2$ ) were used to measure “real-time” internal and external temperatures of wheat during infrared exposure. For measuring internal temperature,  $T_1$  was inserted into the grain through a hole on the germ end made using a 0.24 mm Titex<sup>®</sup> micro drill (MSC Industrial Supply Co., Melville, NY, USA).  $T_2$  was placed on the

surface of a wheat kernel and held in place with a small piece of tape to measure the external temperature. In order to avoid direct heat from the infrared emitter,  $T_2$  was placed underside of the kernel. The wheat kernel was placed in the center of the pan, because this is the area that consistently has temperatures much higher than locations away from the center (Khamis et al., 2010).  $T_1$  and  $T_2$  were connected to input module at the channels of a Fluke Hydra Series II Data Logger (Fluke Corporation, Everett, WA, USA) to record temperatures in real time. The internal and external grain temperatures were continuously sampled at 1 sec interval, and the stored data was transferred to a computer using a RS-232 cable via the USB port. Additionally, a non-contact infrared thermometer (Raynger MX4 model 4TP78, Raytek<sup>®</sup>, Santacruz, CA, USA), mounted on a tripod 85 cm away from the infrared emitter, was used to measure the external surface temperature of the wheat being exposed to infrared radiation. The thermometer works in the 8-14  $\mu\text{m}$  range and has a response time of 250 milliseconds. A RS-232 cable was used to connect the thermometer to a laptop computer. The thermometer was directed towards the center of wheat sample in the pan, and the external temperature of wheat was recorded every second in real time using the data acquisition program developed in LABView software program (National Instruments Corporation, Austin, TX, USA). The experiment was replicated three times.

#### ***2.2.4. Calculation of thermal conductivity and diffusivity***

Thermal conductivity and diffusivity were determined at 2 min exposures for 11% (control), 16% and 18% moisture wheat tempered for 24 h. Thermal conductivity,  $K$  was calculated according to equation 1 as:

$$K = Qx/A(T_a - T_0) \quad (\text{Eq. 1})$$

Where,  $Q$  = energy absorbed by wheat kernel in kilojoules (KJ);  $x$  = thickness of wheat kernel (m);  $A$  = surface area of wheat kernel ( $\text{m}^2$ );  $T_0$  = temperature at center of wheat kernel ( $^{\circ}\text{K}$ );  $T_a$  = temperature at distance 'a' from the center of wheat kernel ( $^{\circ}\text{K}$ ). As stated above,  $T_a$  was measured at a distance of 8 cm from the center of wheat kernel. Thickness of wheat kernel was 0.002 m. Surface area of wheat kernel was  $0.00004 \text{ m}^2$ . Energy absorbed by wheat kernel,  $Q$  was calculated using the following equation 2 as:

$$Q = \varepsilon \sigma A [(T_a)^4 - (T_0)^4] \quad (\text{Eq. 2})$$

where  $\varepsilon$  = emissivity constant, 1.92 (no unit);  $\sigma$  = Stefan-Boltzmann constant,  $5.67 \times 10^{-8} \text{ W/m}^2 \text{ K}^4$ )

Thermal diffusivity  $\alpha$  was calculated using equation 3 as:

$$\alpha = A_0 (a^2)/4(T_a - T_0) \quad (\text{Eq. 3})$$

where  $a$  = radius of wheat kernel (m);  $T_0$  = temperature at center of wheat kernel ( $^{\circ}\text{K}$ );  $T_a$  = temperature at distance 'a' from the center of wheat kernel ( $^{\circ}\text{K}$ );  $A_0$  = constant =  $(Ta_2 - Ta_1)/(t_2 - t_1)$ , where  $Ta_1$  = temperature at time  $t_1$  ( $^{\circ}\text{K}$ );  $Ta_2$  = temperature at time  $t_2$  ( $^{\circ}\text{K}$ ). In this experiment, radius of wheat kernel measured was 0.001 m. The experiment was replicated three times.

### 2.2.5. Data analysis

Linear and/or nonlinear models were fit to external and internal grain temperatures as well as temperatures measured by infrared thermometer for all treatment combinations using TableCurve 2D<sup>®</sup> software (Jandel Scientific, San Rafael, CA, USA). Non-linear model  $y = a + bx^{0.5}$  was fit to the grain temperatures recorded over time (Fig 2.1). Linear model  $y = a + bx$  was fit to grain external temperatures (measured by infrared thermometer and thermocouple) vs. internal temperature (Fig 2.2).

All possible pair-wise comparisons were made by comparing individual models to a pooled model (Draper and Smith, 1981). Models were considered to be significantly different ( $P < 0.05$ ) if the  $F$ -test showed the pooled model to be different from the individual models. The  $F$ -test statistic used is shown below (Draper and Smith, 1981).

$$F = A/B$$

where,  $A$  = [Pooled error sum of squares - (Error sum of squares of 1<sup>st</sup> model + Error sum of squares of 2<sup>nd</sup> model)]/Numerator degrees of freedom (df);  $B$  = [Error sum of squares of 1<sup>st</sup> model + Error sum of squares of 2<sup>nd</sup> model]/Denominator df.

The numerator and denominator df were calculated as follows:

Numerator df = Pooled error degrees of freedom - [Error df of 1<sup>st</sup> model + Error df of 2<sup>nd</sup> model];

Denominator df = Error df of 1<sup>st</sup> model + Error df of 2<sup>nd</sup> model.

In addition, data over each exposure time for 16 and 18% moisture at each of the three holding times was averaged for each replicate. These data were subjected to three-way analysis of variance (ANOVA) to determine significant differences in mean temperatures attained between 16 and 18% tempered wheat, among the three holding times, and the three infrared exposures (SAS Institute, 2008). The data for thermal conductivity and diffusivity determined at

2 min exposures for 11% (control), 16% and 18% moisture wheat tempered for 24 h, was subjected to one-way ANOVA to determine significant differences among grain moistures (SAS Institute, 2008).

### 2.3. Results

As the wheat grains were exposed to catalytic infrared radiation, both the internal and external temperature of the kernels increased. Table 2.1 and 2.2 shows the mean internal and external temperatures attained by wheat as measured by thermocouples  $T_1$  and  $T_2$ , and the infrared thermometer. As expected the temperatures attained by wheat were greater at longer exposure times. The internal grain temperature of the kernels was relatively lower when compared to the external surface temperature. From Table 2.3, 2.4 and 2.5, three-way ANOVA results showed that grain moisture ( $F = 123.09$ ;  $df = 1, 36$ ;  $P < 0.0001$ ), tempering time ( $F = 8.95$ ;  $df = 2, 36$ ;  $P = 0.0007$ ) and exposure time ( $F = 513.84$ ;  $df = 2, 36$ ;  $P < 0.0001$ ) had significant effect on mean surface temperatures measured using infrared thermometer. Similarly grain moisture ( $F = 50.38$ ;  $df = 1, 36$ ;  $P < 0.0001$ ), tempering time ( $F = 21.19$ ;  $df = 2, 36$ ;  $P < 0.0001$ ) and exposure time ( $F = 277.73$ ;  $df = 2, 36$ ;  $P < 0.0001$ ) had significant effect on mean surface temperatures measured using thermocouple. Grain moisture ( $F = 0.02$ ;  $df = 1, 36$ ;  $P = 0.894$ ) and tempering time ( $F = 0.12$ ;  $df = 2, 36$ ;  $P = 0.885$ ) did not have significant effect, while exposure time ( $F = 165.38$ ;  $df = 2, 36$ ;  $P < 0.0001$ ) had significant effect on grain internal temperatures measured using thermocouple.

Representative temperature profiles for internal and external temperature of wheat (11% [control], 16% and 18% moisture) exposed for 1, 1.5 and 2 min of infrared radiation is illustrated in Fig. 2.1. Non-linear model  $y = a + bx^{0.5}$  was fit to the internal and external temperatures of grain, over time, measured by infrared thermometer and thermocouples. The non-linear model  $R^2$  values were 0.97 and greater which indicates good fit of the model to the data. The parameter estimates are shown in Table 2.6 and 2.7. The linear model  $y = a + bx$  fit to external and internal grain temperatures over time is illustrated in Fig. 2.2. The model described the data well ( $R^2 \geq 0.99$ ).

Model comparison of temperature profiles between infrared exposure times (1, 1.5, and 2 min) showed significant differences ( $F = 0.5$  to  $1.7$ ;  $df = 2, 118$ ;  $P < 0.00001$ ). Similarly, pair-wise model comparisons between internal and external grain temperatures over time indicated

that there were significant differences among majority of the pairs ( $F = 0.9$  to  $3.8$ ;  $df = 2, 118$ ;  $P < 0.00001$ ).

The average thermal conductivity of wheat at 11%, 16% and 18% moisture for 1, 1.5 and 2 min exposure were found to be in the range of  $9.77$  to  $10.03 \times 10^{-2}$  W/mK. The average thermal diffusivity at 11%, 16% and 18% moisture for 1, 1.5 and 2 min exposure were found to be in the range of  $4.83$  to  $5.03 \times 10^{-3}$  m<sup>2</sup>/h. Among treatment combinations, there was no significant effect of grain moisture on thermal conductivity ( $F = 0.13$ ;  $df = 2, 18$ ;  $P = 0.883$ ) and diffusivity ( $F = 0.60$ ;  $df = 2, 18$ ;  $P = 0.559$ ).

## 2.4. Discussion

Of the factors examined, the duration of infrared exposure greatly influenced the mean temperatures attained, both in the interior and on the surface of wheat kernels. As the exposure time increased the mean grain temperature also increased. The internal grain temperature being considerably lower by about 20°C than external grain temperature during infrared heating can be explained by the fact that infrared energy has low penetrative power. This makes infrared radiation suitable for several surface treatment applications like sterilization, drying, blanching and dehydration (Sakai and Hanzawa, 1994; Rosenthal et al., 1996).

The mean temperature attained was affected by moisture content of the wheat. The mean surface temperature of wheat was slightly lower by about 4°C for higher moisture content of wheat i.e. 18% when compared to the control (11%) and 16% moisture samples. This could be due to a lower evaporative cooling effect in the low moisture wheat than the high moisture wheat, under constant radiation heat supply (Pan et al., 2008). The pattern of increase in grain temperatures during infrared exposures of 1, 1.5 and 2 minutes were shown by the fitted regression curves. The linear regression models fit to the external vs. internal grain temperature profiles with high  $R^2$  values ( $\geq 98\%$ ) indicate that the temperatures followed a similar pattern of increase over time. The corresponding parameter estimates give us the relationship between the two temperatures, and this helps in predicting the internal temperature when the external surface temperature is known and vice versa.

The model comparison results showed differences among temperature profiles of 1, 1.5 and 2 min infrared exposure. This could be due to differences in initial temperature of grain during infrared exposure. The experiment was conducted over a period of several days. While

exposing the grains to infrared radiation, the average initial ambient temperature (measured using HOBO<sup>®</sup> data logger [Onset Computer Corporation, Bourne, MA]) was 19.6°C. However an increase in ambient temperature was observed, up to 29°C, due to heat generated from the infrared emitter as it ran continuously during experiments. A steel pan was used to expose the wheat grains in several lots. The pan became hot after multiple exposures, and it also reflected radiative heat which increased the initial temperature of the grain. Also, the temperatures increased steadily and faster during the first 1 min, whereas after that the temperature increase was relatively slower. A range of variation in initial temperatures for each treatment combination is shown in Table 2.9.

According to ASABE standards (2006), thermal conductivity of wheat ranges from 0.12 to 0.16 W/mK, and thermal diffusivity of wheat ranges from 3.3 to  $4.1 \times 10^{-4}$  m<sup>2</sup>/h. Thermal conductivity values obtained in this study were slightly lower (0.10 W/mK) and thermal diffusivity values were higher (0.01 m<sup>2</sup>/h) when compared to the standard values. These differences could be due to the fact that the standard conductivity and diffusivity values could be determined using conventional source of heating (by conduction, convection and radiation) unlike the catalytic infrared radiation used in this study. The lesser conductivity shows that flameless catalytic infrared radiation has less penetrating power and hence is mostly used for surface heating of food materials. The higher diffusivity shows that infrared radiation can heat biological materials rapidly that conventional heating methods. In conclusion, this study gave an insight into the pattern of temperature changes in wheat, both internally and externally, during infrared heating. A mathematical relationship established between the internal and external temperatures helps in developing calibration curves for the same, which in turn helps in predicting the temperatures. This would save valuable time, and help optimize infrared process temperatures and exposure times without adversely affecting product quality.

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**Table 2.1 Mean temperature attained by wheat tempered to 16% and 18% moisture for 8, 16 and 24 h on infrared (IR) exposure.**

Moisture (%)	Tempering time (h)	IR exposure time (min)	Mean temperature $\pm$ SE <sup>a</sup> (°C)		
			External temperature	External temperature	Internal temperature
			(IR thermometer)	(T <sub>1</sub> )	(T <sub>2</sub> )
16	8	1.0	85.3 $\pm$ 2.1	77.4 $\pm$ 2.0	67.3 $\pm$ 2.2
		1.5	101.2 $\pm$ 2.1	93.7 $\pm$ 2.3	80.0 $\pm$ 2.1
		2.0	110.4 $\pm$ 1.9	98.3 $\pm$ 2.1	93.2 $\pm$ 2.1
	16	1.0	92.1 $\pm$ 2.3	83.1 $\pm$ 2.3	75.2 $\pm$ 2.2
		1.5	104.2 $\pm$ 2.2	101.2 $\pm$ 2.3	85.6 $\pm$ 2.2
		2.0	110.2 $\pm$ 2.7	91.2 $\pm$ 2.8	81.3 $\pm$ 0.0
	24	1.0	84.4 $\pm$ 2.0	80.7 $\pm$ 2.2	76.4 $\pm$ 2.2
		1.5	98.8 $\pm$ 1.9	100.5 $\pm$ 2.3	85.6 $\pm$ 2.2
		2.0	108.0 $\pm$ 1.8	91.5 $\pm$ 1.9	81.6 $\pm$ 2.0
18	8	1.0	83.7 $\pm$ 2.0	80.7 $\pm$ 2.2	75.4 $\pm$ 2.2
		1.5	95.1 $\pm$ 2.1	100.3 $\pm$ 2.3	85.6 $\pm$ 2.2
		2.0	105.8 $\pm$ 2.2	91.6 $\pm$ 2.0	82.5 $\pm$ 2.0
	16	1.0	83.3 $\pm$ 2.0	74.0 $\pm$ 2.0	75.2 $\pm$ 2.2
		1.5	90.9 $\pm$ 2.5	98.3 $\pm$ 2.3	85.6 $\pm$ 2.2
		2.0	105.2 $\pm$ 1.8	91.6 $\pm$ 2.0	83.6 $\pm$ 2.0
	24	1.0	81.3 $\pm$ 2.0	74.0 $\pm$ 2.0	69.8 $\pm$ 2.1
		1.5	91.3 $\pm$ 2.2	101.4 $\pm$ 2.3	85.6 $\pm$ 2.2
		2.0	101.5 $\pm$ 1.9	91.5 $\pm$ 1.9	82.4 $\pm$ 2.0

<sup>a</sup> Values are based on  $n = 3$  replications.

**Table 2.2 Mean temperature attained by untempered wheat (11% mc) on infrared exposure.**

IR exposure time (min)	Mean temperature $\pm$ SE <sup>a</sup> (°C)		
	External temperature (IR thermometer)	External temperature (T <sub>1</sub> )	Internal temperature (T <sub>2</sub> )
1.0	99.8 $\pm$ 2.4	79.5 $\pm$ 2.4	68.9 $\pm$ 2.0
1.5	104.0 $\pm$ 2.5	93.3 $\pm$ 2.5	81.0 $\pm$ 2.2
2.0	109.2 $\pm$ 2.1	99.3 $\pm$ 2.1	94.3 $\pm$ 2.1

<sup>a</sup> Values are based on  $n = 3$  replications.

**Table 2.3 Three-way ANOVA statistics for mean surface temperature of grain measured using infrared thermometer.**

Source	df	Mean Square	<i>F</i> value	<i>P</i> value
Grain moisture	1,36	510.79	123.09	<0.0001*
Tempering time	2,36	37.13	8.95	0.0007*
Exposure time	2,36	2132.37	513.84	<0.0001*
Grain moisture × Tempering time	2,36	26.11	6.29	0.0045*
Grain moisture × Exposure time	2,36	23.63	5.69	0.0071*
Tempering time × Exposure time	4,36	9.46	2.28	0.0796
Grain moisture × Tempering time × Exposure time	4,36	10.55	2.54	0.0564

\* Significant ( $P < 0.05$ )

**Table 2.4 Three-way ANOVA statistics for mean surface temperature of grain measured using thermocouple.**

Source	df	Mean Square	<i>F</i> value	<i>P</i> value
Grain moisture	1,36	218.11	50.38	<0.0001*
Tempering time	2, 36	91.75	21.19	<0.0001*
Exposure time	2, 36	1202.37	277.73	<0.0001*
Grain moisture × Tempering time	2, 36	21.26	4.91	0.0130*
Grain moisture × Exposure time	2, 36	10.75	2.48	0.0976
Tempering time × Exposure time	4, 36	48.56	11.22	<0.0001*
Grain moisture × Tempering time × Exposure time	4, 36	54.32	12.55	<0.0001*

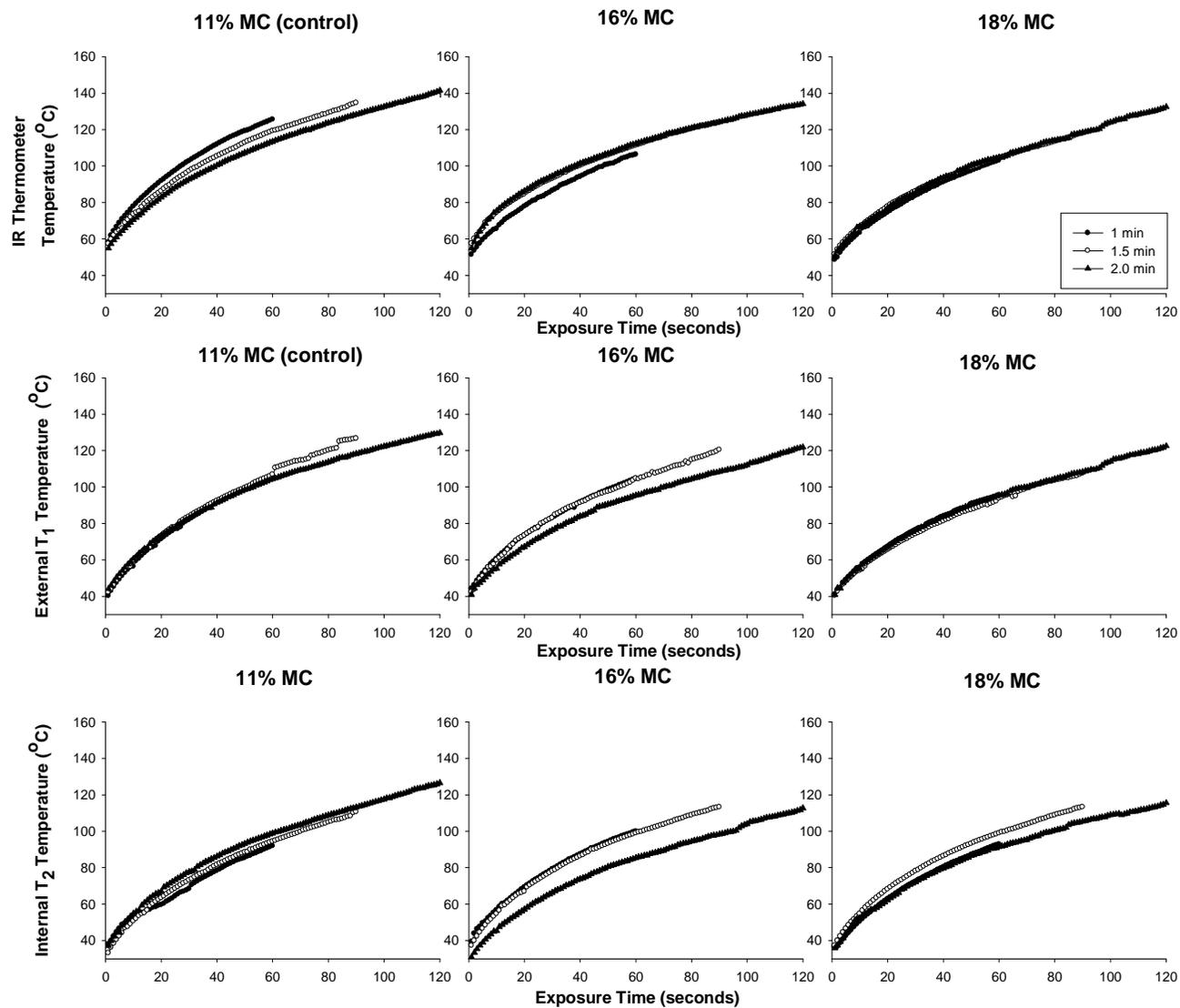
\* Significant ( $P < 0.05$ )

**Table 2.5 Three-way ANOVA statistics for mean internal temperature of grain at 2 mm depth measured using thermocouple.**

Source	df	Mean Square	<i>F</i> value	<i>P</i> value
Grain moisture	1, 36	0.08	0.02	0.8942
Tempering time	2, 36	0.55	0.12	0.8850
Exposure time	2, 36	741.14	165.38	<0.0001*
Grain moisture × Tempering time	2, 36	0.50	0.11	0.8952
Grain moisture × Exposure time	2, 36	20.22	4.51	0.0178*
Tempering time × Exposure time	4, 36	49.04	10.94	<0.0001*
Grain moisture × Tempering time × Exposure time	4, 36	108.35	24.18	<0.0001*

\* Significant ( $P < 0.05$ )

**Figure 2.1 Temperature profiles for external temperature (IR thermometer,  $T_1$ ) and internal temperature ( $T_2$ ) of wheat (11%, 16% and 18%) tempered for 24 h and exposed for 1.0, 1.5 and 2.0 minutes to infrared radiation.**



**Table 2.6 Regression estimates of external temperature (IR thermometer, T<sub>1</sub>) and internal temperature (T<sub>2</sub>) of wheat (16% and 18% mc) tempered for 24 h and exposed for 1.0, 1.5 and 2.0 minutes to infrared radiation.**

Temperature	Grain moisture (%)	IR exposure time (min)	Parameter estimates <sup>a</sup>			
			Intercept	Slope	R <sup>2</sup>	
IR thermometer	16	1.0	39.8 ± 0.3	8.5 ± 0.1	0.99	
		1.5	48.3 ± 0.3	8.1 ± 0.0	0.99	
		2.0	51.3 ± 0.3	7.7 ± 0.0	0.99	
	18	1.0	36.9 ± 0.3	8.5 ± 0.1	0.99	
		1.5	33.0 ± 1.7	9.2 ± 0.2	0.99	
		2.0	40.6 ± 0.3	8.3 ± 0.0	0.99	
	External T <sub>1</sub>	16	1.0	31.4 ± 0.2	9.4 ± 0.0	0.99
			1.5	39.5 ± 1.3	9.5 ± 0.2	0.97
			2.0	30.5 ± 0.3	8.3 ± 0.0	0.99
18		1.0	29.8 ± 0.3	8.5 ± 0.1	0.99	
		1.5	39.5 ± 1.3	9.5 ± 0.2	0.97	
		2.0	30.5 ± 0.3	8.3 ± 0.0	0.99	
Internal T <sub>2</sub>	16	1.0	24.9 ± 0.3	9.6 ± 3.1	0.99	
		1.5	26.4 ± 0.3	9.3 ± 0.0	0.99	
		2.0	19.6 ± 0.6	8.4 ± 0.1	0.99	
	18	1.0	22.3 ± 0.3	9.1 ± 0.1	0.99	
		1.5	26.4 ± 0.3	9.3 ± 0.0	0.99	
		2.0	19.6 ± 0.6	8.4 ± 0.1	0.99	

<sup>a</sup> Values are means ± SE.

**Table 2.7 Regression estimates of external temperature (IR thermometer, T<sub>1</sub>) and internal temperature (T<sub>2</sub>) of untempered wheat (11% mc) tempered for 24 h and exposed for 1.0, 1.5 and 2.0 minutes to infrared radiation.**

Temperature	IR exposure time (min)	Parameter estimates <sup>a</sup>		
		Intercept	Slope	R <sup>2</sup>
IR thermometer	1.0	45.6 ± 0.2	10.4 ± 0.0	0.99
	1.5	36.6 ± 1.4	10.6 ± 0.2	0.97
	2.0	43.3 ± 0.2	9.0 ± 0.0	0.99
External T <sub>1</sub>	1.0	26.2 ± 0.4	10.2 ± 0.1	0.99
	1.5	26.3 ± 0.4	10.5 ± 0.1	0.99
	2.0	34.0 ± 0.3	8.9 ± 0.0	0.99
Internal T <sub>2</sub>	1.0	25.4 ± 0.8	8.3 ± 0.1	0.98
	1.5	21.9 ± 0.2	9.3 ± 0.0	0.99
	2.0	27.4 ± 0.3	9.1 ± 0.0	0.99

<sup>a</sup> Values are means ± SE.

**Table 2.8 Range of initial external and internal temperatures of wheat exposed to infrared radiation, measured by infrared thermometer, and thermocouples.**

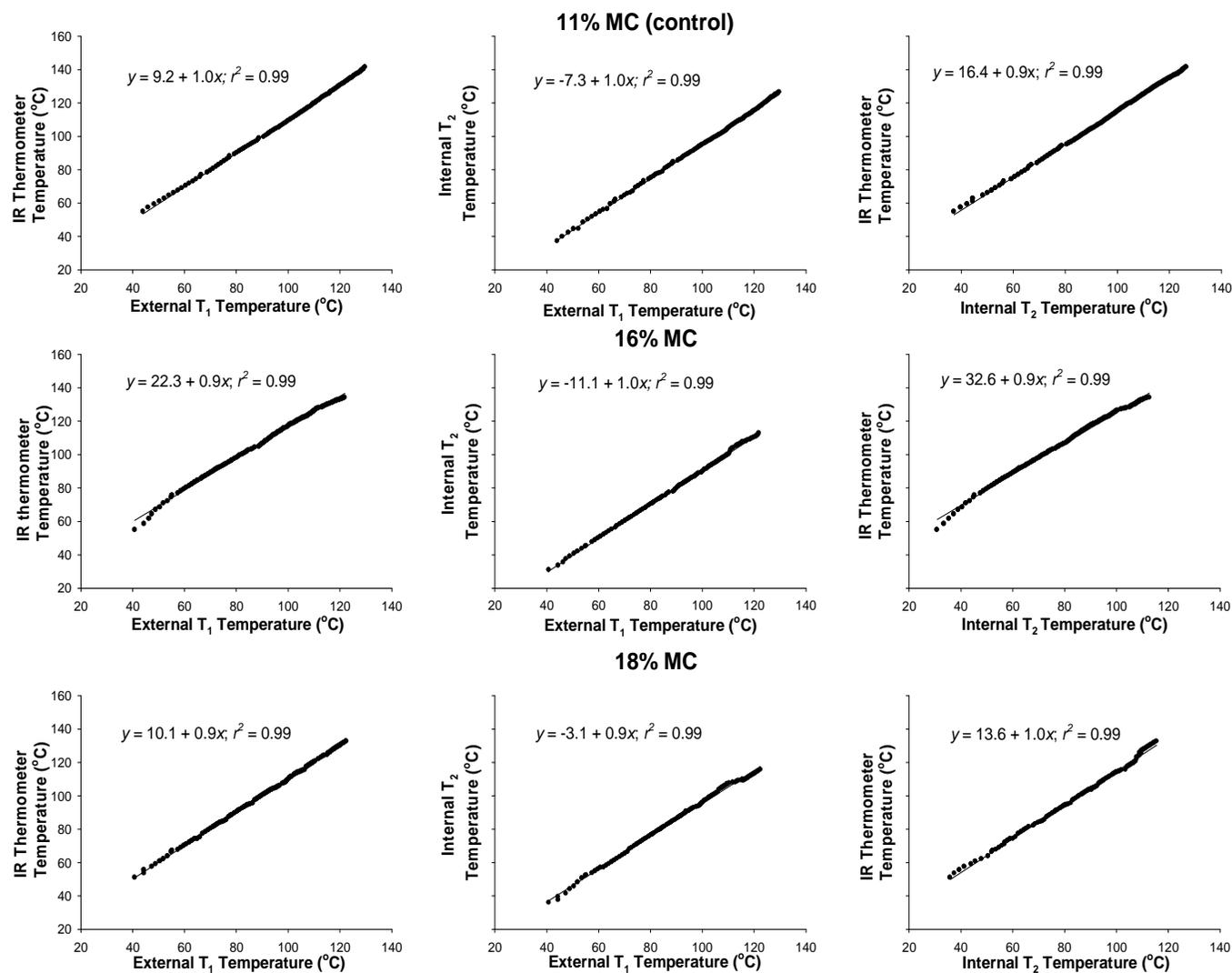
Temperature measurement	IR exposure time (min)	Range of initial temperatures (°C)
Surface temperature (infrared thermometer)	1.0	42.3 - 58.0
	1.5	36.4 - 48.5
	2.0	45.6 - 61.4
External temperature (thermocouple T <sub>1</sub> )	1.0	40.3 - 45.2
	1.5	42.5 - 51.3
	2.0	42.3 - 55.2
Internal temperature (thermocouple T <sub>2</sub> )	1.0	32.6 - 44.4
	1.5	33.4 - 44.6
	2.0	40.2 - 46.7

**Table 2.9 Thermal conductivity and diffusivity of wheat (11% [control], 16% and 18% mc) tempered for 24 h and exposed to infrared radiation for 1, 1.5 and 2 min.**

Grain moisture (%)	IR exposure time (min)	Mean $\pm$ SE <sup>a</sup>	
		Thermal conductivity, $\times 10^{-2}$ (W/mK)	Thermal diffusivity, $\times 10^{-3}$ (m <sup>2</sup> /h)
11 (control)	1.0	9.70 $\pm$ 0.06	5.03 $\pm$ 0.03
	1.5	9.90 $\pm$ 0.06	4.90 $\pm$ 0.00
	2.0	10.03 $\pm$ 0.07	4.83 $\pm$ 0.03
16	1.0	9.73 $\pm$ 0.07	5.03 $\pm$ 0.03
	1.5	9.90 $\pm$ 0.06	4.90 $\pm$ 0.00
	2.0	9.97 $\pm$ 0.07	4.87 $\pm$ 0.03
18	1.0	9.77 $\pm$ 0.03	5.03 $\pm$ 0.03
	1.5	9.97 $\pm$ 0.03	4.90 $\pm$ 0.00
	2.0	9.93 $\pm$ 0.03	4.90 $\pm$ 0.00

<sup>a</sup> Values are based on  $n = 3$  replications.

**Figure 2.2 Linear regression models fit to external temperatures (IR thermometer,  $T_1$ ) and internal temperature ( $T_2$ ) of wheat exposed to 1.0, 1.5 and 2.0 minutes of infrared radiation.**



**Chapter 3 - Effects of infrared heating of tempered wheat on  
microbial loads of whole wheat and milled flour**

## Abstract

Reducing microbial loads on wheat prior to milling may reduce microbial loads in the milled flour. The efficacy of infrared radiation in reducing aerobic bacterial and fungal loads on untempered and tempered whole wheat and flour was investigated. Organic, hard red winter wheat of 11% moisture (wet basis) tempered to 16 and 18% mc and held for 8, 16, and 24 h at 28°C and 65% RH was exposed to a bench-top flameless catalytic infrared emitter. Wheat (113.5 g) was exposed to infrared radiation at a distance of 8 cm from the emitter for 1, 1.5, and 2 min. On untempered wheat that was not exposed to infrared radiation, the bacterial and fungal counts were  $6.5 \times 10^4$  and  $4.1 \times 10^3$  CFU/g, respectively. Wheat tempered similarly but unexposed to infrared radiation (control treatment) had  $4.3$  to  $5.9 \times 10^4$  and  $2.9$  to  $3.7 \times 10^3$  CFU/g, respectively. Across all moisture contents and holding times, infrared exposures of 1, 1.5, and 2 min, on average, reduced bacterial counts by 49.9, 97.3 and 98.7% and fungal loads by 53.7, 96.4 and 97.8%, respectively, relative to tempered and unexposed wheat. Linear regression models were fit to the logarithmic decrease of bacterial and fungal counts, and the mean *D*-values (infrared exposure time required for 1 log reduction of microbial load) for bacterial and fungal counts were determined. Model comparison of logarithmic decrease of bacterial and fungal counts over exposure time between the two moisture contents (16 and 18%) and three holding times (8, 16, 24 h) showed no significant differences in whole wheat and flour. Despite these lack of differences, infrared radiation had pronounced antimicrobial effect on wheat during processing and helps reduce microbial loads in the milled flour.

### 3.1. Introduction

Sterilization of stored grains or grain products has been an intensive research area because of increasing concerns about food safety (Jun et al., 2003). Stored wheat can be contaminated with fungi and other microorganisms that produce off odors and change the chemical composition of wheat and make it unsuitable as food (Cornell and Hoveling, 1998). Enteric pathogens such as *Salmonella* spp. may occur in wheat due to fecal contamination from birds and rodents prior to harvest through storage. Spore-forming pathogens such as *Bacillus cereus* and *Clostridium botulinum* may be present due to soil contamination (Manthey et al., 2003). Toxin-producing fungi like *Aspergillus flavus*, *Alternaria* spp., and *Fusarium* spp. are also found to occur in stored wheat when there is higher water activity (Saleh et al., 1988). Chlorinated water (600 to 700 mg/L) has been used to control mold and bacterial loads in wheat milling, and is commonly used to disinfect wheat grain during tempering (EPRI, 2000). However, there are growing safety concerns about the use of chemicals like chlorine since it could react with organic residues to form potentially carcinogenic reaction products (Beltran et al., 2005). Hence, there is a need for, and interest in, developing practical and effective antimicrobial treatments for the inactivation of pathogenic microorganisms on cereal grains.

Infrared heating can be used to inactivate bacteria, spores, and fungi in both solid and liquid foods (Krishnamurthy et al., 2008). Application of far infrared radiation (FIR) to food pasteurization processes has been studied by several researchers (Hashimoto et al., 1993; Sakai and Hanzawa, 1994; Rosenthal et al., 1996). Hamanaka et al. (2000) examined the disinfection effect of infrared radiation on bacterial count on wheat exposed to a 2 kW infrared heater for 60 sec decreased the bacterial count from  $5.7 \times 10^4$  to  $0.73 \times 10^3$  CFU/g. Uchino et al. (2000) exposed wheat to 2 kW infrared radiation for 28, 37, and 63 sec and obtained microbial load reduction. However, sterilization effects of infrared treatment for extended exposure times have not been studied. These studies used infrared lamps or gas-fired infrared emitters for treatments.

Khamis et al. (2010, 2011a,b) exposed wheat to flameless catalytic infrared radiation for 60 sec at a distance of 8 cm from emitter and obtained complete mortality of three major stored product insects without adversely affecting wheat quality (Khamis et al., 2011c). Khamis et al. (2010, 2011a,b) exposed wheat of 12% moisture (wet basis, wb) to infrared radiation and then tempered it to 16% moisture which was used for milling. In a study by Macaluso (2007) flameless catalytic infrared radiation was used to inactivate *Salmonella* spp., in processed

almonds after a 30-90 sec exposure. We hypothesized that flameless catalytic infrared radiation can be used effectively to reduce microbial loads (bacteria and fungi) of tempered wheat without adversely affecting wheat quality.

In this study the efficacy of flameless catalytic infrared radiation in reducing microbial loads (aerobic bacteria and fungi) on whole wheat and flour was investigated and evaluated at different moisture contents and tempering times.

## **3.2. Materials and Methods**

### ***3.2.1. Tempering of wheat samples***

Uninfested, organic hard red winter wheat of 11% moisture (wb) was procured from Heartland Mills, Marienthal, KS, USA. About 200 g of the wheat was placed in sterile polythene Ziploc bag (Fisher Scientific, Pittsburg, PA), and distilled water was added to it to increase the grain moisture to 16 or 18%. The amount of water added was determined using the formula below (Harris and Lindblad, 1978):

$$\text{Amount of water added (in ml)} = \left[ \frac{100 - \text{Initial moisture}}{100 - \text{Final moisture}} - 1 \right] \times \text{Amount of wheat (in g)}$$

After adding the required amount of water, the wheat bag was shaken vigorously for uniform distribution of moisture and was allowed to temper for 8, 16 or 24 h in a growth chamber (Percival, Perry, IA, USA) at 28°C and 65% RH. Tempered wheat unexposed to infrared radiation served as the control treatment. A total of 140 such bags were prepared for the various treatment combinations (Table 3.1).

### ***3.2.2. Infrared exposure***

A bench-top flameless catalytic infrared emitter (Catalytic Drying Technologies LLC, Independence, KS, USA) was used for infrared treatment of wheat. The unit has a circular infrared emitting surface of 0.0613 m<sup>2</sup>, and is fuelled by propane gas at 28 cm of water column pressure. The total heat energy output of the unit is 1.5 kW/h (5000 BTU/h). A grain quantity of 113.5 g was exposed at 8 cm from the emitter surface in a single layer thickness. Wheat was placed in a steel pan of 28 cm diameter and exposed for 1, 1.5 or 2 min to infrared radiation.

### **3.2.3. Milling**

The tempered wheat samples (except control, and untempered-infrared treated) were milled in a Quadrumat Senior mill (Brabender GmbH and Co., Duisburg, Germany) in the Department of Grain Science and Industry, Kansas State University, Manhattan, KS. Before milling, the sieves were cleaned and rolls were wiped with 95% ethanol. The machine was warmed for 30 min by milling wheat. The feed rate was set at 150 g of wheat/min. After milling, the break and reduction flours were combined. The ambient temperature and relative humidity during milling were 18°C and 40%, respectively. Each treatment combination was replicated five times.

### **3.2.4. Microbial plating**

Microbial analyses were initiated by aseptically weighing a 10 g sample (whole wheat or flour) and suspending in 100 ml of distilled water. Peptone 0.1% dilution blanks were prepared, and serial dilutions from  $10^{-1}$  to  $10^{-4}$  levels were made for the samples (AACC Method 42-11; 42-50). For total bacterial counts, 1 ml each of the dilutions  $10^{-4}$ ,  $10^{-3}$  and  $10^{-2}$  were plated on Trypticase Soy Broth (TSB) nutrient agar using the surface spread method. Using the same procedure, for fungal counts, 1 ml each of the dilutions  $10^{-3}$ ,  $10^{-2}$  and  $10^{-1}$  were plated on Potato Dextrose Agar (PDA) medium. Chloramphenicol (75 mg/L) was added to PDA to prevent the growth of bacteria. Three sub-replicates were plated for each replicate of all the samples and the plates were incubated at 25°C for 5 d. Enumeration of total bacterial and fungal loads was done by colony count method and the results were expressed in colony forming units per gram (CFU/g).

### **3.2.5. Statistical analysis**

A completely randomized design was used for all experiments. Before statistical analysis, the total bacterial and fungal counts of whole wheat and flour treated with infrared radiation were transformed to  $\log_{10}(x+1)$  scale to normalize heteroscedastic treatment variances and due to zero counts obtained in the 2 min infrared exposure data.

The logarithmic decrease of total bacteria and fungal counts in untempered and tempered wheat as a function of infrared exposure time (0, 1, 1.5, and 2 min) was described by linear regression (SAS Institute, 2008),  $y = a + bx$ , where  $y$  = total bacterial or mold count ( $\log_{10}$  CFU/g),  $x$  = infrared exposure time in min, and  $a$  and  $b$  are estimated parameters from the regression. The inverse of the slope gave a  $D$ -value which represented the number of minutes of

infrared exposure required for a 1-log reduction of bacterial or fungal counts. Linear regressions were also fit to data on bacterial and fungal counts in flour milled from tempered wheat held for various time periods.

All possible two way comparisons of linear regressions for tempered wheat were made by comparing individual models to a pooled model (Draper and Smith, 1981). Models were considered to be significantly different ( $P < 0.05$ ) if the  $F$ -test showed the pooled model to be different from the individual models.

### 3.3. Results

The mean bacterial and fungal counts of untempered wheat are shown in Table 3.2 and that of tempered wheat and flour from infrared-treated wheat is shown in Table 3.3. The untempered wheat which was not exposed to infrared radiation had the highest mean total bacterial ( $6.47 \times 10^4$  CFU/g) and mold count ( $4.13 \times 10^3$  CFU/g). These loads decreased in a logarithmic fashion as a function of infrared exposure time (Fig 3.1). The time required for 1-log reduction of bacterial counts on untempered wheat was 1 min whereas similar time for reduction of mold counts was 1.2 min. Generally, bacterial counts were consistently higher than fungal counts in both wheat and, irrespective of tempering moisture, the microbial loads of samples in all treatment combinations decreased consistently as the exposure time increased. Control wheat (11% moisture) that was not tempered with distilled water, on exposure to infrared radiation, showed a considerable decrease in bacterial loads. Bacterial loads decreased by 53.6, 97.0, and 98.7% after exposure to infrared radiation for 1, 1.5 and 2 min, respectively. Similarly, fungal loads decreased by 54.7, 95.8 and 97.1% for 1, 1.5 and 2 min of infrared exposure, respectively. Tempered wheat that was not exposed to infrared radiation had lower microbial loads when compared to the untempered wheat (20.8% less bacterial and 19.3% less fungal loads). A slightly higher decrease in microbial load was obtained in the case of tempered wheat exposed to infrared radiation. Bacterial loads decreased by 46.2, 97.6 and 98.6% after 1, 1.5 and 2 min of infrared exposure, respectively, and fungal loads decreased by 52.6, 96.9 and 98.5% for 1, 1.5 and 2 min of infrared exposure, respectively. Microbial loads in flour were consistently lower compared to whole wheat in all treatment combinations.

The logarithmic reduction of total bacterial and fungal counts of whole wheat and flour for the various treatment combinations as a function of infrared exposure time is illustrated in

Fig 3.1 only for samples held for 24 h. Regression estimates for  $a$  and  $b$  parameters for all treatment combinations are shown in Table 3.4 and Table 3.5. The  $R^2$  values ranged from 0.61 to 0.86 for whole wheat and 0.64 to 0.91 for flour indicating that 61 to 91% of the decrease in bacterial and fungal counts was predicted by the linear regression model. In whole wheat and flour, the logarithmic reduction in total bacterial counts was more rapid than the logarithmic reduction in fungal counts as indicated by the steeper slope values in bacterial count than in mold counts.

In the case of tempered wheat, pairwise comparisons of fitted regression models between the treatment combinations indicated that there were no significant differences in logarithmic decrease of bacterial counts ( $F$ , range among treatment combinations = 0.04 to 3.70;  $df = 2, 36$  (for each pair being compared);  $P$ , range = 0.18 to 0.95) and fungal counts ( $F = 0.10$  to 4.20;  $df = 2, 36$ ;  $P = 0.20$  to 0.83) in whole wheat. Similarly, there were no significant differences in logarithmic decrease of bacterial counts ( $F = 0.03$  to 4.40;  $df = 2, 36$ ;  $P = 0.14$  to 0.89) and fungal counts ( $F = 0.10$  to 5.44;  $df = 2, 36$ ;  $P = 0.10$  to 0.90) in flours milled from infrared exposed tempered wheat.

The time required for 1-log reduction ( $D$ -value) was consistently lower for total bacterial counts than mold counts on both wheat and flour, and ranged from 0.85-1.20 for wheat and 0.86-1.22 for flour. These values indicated rapid inactivation of bacteria and fungi on whole wheat exposed to infrared radiation.

### 3.4. Discussion

Food components and microorganisms readily absorb infrared radiation, especially in the far infrared region (Sakai and Hanzawa, 1994; Fasina and Thomas, 2001; Krishnamurthy et al., 2008; Erdogdu et al., 2010). When the infrared energy impinges upon a surface, it may induce changes in the vibrational state of molecules, and dissipation of infrared energy as heat results in rapid increases in surface temperature (Sakai and Hanzawa, 1994; Fasina and Thomas, 2001; Krishnamurthy et al., 2008). Since the heat is conducted to the interior by conduction and that the food products have lower thermal conductivity ( $<0.6 \text{ W/m}^2\text{K}$ ), the rate of heat transfer through the food products under infrared radiation is rather slow. Therefore, intense heat might accumulate on the surface causing the surface temperature to increase rapidly (Erdogdu et al., 2010). If the infrared exposure time is properly controlled, surface temperature can be

preferentially raised to a degree where target pathogenic microorganism can be inactivated without substantially increasing the interior temperature (Fasina and Thomas, 2001; Huang, 2004).

In our study, microbial loads of whole wheat that was untempered and tempered decreased in a logarithmic fashion after exposure to infrared radiation. Greater reductions were observed after a 2 min exposure. Slightly higher microbial reduction was observed in wheat tempered to 18% moisture when compared with untempered 11% moisture wheat. However, in tempered wheat held at different time periods, the logarithmic decrease in bacterial or fungal counts was similar irrespective of initial moisture content or holding time. Hamanaka et al. (2007) reported increased microbicidal effect of infrared radiation on wheat at higher moisture content (24% wb) and little microbicidal effect in low moisture content (14% wb) wheat. As the majority of microbial population is on the surface of the wheat, milling process itself reduces surface microbial load to some extent. Hence the bacterial and fungal counts of flour were consistently lesser than that of whole wheat.

Sterilization of wheat surface was investigated by Hamanaka et al. (2000). Surface temperature increased rapidly as infrared rays directly heated the surface without the need of any conductors. Therefore, irradiating powers of 0.5, 1.0, 1.5 and 2.0 kW resulted in 60, 80, 125 and 195°C inside the experimental device, and 45, 65, 95, and 120°C on the surface of wheat stack, obtaining 0.83, 1.14, 1.18, and 1.90 log<sub>10</sub> CFU/g total bacteria after a 60 sec treatment, respectively.

Inactivation of microorganisms by infrared heating may include inactivation mechanism similar to that of ultra-violet light (DNA damage) and microwave heating (induction heating) in addition to thermal effect, as infrared wavelength is located between ultraviolet and microwave on the electromagnetic spectrum (Hamanaka et al., 2000) Thermal inactivation can damage DNA, RNA, ribosome, cell envelope, and proteins in microbial cell. Transmission electron microscopic observation and infrared spectroscopy of infrared treated *Staphylococcus aureus* cells clearly verified cell wall damage, cytoplasmic membrane shrinkage, cellular content leakage and mesosome disintegration (Krishnamurthy, 2006).

In conclusion, this study showed that infrared radiation has a pronounced antimicrobial effect on wheat and milling of infrared exposed wheat may enhance food safety. However, a set of experiments done in our laboratory showed that infrared radiation of tempered wheat had

adverse effects on wheat flour quality and functionality (Deliephan, 2013). Taking this into account, the use of infrared radiation on tempered wheat may not be recommended. There was a logarithmic decrease in bacterial and fungal counts in untempered wheat (11% mc) when exposed to infrared radiation (Table 3.3), and the values were comparable to those observed on tempered wheat (Table 3.2). Khamis et al. (2011c) reported that treatment of untempered wheat for 1 min followed by tempering and milling had no adverse effects on milling yield, flour functionality and end use properties. Therefore, exposure of untempered wheat to infrared radiation may result in reducing microbial loads on wheat and subsequently in flour without adversely affecting wheat quality. However, further study is needed to extend findings by Khamis et al. (2011c) on wheat and flour quality by exposing untempered wheat to 1.5 and 2 min followed by tempering and milling to determine if these times also do not adversely affect the milling yield and flour quality.

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Engineering and Agro Products Processing toward Mechanization and Modernization in Agriculture and Rural Areas.

**Table 3.1 Treatment combinations used for untempered and tempered wheat in this study.**

Treatment	Factors:		
	Grain moisture (%)	Tempering time (h)	Infrared exposure time (min)
Untempered; untreated	11	0	0
Untempered; infrared treated	11	0	1.0, 1.5, 2.0
Tempered; untreated	16, 18	8, 16, 24	0
Tempered; infrared treated	16, 18	8, 16, 24	1.0, 1.5, 2.0

**Table 3.2 Total bacterial and fungal counts in tempered and infrared treated whole wheat and flour milled from treated wheat.**

Grain moisture (%)	Tempering time (h)	Infrared exposure time (min)	Mean $\pm$ SE <sup>a</sup>			
			Total bacterial counts (CFU/g)		Fungal counts (CFU/g)	
			Whole wheat	Flour	Whole wheat	Flour
16	8	0.0	$(4.87 \pm 0.08) \times 10^4$	$(2.53 \pm 0.08) \times 10^4$	$(3.53 \pm 0.25) \times 10^3$	$(1.73 \pm 0.12) \times 10^3$
		1.0	$(2.73 \pm 0.12) \times 10^4$	$(1.40 \pm 0.07) \times 10^4$	$(1.07 \pm 0.12) \times 10^3$	$(4.73 \pm 0.56) \times 10^2$
		1.5	$(1.40 \pm 0.25) \times 10^3$	$(7.13 \pm 0.52) \times 10^2$	$(1.47 \pm 0.08) \times 10^2$	$(7.13 \pm 0.44) \times 10^1$
		2.0	$(7.33 \pm 1.24) \times 10^2$	$(3.93 \pm 1.26) \times 10^2$	$(1.07 \pm 0.27) \times 10^2$	$(5.53 \pm 1.46) \times 10^1$
	16	0.0	$(5.60 \pm 0.22) \times 10^4$	$(2.93 \pm 0.24) \times 10^4$	$(3.73 \pm 0.44) \times 10^3$	$(2.07 \pm 0.22) \times 10^3$
		1.0	$(2.73 \pm 0.07) \times 10^4$	$(1.40 \pm 0.12) \times 10^4$	$(1.60 \pm 0.29) \times 10^3$	$(8.00 \pm 1.51) \times 10^2$
		1.5	$(1.00 \pm 0.24) \times 10^3$	$(3.40 \pm 0.95) \times 10^2$	$(9.33 \pm 1.63) \times 10^1$	$(1.11 \pm 0.64) \times 10^2$
		2.0	$(6.67 \pm 1.49) \times 10^2$	$(3.20 \pm 0.95) \times 10^2$	$(5.33 \pm 1.70) \times 10^1$	$(2.53 \pm 0.76) \times 10^1$
	24	0.0	$(4.33 \pm 0.49) \times 10^4$	$(2.13 \pm 0.33) \times 10^4$	$(3.53 \pm 0.37) \times 10^3$	$(1.77 \pm 0.19) \times 10^3$
		1.0	$(2.20 \pm 0.17) \times 10^4$	$(1.14 \pm 0.13) \times 10^4$	$(1.53 \pm 0.25) \times 10^3$	$(6.47 \pm 1.01) \times 10^2$
		1.5	$(1.07 \pm 0.12) \times 10^3$	$(4.60 \pm 0.78) \times 10^2$	$(8.00 \pm 1.33) \times 10^1$	$(3.47 \pm 0.52) \times 10^1$
		2.0	$(6.67 \pm 1.83) \times 10^2$	$(3.13 \pm 0.59) \times 10^2$	$(6.67 \pm 1.05) \times 10^1$	$(3.00 \pm 0.56) \times 10^1$
18	8	0.0	$(4.40 \pm 0.27) \times 10^4$	$(2.40 \pm 0.29) \times 10^4$	$(3.33 \pm 0.32) \times 10^3$	$(1.60 \pm 0.19) \times 10^3$
		1.0	$(2.73 \pm 0.16) \times 10^4$	$(1.40 \pm 0.12) \times 10^4$	$(1.67 \pm 0.38) \times 10^3$	$(6.93 \pm 1.58) \times 10^2$
		1.5	$(1.27 \pm 0.19) \times 10^3$	$(5.93 \pm 1.32) \times 10^2$	$(1.00 \pm 0.11) \times 10^2$	$(4.53 \pm 0.50) \times 10^1$
		2.0	$(8.00 \pm 1.70) \times 10^2$	$(3.60 \pm 0.90) \times 10^2$	$(6.67 \pm 1.05) \times 10^1$	$(2.87 \pm 0.49) \times 10^1$
	16	0.0	$(5.67 \pm 0.41) \times 10^4$	$(2.93 \pm 0.24) \times 10^4$	$(3.00 \pm 0.33) \times 10^3$	$(1.40 \pm 0.16) \times 10^3$

	1.0	$(2.93 \pm 0.12) \times 10^4$	$(1.47 \pm 0.08) \times 10^4$	$(1.73 \pm 0.19) \times 10^3$	$(7.07 \pm 0.78) \times 10^2$
	1.5	$(1.20 \pm 0.25) \times 10^3$	$(5.67 \pm 1.59) \times 10^2$	$(8.00 \pm 0.82) \times 10^1$	$(3.33 \pm 0.64) \times 10^1$
	2.0	$(7.33 \pm 1.25) \times 10^2$	$(2.80 \pm 0.87) \times 10^2$	$(4.67 \pm 0.82) \times 10^1$	$(2.07 \pm 0.41) \times 10^1$
24	0.0	$(5.87 \pm 0.25) \times 10^4$	$(2.80 \pm 0.20) \times 10^4$	$(2.87 \pm 0.13) \times 10^3$	$(1.40 \pm 0.07) \times 10^3$
	1.0	$(3.20 \pm 0.25) \times 10^4$	$(1.47 \pm 0.13) \times 10^4$	$(1.87 \pm 0.31) \times 10^3$	$(8.20 \pm 1.44) \times 10^2$
	1.5	$(1.33 \pm 0.30) \times 10^3$	$(5.60 \pm 1.27) \times 10^2$	$(9.33 \pm 2.45) \times 10^1$	$(4.33 \pm 1.36) \times 10^1$
	2.0	$(6.00 \pm 1.25) \times 10^2$	$(1.93 \pm 0.45) \times 10^2$	$(3.33 \pm 0.00) \times 10^1$	$(1.60 \pm 0.07) \times 10^1$

<sup>a</sup> Each mean is based on  $n = 5$  replications.

**Table 3.3 Total bacterial and fungal counts on untempered (11% moisture, wb) whole wheat unexposed and exposed to infrared radiation.**

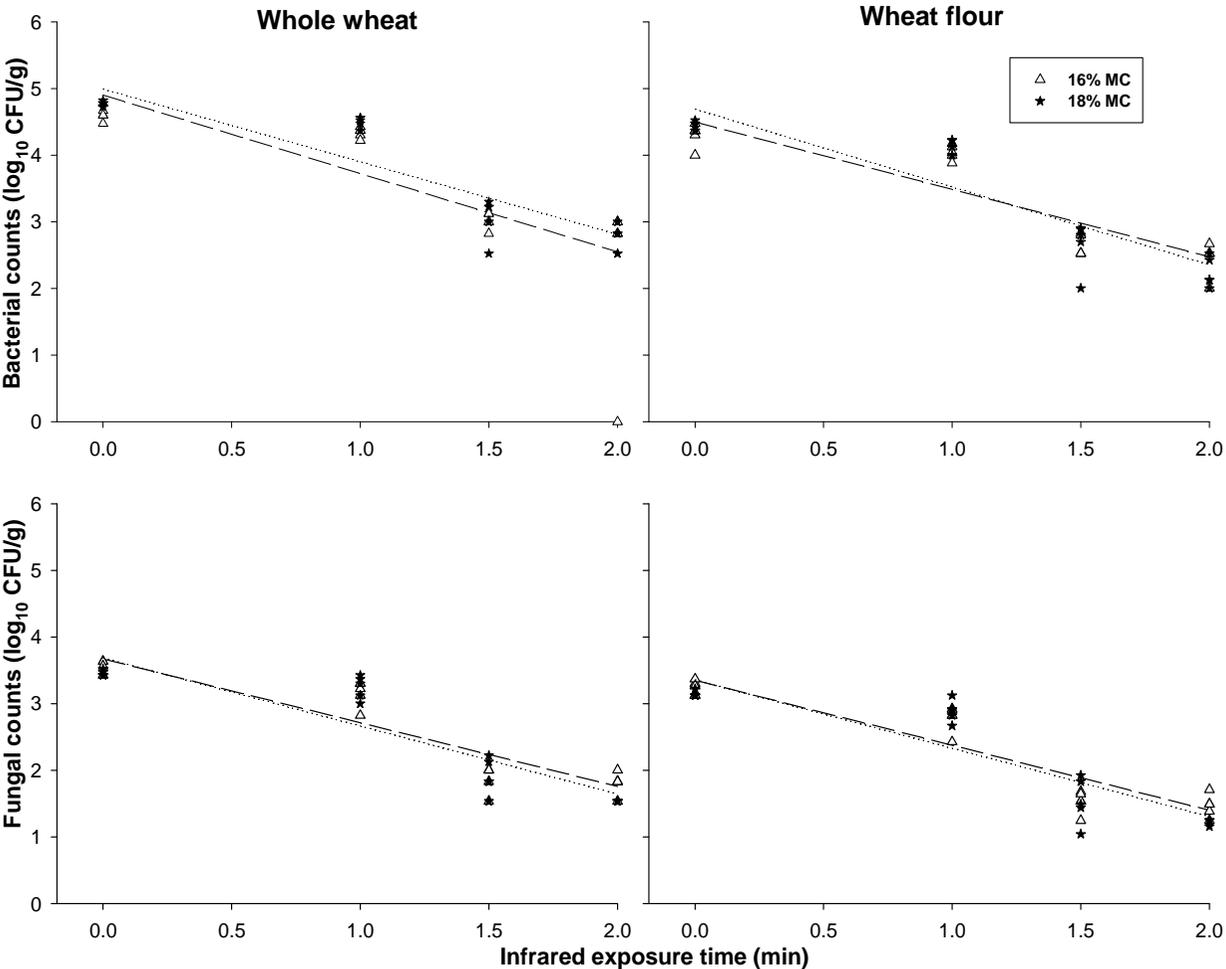
Infrared exposure time (min)	Mean $\pm$ SE <sup>a</sup>	
	Total bacterial counts (CFU/g) <sup>b</sup>	Fungal counts (CFU/g) <sup>c</sup>
	Whole wheat	Whole wheat
0.0	$(6.47 \pm 0.27) \times 10^4$	$(4.13 \pm 0.27) \times 10^3$
1.0	$(3.00 \pm 0.30) \times 10^4$	$(1.87 \pm 0.25) \times 10^3$
1.5	$(1.93 \pm 0.12) \times 10^3$	$(1.73 \pm 0.29) \times 10^2$
2.0	$(8.40 \pm 1.69) \times 10^2$	$(1.20 \pm 0.23) \times 10^2$

<sup>a</sup> Each mean is based on  $n = 5$  replications.

<sup>b</sup> Mean  $\pm$  SE linear regression parameters  $a$  and  $b$  describing logarithmic decrease in bacterial counts as a function of time are  $5.00 \pm 0.36$  and  $-1.00 \pm 0.26$ , respectively. The  $D$ -value ( $1/b$ ) is 1.00.

<sup>c</sup> Mean  $\pm$  SE linear regression parameters  $a$  and  $b$  describing logarithmic decrease in fungal counts as a function of time are  $3.74 \pm 0.30$  and  $-0.83 \pm 0.22$ , respectively. The  $D$ -value ( $1/b$ ) is 1.20.

**Figure 3.1 Linear regressions describing logarithmic reduction in bacterial and fungal counts in whole wheat and flour as a function of infrared exposure time. The wheat tempered to 16 and 18% moisture (wb) and held for 24 h at 28°C and 65% RH was exposed to infrared radiation, followed by milling to extract flour.**



**Table 3.4 Regression estimates showing logarithmic reduction in total bacterial and fungal counts in whole wheat tempered to 16 and 18% moisture and held for 8, 16, and 24 h and exposed to infrared radiation.**

Tempering moisture (%)	Tempering time (h)	Total bacterial counts				Fungal counts			
		$a \pm SE$	$b \pm SE$	$R^2$	$D$ -value (min) <sup>a</sup>	$a \pm SE$	$b \pm SE$	$R^2$	$D$ -value (min) <sup>a</sup>
16	8	4.89 ± 0.15	-1.00 ± 0.11	0.82	1.00	3.61 ± 0.09	-0.83 ± 0.06	0.91	1.20
	16	4.94 ± 0.17	-1.08 ± 0.13	0.80	0.93	3.77 ± 0.21	-1.10 ± 0.16	0.74	0.91
	24	4.90 ± 0.30	-1.18 ± 0.22	0.61	0.85	3.67 ± 0.14	-1.00 ± 0.11	0.82	1.00
18	8	4.84 ± 0.15	-0.97 ± 0.11	0.80	1.03	3.65 ± 0.14	-0.92 ± 0.10	0.82	1.09
	16	4.95 ± 0.16	-1.04 ± 0.12	0.81	0.96	3.66 ± 0.15	-0.98 ± 0.11	0.82	1.02
	24	4.99 ± 0.17	-1.09 ± 0.13	0.81	0.92	3.68 ± 0.16	-1.02 ± 0.12	0.81	0.98

<sup>a</sup> $D$ -value (1/ $b$ ) shows time in min for 1-log reduction of bacterial and fungal counts.

**Table 3.5 Regression estimates showing logarithmic reduction in total bacterial and fungal count in wheat flour obtained by milling wheat tempered to 16% or 18% moisture for 8, 16, and 24 h, and exposed to infrared radiation.**

Tempering moisture (%)	Tempering time (h)	Total bacterial count				Mold count			
		$a \pm SE$	$b \pm SE$	$R^2$	$D$ -value (min) <sup>a</sup>	$a \pm SE$	$b \pm SE$	$R^2$	$D$ -value (min) <sup>a</sup>
16	8	4.69 ± 0.28	-1.16 ± 0.21	0.64	0.86	3.28 ± 0.08	-0.82 ± 0.06	0.91	1.22
	16	4.65 ± 0.20	-1.14 ± 0.15	0.77	0.88	3.50 ± 0.20	-1.07 ± 0.15	0.75	0.93
	24	4.50 ± 0.16	-1.01 ± 0.12	0.80	0.99	3.35 ± 0.14	-0.98 ± 0.10	0.84	1.02
18	8	4.58 ± 0.17	-1.02 ± 0.12	0.79	0.98	3.31 ± 0.13	-0.93 ± 0.10	0.84	1.08
	16	4.69 ± 0.17	-1.13 ± 0.13	0.81	0.88	3.31 ± 0.15	-0.99 ± 0.11	0.82	1.01
	24	4.69 ± 0.18	-1.17 ± 0.14	0.81	0.85	3.36 ± 0.16	-1.03 ± 0.12	0.80	0.97

<sup>a</sup> $D$ -value ( $1/b$ ) shows time in min for 1-log reduction of bacterial and fungal counts.

## **Chapter 4 - Effects of infrared heating on wheat physical properties, milling yield, and flour quality**

## Abstract

Infrared radiation is used for grain drying, disinfestation, and disinfection. In a laboratory study, the effects of flameless catalytic infrared radiation on tempered wheat physical properties, milling yield, and flour quality were characterized. Organic, hard red winter wheat of 11% moisture (wb) was tempered with distilled water to moisture levels of 16 and 18% and held for 8, 16, and 24 h at 28°C and 65% RH. At each moisture and holding time, wheat was unexposed (control) or exposed to infrared radiation for 1, 1.5, and 2 min using a bench-top flameless catalytic infrared emitter. Physical properties of wheat kernels were analyzed using the Single Kernel Characterization System. Up to 2% moisture loss was observed after a 2 min exposure to infrared radiation. Flour yield during milling increased by 9% for infrared treated wheat when compared with unexposed wheat. Color of infrared-treated wheat flour was slightly darker (color change,  $\Delta E = 0.31$ ). Differential scanning calorimetry showed that flour from infrared-exposed wheat had lower enthalpy (3.0 J/g) when compared with flour (3.3 J/g) from unexposed wheat. Falling numbers of the infrared-exposed and unexposed wheat samples were essentially similar. Infrared-exposed flours had poor mixing properties due to the presence of high amount of insoluble polymeric protein (up to 60%) and the flour failed to form into a dough. Since the flour functionality was adversely affected, the exposure of tempered wheat to infrared radiation cannot be recommended despite an increase in milling yield.

## 4.1. Introduction

Infrared radiation is used for grain drying (Bradbury et al., 1960; Schroeder, 1960; Faulkner and Wratten, 1969; Pan et al., 2008) and for controlling various life stages of insects developing within and outside kernels of stored grains (Tilton and Schroeder 1963; Cogburn et al., 1971; Kirkpatrick and Tilton, 1972; Tilton et al., 1983). In these studies, the grain temperatures were measured after infrared exposure and not in real time, which resulted in under-reporting actual temperatures attained by the grain. Infrared radiation sources used were lamps or tubes with low power (0.5 kW), or gas-fired emitters with high power (14 kW) resulting in production of very high temperatures (900°C). Except for the study by Pan et al. (2008), the infrared emitters were fueled by natural gas or propane combusted over ceramic panels in the presence of oxygen and had open flames.

Unlike gas-fired emitters, the flameless catalytic infrared emitters developed by Catalytic Industrial Technologies LLC, Independence, KS, use propane or natural gas. In the presence of a platinum catalyst and oxygen these emitters produce infrared energy in the 3 to 7  $\mu\text{m}$  wavelength range resulting in temperatures below 500°C. The catalytic infrared heaters are environmentally friendly and since they do not use any flame these heaters do not produce any  $\text{NO}_x$  or CO. The co-products of catalytic oxidation-reduction reaction are carbon dioxide, water vapor, and infrared radiation.

Khamis et al. (2010; 2011a,b) studied the effectiveness of flameless catalytic infrared radiation against eggs, larvae, pupae, and adults of two internal insect species, the lesser grain borer, *Rhyzopertha dominica* (F.) and rice weevil, *Sitophilus oryzae* (L.); and one external insect species, the red flour beetle, *Tribolium castaneum* (Herbst). They reported that a 1 min exposure of 113.5 g of wheat (12% moisture, wb) at a distance of 8 cm from the emitter surface, which resulted in producing mean temperatures of 90-114°C were necessary to assure complete disinfestations of all stages. Pan et al. (2008) studied the feasibility of using infrared heating in rough rice drying and disinfestation by exposing 20.6 and 25.0% moisture (wb) paddy rice to infrared radiation for 25, 40, 60 and 90 s, and reported 0.7-1.9% higher head rice yield up on milling following the exposures.

Deliephan (2013) evaluated flameless catalytic infrared radiation to be effective in reducing microbial loads of tempered wheat (up to 3 log reductions in bacterial loads, and 2 log reductions in fungal loads) after brief exposure of 1, 1.5 and 2 min. To our knowledge there is

little information available in the literature on the effects of flameless catalytic infrared radiation on quality and end-use characteristics of tempered wheat. Khamis et al. (2011c) studied the effects of flameless catalytic infrared radiation on physical, chemical and rheological properties of wheat, after short exposure times (45 and 60 sec). Wheat exposed to flameless catalytic infrared radiation for 45 or 60 sec and then tempered to 16% moisture for 16 h was milled to evaluate wheat quality. They reported that test weight of wheat after infrared exposure was unaffected. They found no significant changes in milling yield of the various wheat fractions, and the chemical properties (protein and ash) of flour. Mixograph and bake test results showed no adverse effects on quality and end-use properties of flour. These interesting results prompted us to investigate the effects of extended exposures to infrared radiation on wheat physical properties, milling quality, and flour functionality after tempering.

The objectives of this study are to evaluate the effects of flameless catalytic infrared radiation on physical properties, germination, milling yield, flour color, gelatinization property, falling number, and end-use quality of tempered wheat.

## **4.2. Materials and methods**

### ***4.2.1. Tempering of wheat samples***

Uninfested, organic, hard red winter wheat of initial moisture content 11% (wb) was procured from Heartland Mills, Marienthal, KS. About 200 g of the wheat was placed in sterile polythene Ziploc bag (Fisher Scientific, Pittsburg, PA, USA), and distilled water was added to it to increase the grain moisture to 16% or 18%. The amount of water added was determined according to Harris and Lindblad (1978) using the following formula:

$$\text{Amount of water added (in ml)} = \left[ \frac{100 - \text{Initial moisture}}{100 - \text{Final moisture}} - 1 \right] \times \text{Amount of wheat (in g)}$$

The wheat bag after addition of water was shaken vigorously for uniform distribution of moisture and was allowed to temper for 8, 16 or 24 h at 28°C and 65% RH in a growth chamber (Percival, Perry, IA). Tempered wheat that was not exposed to infrared radiation served as the control treatment. Untempered wheat unexposed and exposed to infrared radiation was used for comparative purposes. A total of 140 such bags were prepared for the various treatment combinations.

#### ***4.2.2. Infrared exposure***

A bench-top flameless catalytic infrared emitter from Catalytic Drying Technologies LLC, was used for infrared treatment of the wheat samples. The unit was described in detail by Khamis et al. (2010). The unit has a circular infrared emitting surface of 613.4 cm<sup>2</sup>, and is fueled by propane at 28 cm of water column pressure. The total heat energy output of the unit is 1.5 kW/h (5000 BTU/h). A grain quantity of 113.5 g spread as a single layer thickness in a steel pan and was exposed to infrared radiation at 8 cm from the emitter surface for 1, 1.5 or 2 min. Each treatment combination was replicated five times.

#### ***4.2.3. SKCS analysis***

The Perten 4100 Single Kernel Characterization System (SKCS, Perten Instruments North America Inc., Springfield, IL) was used to determine hardness index (a dimensionless index), moisture (% wb), weight (mg) and diameter (mm) of the wheat kernels that were unexposed or exposed to infrared radiation. The SKCS tests 300 kernels per sample, and as explained above each treatment combination was evaluated five times.

#### ***4.2.4. Germination test***

Germination assays were performed by placing 10 whole wheat kernels on filter paper moistened with distilled water in sterile glass Petri dishes. A total of 10 such Petri dishes were prepared for each replication. The dishes were held at 25°C and 65% RH for 6 d to determine kernel germination. Germination was expressed as a percentage, after averaging germination across all 10 dishes in each replication ( $n = 5$  replications per treatment).

#### ***4.2.5. Milling***

Tempered wheat of 18% moisture (wb) held for 24 h (untreated and infrared-treated) were milled in a Quadrumat Senior mill (Brabender GmbH and Co., Duisburg, Germany) in the Department of Grain Science and Industry, Kansas State University, Manhattan, KS. Wheat tempered to 16% moisture was not milled because on infrared exposure the moisture content dropped down to 14% (wb) which is not suitable for milling hard red winter wheat. Untempered wheat (11% moisture) was also not milled. Before milling, the sieves and rolls were cleaned. The machine was warmed for 30 min by initially milling wheat. The feed rate was set at 150 g of wheat/min. After milling, the break and reduction flours were combined. The ambient

temperature and relative humidity during milling were 18°C and 40%, respectively. Total flour, bran, and shorts yields, and yield loss were calculated and reported on a percentage basis based on the weight of original wheat and the weights of each of the three fractions. Each treatment combination was replicated five times.

#### **4.2.6. Color analysis**

Color values of the flour samples from each treatment replication were measured using a CM-3500d Spectrophotometer (Minolta, Tokyo, Japan) to obtain the color parameters ( $L$ ,  $a$ , and  $b$  values). According to Commission Internationale de l'Eclairage (CIE), an international color standardization body, ' $L$ ' represents lightness, ' $a$ ' represents redness or green-ness while ' $b$ ' represents blueness or yellowness (Minolta, 1999). The Hunter color instrument was calibrated with white and black ceramic plates. The changes in the individual color parameters were calculated as follows:

$$\Delta L = L - L_0; \quad \Delta a = a - a_0; \quad \Delta b = b - b_0$$

The subscript '0' refers to the initial color parameter of each flour sample that is unexposed to infrared radiation. The total color difference ( $\Delta E$ ) was determined by the following equation (Nsonzi and Ramaswamy, 1998):

$$\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{0.5}$$

#### **4.2.7. Differential Scanning Calorimetry**

Differential scanning calorimetry analyses were performed using a DSC-Q100 (TA Instruments, New Castle, DE) equipped with a TA<sup>®</sup> Universal Analysis<sup>®</sup> 2000 workstation. The moisture content of the flour samples was increased by 1:2, solid to water ratio. The samples were weighed and hermetically sealed into standard aluminum pans, and were heated from 30 to 130°C at the rate of 10°C/min. An empty pan was used as a reference. The gelatinization profiles of the various samples during heating were obtained, and the enthalpy of gelatinization  $\Delta H$  (J/g of sample) was determined. Each treatment was replicated five times. Degree of gelatinization (%DG) was calculated as follows:

$$\%DG = \frac{\Delta H_0 - \Delta H_1}{\Delta H_0}$$

Where,  $\Delta H_0$  is gelatinization enthalpy of flour from untreated wheat and  $\Delta H_1$  is gelatinization enthalpy of flour from infrared-exposed wheat. It follows from the equation that %DG of untreated wheat flour is 0%.

#### **4.2.8. Falling Number**

Falling Number of the flour samples was determined according to standard AACC method 56-81. It correlates to  $\alpha$ -amylase activity of the flour, which in turn is associated with kernel sprouting. Each treatment combination was replicated five times.

#### **4.2.9. Insoluble Polymeric Protein Analysis**

The amount of insoluble polymeric protein was determined for flour samples from unexposed wheat and wheat exposed for 1 and 2 min to infrared radiation. Flour samples (100 mg) were mixed with 1 ml of solvent (1-propanol) and placed in a vortex stirrer (Vortex Genie-2, Scientific Industries, Bohemia, NY) and vortexed continuously for 5 min. Samples were then centrifuged at  $12,000 \times g$  for 5 min and the supernatant was discarded. The procedure was repeated two more times and the supernatants were discarded as they contain monomeric and soluble polymeric proteins. After extraction, the pellets containing the insoluble polymeric protein were freeze dried. The pellet protein content was determined by nitrogen combustion (LECO analysis), and the insoluble polymeric protein percentage was calculated by multiplying nitrogen values by a conversion factor of 5.7 and dividing by total flour protein. Each treatment was replicated two times in duplicates.

#### **4.2.10. Statistical Analysis**

Data from various experiments were subjected to three-way analysis of variance (ANOVA) to determine significant differences among the treatments at  $\alpha = 0.05$  using the GLM procedure (SAS Institute, 2008). Data at 16 and 18% moisture and 8, 16, and 24 h holding times on kernel hardness, moisture, and weight among infrared exposure times were subjected to one-way ANOVA. If one-way ANOVA showed significant differences means for each variable among infrared exposure times was separated using Bonferroni *t*-tests (SAS Institute, 2008). Kernel hardness frequency distributions were plotted using SigmaPlot<sup>®</sup> software (Scientific Graphing Software, Jandel Corporation, San Rafael, CA). Data on insoluble polymeric protein percentage were regressed over infrared exposure times using a linear regression,  $y = a + bx$ ,

where  $y$  = amount of insoluble polymeric protein (%);  $x$  = infrared exposure time (min), and  $a$  and  $b$  are estimated parameters from the regression.

### 4.3. Results

Kernel hardness was 10-20 times higher for wheat at 11% moisture compared to unexposed wheat at this moisture. Exposure to infrared radiation slightly increased kernel hardness probably due to loss of kernel moisture. On average, the mean kernel hardness increased by 1.2, 2.1 and 3.0% and the mean kernel weight decreased by 1.4, 2.4 and 3.7% after exposure to infrared for 1, 1.5 and 2 min, respectively. Infrared exposure of 1 min decreased the kernel moisture by 1.1%, while infrared exposures of 1.5 and 2 min decreased kernel moisture by 1.6 and 2.2% respectively (Table 4.1). Three-way ANOVA results showed that tempering moisture, tempering time, and infrared exposure time had significantly influenced kernel hardness ( $F$ , range between tempering moistures, holding times, and infrared exposures = 8.77-3508.10;  $P \leq 0.0003$ ), kernel weight ( $F$ , range = 5.00-116.51;  $P \leq 0.0085$ ), and kernel moisture ( $F$ , range= 11.21 to 1803.57;  $P < 0.0001$ ).

Germination percentages of untempered and tempered wheat ranged from 78 to 87% (Table 4.2). Grain moisture ( $F = 42.85$ ;  $df = 1, 102$ ;  $P < 0.0001$ ) and tempering time ( $F = 6.37$ ;  $df = 2, 102$ ;  $P \leq 0.0025$ ) had significant effect on germination percentage, whereas infrared exposure time ( $F = 1.92$ ;  $df = 3, 102$ ;  $P = 0.130$ ) did not have significant effect.

Infrared exposure increased milling yield (up to 8.8%) and decreased the bran yield (up to 6.8%) when compared with similar values from tempered and unexposed wheat. Yield of shorts was reduced by 3.9%, and flour loss increased by 2% following infrared treatment (Table 4.3). Yield of flour, bran and shorts, and flour loss were significantly affected by infrared exposure time ( $F$  range = 5.17-180.40;  $df = 3, 16$ ;  $P < 0.0001$ ).

The color analysis ( $L^*$ ,  $a^*$ ,  $b^*$  values) of the flour showed an overall color change ( $\Delta E$ ) of 0.31 to 0.40 for infrared treated flour compared with untreated flour ( $\Delta E = 0$ ) (Table 4.4).

Measurements using Differential Scanning Calorimetry (DSC) did not show any consistent difference between gelatinization temperatures among the treatment combinations; however there is a slight decrease in enthalpy (up to 0.5 J/g) and an increase in degree of gelatinization (up to 15.2%) for infrared treated flours when compared to untreated ones (Table 4.5).

The Falling number of the flour samples ranged from 387 to 514 for the infrared treated samples, and 384 to 475 for the untreated ones (Table 4.6). This showed that the flours were unaffected (high falling number).

Preliminary mixing experiments (mixograph) for the flour samples were done using full formula bread doughs. Infrared treatment did not affect water absorption which was about 61% (flour weight basis, fwb) for all samples. Mixing time was significantly and negatively affected. Mix time of flour from tempered and unexposed wheat was 4 min, whereas flour from 1 min-infrared exposed wheat had a mixing time of 6.5 min. Flours from 1.5 and 2 min-infrared-treated wheat did not develop into dough even after 15 min of mixing. The dough formed was hard and biscuit-like. These results indicated that infrared treatment negatively affected the functional dough-making properties of gluten in the flour. Due to the fact that dough could not be formed, the bread was not baked.

The percentage of insoluble polymeric protein determined for flours indicated that longer exposure to infrared radiation resulted in flour with higher amount of insoluble polymeric protein. The mean insoluble polymeric protein percentages were 44.6, 47.6 and 60.4% for flours obtained from untreated wheat, and wheat treated with infrared radiation for 1 and 2 min, respectively. The linear regression satisfactorily described an increase in insoluble polymeric protein as a function of infrared exposure time ( $R^2 = 0.72$ ) (Fig. 4.1). There was 8% increase in insoluble polymeric protein for every second of infrared exposure.

#### **4.4. Discussion**

Infrared radiation is used for drying of cereal grains (Bradbury et al., 1960; Pan et al., 2008). Loss of kernel moisture leads to an increase in kernel hardness which is supported by the SKCS results in our study and those reported by Khamis et al. (2011c). Kirkpatrick and Cagle (1978) reported a decrease of 0.5% from initial moisture content of wheat (13.5%) when exposed to infrared radiation. In our study a decrease of up to 2.2% moisture was observed after a 2 min infrared exposure. This decrease in moisture is the reason for a decrease in kernel weight as well.

The germination percentage was not significantly affected by infrared exposure of the wheat grains. Ghaly and Touw (1982) evaluated the level of heat damage to wheat samples in a small batch fluidized-bed rig, and determined that the effects of temperature and initial moisture content were highly significant, but exposure time had little effect on quality deterioration of

wheat. Since infrared heating is rapid, and the product loses heat relatively faster (Sakai and Hanzawa, 1994) it would not have adversely affected the germination potential of the wheat kernels.

During milling, flour yield is influenced by several factors such as milling conditions, condition of the machine, and type of wheat (Maghirang et al., 2006). Hard red winter wheat is normally tempered to 16% moisture before milling. In our study, wheat that was unexposed to infrared radiation had moisture content of 18% which is higher than the recommended level. This could be the reason that the mean flour yield was low in this treatment (64.7%). On exposure to infrared radiation there was moisture loss, and the moisture content of wheat came down to 16% especially for the 2 min infrared exposure. Hence, the flour yield was much higher (73.5%). Another reason for higher flour yield could be the uniform heating of kernels when exposed to infrared radiation, because water molecules have the maximum absorption of the 3-7  $\mu\text{m}$  infrared energy emitted by the flameless catalytic infrared units. Pan et al. (2008) reported that paddy rice dried with heated air (conventional heating), had more moisture near the surface of the rice kernel relative to the kernel center thus creating a moisture gradient in the rice kernel. The moisture gradient could induce tensile and compressive stresses resulting in fissures after cooling and lowering of milling quality (Ban, 1971; Kunze and Choudhury, 1972; Kunze, 1979). Using infrared radiation, Pan et al. (2008) obtained higher head rice yield due to uniform heating which caused reduced moisture gradient in kernels and faster moisture removal.

In the color values of flour samples there was a slight decrease in  $L^*$  value or brightness of the sample as the infrared exposure time increased. No consistent trend was observed in the case of  $a^*$  (green-ness) and  $b^*$  (yellowness) values. Visual comparison with the untreated sample indicated a slight darkness in the flour from 2 min-infrared treated wheat, which could be due to Maillard browning. Arntfield et al. (2001) reported infrared treated lentils to be slightly darker than raw lentils.

Differential Scanning Calorimetry indicated a slight decrease in enthalpy for the 2 min infrared treated sample when compared to the untreated one. It could be due to the fact that some extent of gelatinization had occurred during infrared exposure of 2 min, which may have lowered the enthalpy. This is supported by the fact that a slightly higher degree of gelatinization (15.2%) occurred for wheat exposed for 2 min to infrared radiation when compared with those exposed for 1.0 min (9.1%) and 1.5 min (6.1%).

Falling numbers for wheat flour can range from 278 to 861 (Maghirang et al. 2006). Falling number values below 278 may indicate sprout damage or increased enzymatic activity (Atwell, 2001). Falling number values of flours observed in our study fell within these ranges specified by Maghirang et al (2006). There was a slight increase in falling number for 2 min-infrared treated wheat flour when compared to the flour from unexposed wheat and wheat exposed for 1 min and 1.5 min to infrared radiation. This could be due to inactivation of alpha amylase enzyme during infrared treatment.

Glutenin polymers are strongly correlated with dough properties and breadmaking quality (Gupta et al., 1993). When gluten is heat-denatured it forms a network which resists water penetration and protects the starch granules from water uptake. A number of studies have found that the amount of insoluble polymeric protein left behind after removal of all other soluble proteins is directly related to flour quality (Orth and Bushuk, 1972; MacRitchie, 1978; Chakraborty and Khan, 1988). Presence of higher amount of insoluble polymeric protein decreases the extensibility of the dough (Gupta et al., 1993). In our study, the amount of insoluble polymeric protein increased as a function of infrared exposure time, which indicated protein denaturation. This in turn affected the bread-making property of the dough.

In conclusion, despite an increase in flour yield, infrared treatment of tempered wheat adversely affected flour quality and functionality. Hence, we do not recommend exposing tempered wheat to infrared radiation, even though there are some beneficial effects such as increased milling yield and decreased microbial loads.

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**Table 4.1 Effects of infrared treatment on physical properties of wheat tempered to different moisture and held for different tempering times at 28°C and 65% RH.**

Moisture (%)	Tempering time (h)	Exposure time (min)	Mean ± SE <sup>a</sup>			
			Hardness Index (HI) <sup>b</sup>	Weight (mg) <sup>b</sup>	Moisture (%) <sup>b</sup>	Diameter (mm) <sup>c</sup>
11 <sup>d</sup>	0	0	75.3 ± 1.6a	27.8 ± 0.3a	10.8 ± 0.1a	2.6 ± 0.0
		1	76.1 ± 0.4a	27.4 ± 0.1ab	10.3 ± 0.2ab	2.6 ± 0.0
		1.5	76.7 ± 0.5a	27.2 ± 0.3ab	10.5 ± 0.0ab	2.6 ± 0.0
		2	77.1 ± 0.6a	26.9 ± 0.2b	10.5 ± 0.0b	2.6 ± 0.0
16	8	0	66.5 ± 0.2b	28.8 ± 0.2a	16.0 ± 0.0a	2.6 ± 0.0
		1	67.1 ± 0.2ab	28.4 ± 0.2ab	14.4 ± 0.1b	2.6 ± 0.0
		1.5	67.8 ± 0.2ab	28.2 ± 0.2ab	13.7 ± 0.1c	2.6 ± 0.0
		2	68.4 ± 0.6b	27.8 ± 0.2b	12.5 ± 0.1d	2.6 ± 0.0
	16	0	66.6 ± 0.7b	28.4 ± 0.2a	15.9 ± 0.0a	2.6 ± 0.0
		1	67.6 ± 0.4ab	28.1 ± 0.1ab	14.4 ± 0.1b	2.6 ± 0.0
		1.5	68.3 ± 0.1ab	27.9 ± 0.2ab	13.9 ± 0.1c	2.6 ± 0.0
		2	68.8 ± 0.2b	27.4 ± 0.1b	13.3 ± 0.2d	2.6 ± 0.0
	24	0	66.0 ± 0.3b	28.9 ± 0.1a	15.8 ± 0.0a	2.6 ± 0.0
		1	67.0 ± 0.4ab	28.4 ± 0.3ab	14.0 ± 0.1b	2.6 ± 0.0
		1.5	67.7 ± 0.4ab	28.2 ± 0.1ab	13.5 ± 0.1c	2.6 ± 0.0
		2	68.5 ± 0.5b	27.7 ± 0.3b	12.8 ± 0.2d	2.6 ± 0.0
18	8	0	56.9 ± 0.2a	29.6 ± 0.2a	17.9 ± 0.1a	2.6 ± 0.0

	1	57.6 ± 0.3ab	29.2 ± 0.2a	16.8 ± 0.3b	2.6 ± 0.0
	1.5	58.2 ± 0.2ab	28.8 ± 0.2a	16.1 ± 0.1b	2.6 ± 0.0
	2	58.8 ± 0.6b	28.6 ± 0.3a	15.3 ± 0.2c	2.6 ± 0.0
16	0	58.2 ± 0.8a	29.6 ± 0.1a	17.5 ± 0.2a	2.6 ± 0.0
	1	58.8 ± 0.4a	29.2 ± 0.2a	17.0 ± 0.2ab	2.6 ± 0.0
	1.5	59.3 ± 0.2a	28.8 ± 0.3ab	16.6 ± 0.2bc	2.6 ± 0.0
	2	59.8 ± 0.3a	28.3 ± 0.2b	16.2 ± 0.2c	2.6 ± 0.0
24	0	57.8 ± 0.1a	29.8 ± 0.1a	17.8 ± 0.1a	2.6 ± 0.0
	1	58.3 ± 0.2ab	29.4 ± 0.2ab	16.9 ± 0.1b	2.6 ± 0.0
	1.5	58.7 ± 0.2bc	29.0 ± 0.1b	16.0 ± 0.1c	2.6 ± 0.0
	2	59.2 ± 0.2c	28.7 ± 0.1b	15.5 ± 0.2c	2.6 ± 0.0

<sup>a</sup>Each mean is based on  $n = 5$  replications.

<sup>b</sup>For each tempering moisture and holding time combination, means among infrared exposure times followed by different letters are significantly different ( $P < 0.05$ , Bonferroni  $t$ -test).

<sup>c</sup>SE for mean diameter (mm) ranged from 0.006 - 0.037; since the mean values were similar, data were not subjected to one-way ANOVA.

**Table 4.2 Mean germination percentages of untempered wheat and wheat tempered to 16, 18% moisture for 8, 16 and 24 h, and exposed to infrared radiation for 0, 1, 1.5 and 2 min.**

Grain moisture (%)	Tempering time (h)	Exposure time (min)	Germination Mean $\pm$ SE (%) <sup>a</sup>
11	0	0	87.4 $\pm$ 3.2
		1	81.2 $\pm$ 1.4
		1.5	81.8 $\pm$ 0.2
		2	82.3 $\pm$ 0.6
16	8	0	81.0 $\pm$ 0.8
		1	81.9 $\pm$ 0.3
		1.5	81.7 $\pm$ 0.7
		2	81.6 $\pm$ 0.3
	16	0	81.5 $\pm$ 0.5
		1	80.7 $\pm$ 0.8
		1.5	82.0 $\pm$ 0.9
		2	82.0 $\pm$ 0.6
	24	0	82.1 $\pm$ 0.5
		1	81.8 $\pm$ 0.6
		1.5	82.5 $\pm$ 0.7
		2	82.5 $\pm$ 0.4
18	8	0	82.1 $\pm$ 0.5
		1	78.0 $\pm$ 0.4
		1.5	80.1 $\pm$ 0.6
		2	80.1 $\pm$ 0.5
	16	0	81.0 $\pm$ 0.5
		1	79.1 $\pm$ 0.5
		1.5	77.9 $\pm$ 0.5
		2	80.4 $\pm$ 0.4
	24	0	80.2 $\pm$ 0.3
		1	81.8 $\pm$ 0.3

1.5	$81.0 \pm 0.4$
2	$80.7 \pm 0.2$

---

<sup>a</sup>Each mean is based on  $n = 5$  replications.

**Table 4.3 Milling yields of various fractions and flour loss of wheat tempered to 18% moisture for 24 h and exposed to infrared radiation for 0, 1, 1.5 and 2 min.**

Exposure time (min)	Mean $\pm$ SE (%) <sup>a</sup>			
	Flour <sup>b</sup>	Bran <sup>b</sup>	Shorts <sup>b</sup>	Loss <sup>b</sup>
0	64.7 $\pm$ 0.8a	20.4 $\pm$ 0.3a	7.4 $\pm$ 0.8a	7.4 $\pm$ 0.7a
1	68.0 $\pm$ 0.8b	15.9 $\pm$ 0.8b	6.4 $\pm$ 0.6a	9.7 $\pm$ 0.3a
1.5	70.1 $\pm$ 0.8c	15.0 $\pm$ 0.6b	5.2 $\pm$ 0.6b	9.6 $\pm$ 0.8ba
2	73.5 $\pm$ 0.6d	13.6 $\pm$ 0.4c	3.5 $\pm$ 0.8c	9.4 $\pm$ 0.3b

<sup>a</sup> Each mean is based on  $n = 5$  replications.

<sup>b</sup> Means within a column followed by different letters are significantly different ( $P < 0.05$ , Bonferroni  $t$ -test).

**Table 4.4 Color values ( $L^*$ ,  $a^*$ ,  $b^*$ ) and overall color change ( $\Delta E$ ) of flour obtained from wheat tempered to 18% moisture for 24 h and exposed to infrared radiation for 0, 1.0, 1.5, and 2 min.**

Exposure time (min)	Mean $\pm$ SE <sup>a</sup>			
	$L^*$	$a^*$	$b^*$	$\Delta E$
0	97.42 $\pm$ 0.01	1.82 $\pm$ 0.09	0.03 $\pm$ 0.00	0.00 $\pm$ 0.00
1	97.39 $\pm$ 0.06	1.68 $\pm$ 0.12	0.02 $\pm$ 0.00	0.32 $\pm$ 0.15
1.5	97.43 $\pm$ 0.02	1.80 $\pm$ 0.10	0.07 $\pm$ 0.01	0.40 $\pm$ 0.05
2	97.20 $\pm$ 0.01	1.82 $\pm$ 0.06	0.03 $\pm$ 0.00	0.31 $\pm$ 0.11

<sup>a</sup> Each mean is based on  $n = 5$  replications.

**Table 4.5 Gelatinization temperature, enthalpy and degree of gelatinization of flour obtained from wheat tempered to 18% moisture for 24 h and exposed to infrared radiation for 0, 1, 1.5 and 2 min.**

Exposure time (min)	Mean $\pm$ SE <sup>a</sup>		
	Gelatinization temperature ( $^{\circ}$ C)	Enthalpy (J/g)	Degree of gelatinization (%)
0	65.1 $\pm$ 0.6	3.3 $\pm$ 0.3	0.0 $\pm$ 0.0
1	65.7 $\pm$ 1.0	3.0 $\pm$ 0.4	9.1 $\pm$ 0.3
1.5	64.9 $\pm$ 0.3	3.1 $\pm$ 0.3	6.1 $\pm$ 0.3
2	65.4 $\pm$ 0.9	2.8 $\pm$ 0.3	15.2 $\pm$ 0.3

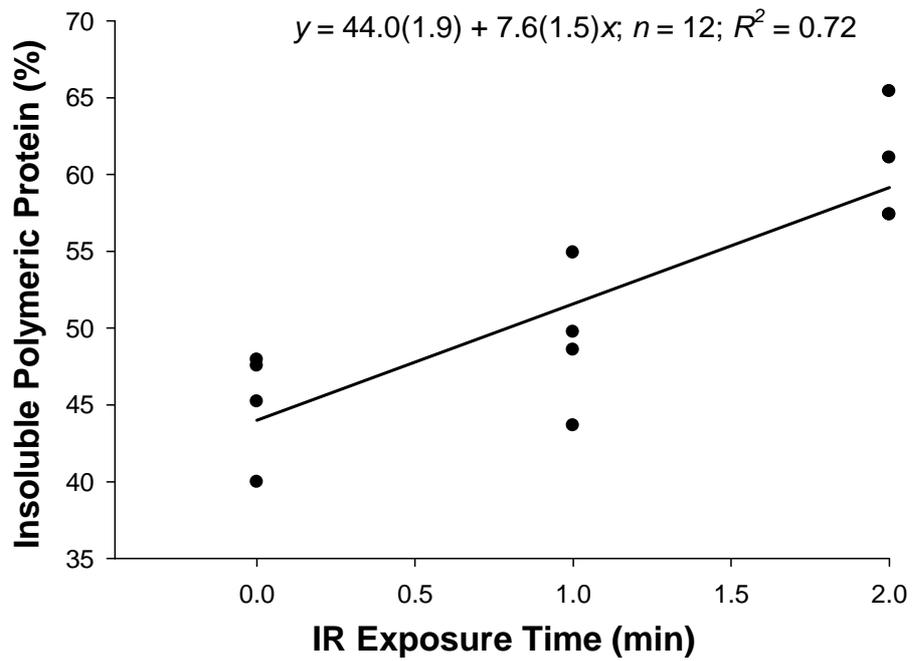
<sup>a</sup>Each mean is based on  $n = 5$  replications.

**Table 4.6 Falling numbers of flours obtained from wheat tempered to 18% moisture for 24 h and exposed to infrared radiation for 0, 1, 1.5 and 2 min.**

Infrared exposure time (min)	Falling number (Mean $\pm$ SE) <sup>a</sup>
0	430 $\pm$ 39
1.0	426 $\pm$ 38
1.5	423 $\pm$ 23
2	484 $\pm$ 36

<sup>a</sup> Means are based on  $n = 5$  replications.

**Figure 4.1 Linear regression describing an increase in insoluble polymeric protein in flour from wheat at 18% moisture held for 24 h that was exposed to infrared radiation for 0, 1, and 2 min. Values in parenthesis in the equation are standard errors of parameter estimates.**



**Chapter 5 - Effects of electrolyzed oxidizing water alone and  
electrolyzed oxidizing water and infrared exposure on microbial  
loads of whole wheat and flour**

## Abstract

The efficacy of electrolyzed oxidizing (EO) water alone for tempering wheat and EO water followed by infrared treatment of tempered wheat on aerobic bacterial and fungal loads was investigated. A bench-top flameless catalytic infrared emitter was used to treat wheat that was tempered with distilled water or EO water to 18% moisture (wb) for 24 h and exposed to infrared radiation for 1, 1.5 and 2 min. The treated wheat was milled, and microbial plating was done for the whole wheat and flour to enumerate microbial loads. Wheat tempered with distilled water and exposed to infrared radiation resulted in reducing bacterial and fungal counts by 98.9% when compared with wheat that was untempered (11% moisture, wb) and unexposed to infrared radiation. Wheat tempered with EO water and exposed to infrared radiation showed about 99.5% reduction in bacterial and fungal counts relative to those found on untempered and unexposed wheat. Tempering wheat with EO water and not exposing to infrared radiation reduced bacterial counts by 56.1% and fungal counts by 66.1%. Linear regression models were fit to the logarithmic decrease of bacterial and fungal counts as a function of infrared exposure time to determine *D*-values (time in min required for 1-log reduction of microbial loads). *D*-values showed that the time for 1-log reduction of microbial loads in wheat and flour took about a minute, irrespective of whether distilled water or EO water was used for tempering wheat. The results show that the use of EO water for tempering wheat will help reduce bacterial and fungal loads on wheat.

## 5.1. Introduction

Food safety and quality has been a high priority over the last decade in light of several food safety recalls of cereal commodities. Cereal grains contain a wide assortment of microflora including bacteria, fungi, actinomycetes, and yeast. Several studies have documented the presence of *Salmonella* spp., *Escherichia coli*, *Bacillus cereus* and other spoilage microorganisms in wheat and flour (Ottogalli and Galli, 1979; Eyles et al., 1989; Richter et al., 1993).

In recent years, the electrolyzed-oxidizing water (EO water) has received much attention as a novel non-thermal antimicrobial agent. EO-water has been extensively studied for use in agricultural and food industrial processes, post-harvest disease control of raw agricultural commodities, and disinfection of food contact surfaces (Grech and Rijkenberg 1992; Kim et al., 2000; Al-Haq et al., 2002; Venkitanarayanan et al., 1999). The application of EO water for microbial control provides many advantages over other chemical agents, including less adverse chemical residue, and it is cost-effective and environmentally friendly (Kroyer 1995; Al-Haq et al., 2002).

EO water is produced by passing a diluted salt solution (0.1% NaCl) through an electrolytic cell, within which the anode and cathode are separated by a membrane (Fig. 5.1) (Huang et al., 2008). By subjecting the electrodes to direct current voltages, negatively charged ions of salt and water,  $\text{Cl}^-$  and  $\text{OH}^-$  respectively, are attracted to the anode where they bind with dissociated water molecules ( $\text{H}^+$  and  $\text{O}_2$ ) to form HCl, HOCl,  $\text{Cl}_2$ ,  $\text{OCl}^-$ , and  $\text{O}_2$ . Positively charged ions such as  $\text{Na}^+$  and  $\text{H}^+$  move to the cathode to take up electrons to form NaOH and  $\text{H}_2$  (Hsu, 2005). EO water is collected at the anode, and has a low pH (2.3-2.7), a high oxidation-reduction potential (ORP >1000 mV), high dissolved oxygen, and a free chlorine concentration of 10-80 ppm (Shimizu and Huruzawa, 1992).

Bonde and Nester (1999), Oomori et al. (2000) and Yoshida et al. (2004) studied the effects of EO water in controlling post-harvest diseases in cereal grains. However there are only very few literature references that focus on the application of EO water in grain processing.

Macaluso (2007) developed a patented process for heating nuts using infrared radiation in combination with EO water to inactivate *Salmonella* spp. Adding EO water to nuts followed by infrared radiation exposure for 20-100 sec inactivated microbial loads without adversely affecting nut quality. Deliephan (2013) reported that flameless catalytic infrared radiation is

effective in reducing microbial loads of tempered wheat by 2-3 logs after 1, 1.5, and 2 min exposures. It is plausible that tempering wheat with EO water alone followed by brief exposure to infrared radiation may further reduce microbial loads. In the present study, the sterilization effects of tempering wheat with EO water followed by infrared radiation exposure was studied to determine microbial loads on wheat and flour milled from wheat.

## **5.2. Materials and methods**

### ***5.2.1. Tempering of wheat samples***

About 200 g of uninfested, organic, hard red winter wheat of 11% moisture (wb) (Heartland Mills, Marienthal, KS, USA) was placed in individual sterile polythene Ziploc bags (Fisher Scientific, Pittsburg, PA). EO water of pH 2.4 and oxidation-reduction potential (ORP) 1,100 mV was procured from RPA Biotech Inc., Las Vegas, NV. A calculated amount of EO water or distilled water was added to wheat to increase the grain moisture to 18%. The amount of water added was determined using the formula below (Harris and Lindblad, 1978):

$$\text{Amount of water added (in ml)} = \left[ \frac{100 - \text{Initial moisture}}{100 - \text{Final moisture}} - 1 \right] \times \text{Amount of wheat (in g)}$$

Each bag with wheat after addition of distilled or EO water was shaken vigorously for uniform distribution of moisture and was allowed to temper for 24 h at 28 °C and 65% RH in a growth chamber (Percival, Perry, IA). Untempered wheat (11% mc) was also included in this study to show microbial loads prior to tempering and the impact of infrared radiation exposure on microbial loads. A total of 60 such bags were prepared for the various treatment combinations (Table 5.1).

### ***5.2.2. Infrared exposure***

Each wheat sample (113.5 g in a single layer) at 11% moisture or 18% moisture was either unexposed or exposed for 1, 1.5, and 2 min to infrared radiation using a bench-top flameless catalytic infrared emitter (Catalytic Drying Technologies LLC, Independence, KS, USA), fueled by propane, at a distance of 8 cm from the emitter surface. The total heat energy output of this unit is 1.5 kW/h. Temperatures attained at each exposure time are reported elsewhere (Deliephan, 2013).

### **5.2.3. Milling**

Only the 18% tempered wheat samples were milled in a Quadrumat Senior mill (Brabender GmbH and Co., Duisburg, Germany) in the Department of Grain Science and Industry, Kansas State University, Manhattan, KS. Before milling, the sieves were cleaned and rolls were wiped with 95% ethanol. The machine was warmed for 30 min by initially milling wheat. The feed rate was set at 150 g of wheat/min. After milling, the break and reduction flours were combined. The ambient temperature and relative humidity during milling were 18°C and 40% respectively. There were five replications for each treatment combination.

### **5.2.4. Microbial plating**

Microbial analyses were initiated by aseptically weighing 10 g of sample (whole wheat or flour) and suspending it in 100 ml of distilled water. Dilution blanks (peptone 0.1%) were prepared, and serial dilutions from  $10^{-1}$  to  $10^{-4}$  were made for the samples. For total bacterial count, 1 ml each of the dilutions  $10^{-4}$ ,  $10^{-3}$  and  $10^{-2}$  were plated using the surface spread method on Trypticase Soy Broth (TSB) nutrient agar. Using the same procedure, for fungal counts, 1 ml each of the dilutions  $10^{-3}$ ,  $10^{-2}$  and  $10^{-1}$  were plated on Potato Dextrose Agar (PDA) medium. Chloramphenicol (75 mg/L) was added to PDA to prevent the growth of bacteria. Three sub-replicates were plated for each replicate of all the samples. The plates were incubated at 25°C for 5 d. Enumeration was done by colony count method for total bacteria and fungi, and the results were expressed in colony forming units per gram (CFU/g).

### **5.2.5. Statistical analysis**

The logarithmic decrease of total bacteria and mold count of whole wheat and flour treated with infrared radiation and/or EO water as function of infrared exposure time (0-2 min) was described by a linear regression,  $y = a + bx$ , where  $y$  = total bacterial or fungal counts ( $\log_{10}$  CFU/g);  $x$  = infrared exposure time in minutes, and  $a$  and  $b$  are estimated parameters from the regression. The inverse of the slope gave a  $D$ -value which represented the minutes of infrared exposure required for a 1-log reduction of bacteria or fungal counts.

## **5.3. Results**

The mean bacterial and mold counts for all treatment combinations are shown in Table 5.2. The control wheat which is untempered and not treated with infrared radiation had the

highest mean total bacteria ( $6.67 \times 10^4$  CFU/g) and fungal counts ( $4.33 \times 10^3$  CFU/g). Among the samples that are not exposed to infrared radiation, wheat tempered with EO water alone had the lowest microbial loads:  $2.93 \times 10^4$  and  $1.47 \times 10^3$  CFU/g for bacterial and fungal counts, respectively. Wheat tempered with distilled water alone had a slightly lower microbial load ( $5.80 \times 10^4$  CFU/g bacterial and  $2.93 \times 10^3$  CFU/g fungal counts) when compared with untempered and unexposed wheat, but higher microbial loads when compared with wheat tempered with EO water alone ( $2.93 \times 10^4$  CFU/g bacterial and  $1.47 \times 10^3$  CFU/g fungal counts). Exposure to infrared radiation reduced both bacterial and fungal counts, and this decrease was logarithmic (Table 5.3).

Wheat at 11% moisture showed 98.7% decrease in microbial loads after infrared exposure. The decrease in microbial loads was greater for tempered wheat samples exposed to infrared radiation. Wheat tempered with distilled water and exposed to infrared radiation showed up to 98.9% decrease in microbial loads while the wheat tempered with EO water and exposed to infrared showed up to 99.5% decrease in microbial loads. Wheat tempered with EO water and not exposed to infrared showed up to 56.1% decrease in microbial loads. Microbial loads in flour was consistently lower compared to whole wheat in all treatment combinations, and the trends were similar to that of whole wheat. The decrease in microbial loads in flour from tempered and infrared exposed wheat also decreased logarithmically (Table 5.4). The time required for 1-log reduction of microbial loads in tempered wheat exposed to infrared radiation and those found in flour from treated wheat was about 1 minute.

## 5.4. Discussion

The typical range of bacterial and fungal counts found in wheat is 3-8 logs and 2-7 log CFU/g, respectively (Manthey et al., 2004). The use of EO water as a tempering agent for whole wheat (without treating with infrared radiation) led to a 99.6% decrease in bacterial counts and a 66.1% decrease in fungal counts. The inhibition of microbes by EO water is based on the low pH and high oxidation-reduction potential (ORP) of EO water causing damage of cell membranes. In general, aerobic bacteria grow in a pH range of 4-9 and mostly at an ORP range of +200 to 80 mV. Oxidation occurs when oxygen reacts with other compounds causing them to lose electrons and further causing the compounds to break down. In the case of microbes, oxidation due to EO water (ORP >1000) damages cell membranes, creates disruption in cell metabolic processes due

to change in electron flow, and eventually kills the cell. The low pH of EO water (pH 2-3) could sensitize the outer membrane of microbial cells to the entry of HOCl (hypochlorous acid) into the cell. HOCl, the most active of chlorine compounds, appears to kill microbial cells through inhibiting glucose oxidation by chlorine-oxidizing sulfhydryl groups of certain enzymes important in carbohydrate metabolism (Marriott and Gravani, 2006; Liao et al., 2007).

An EO water generator is normally used to produce EO water, and various types of these machines are available in the market. Al-Haq and Sugiyama (2004) state that the machine most commonly used in published reports is the ROX-20 (Hoshizaki Electric Inc., Toyoake, Aichi, Japan). This has been used in studies reported by Koseki and Itoh (2001); Park et al. (2001, 2002) and Venkitanarayanan et al. (1999). A machine manufactured by Remotex (Remote Co., Toshima-ku, Tokyo, Japan) was used by Yamaki (1998) and Yamaki and Schoerner (1995). Al-Haq et al. (2002) used a machine manufactured by Mitsubishi Electric Engineering Co., Japan.

Production capacity of a ROX-20 generator is 1.5-3.0 L/min. A ROX-60 generator has a higher production capacity of 3.0-6.0 liters/min. Electricity consumption is 0.34 kW and 0.45 kW for ROX-20 and ROX-60 generators, respectively. According to Viking Pure<sup>®</sup>, a supplier of EO water generators, the price range of larger-scale units range from \$12,000-\$15,000 while that of smaller-scale units is about \$3,500.

In conclusion, the use of infrared radiation on untempered wheat and wheat tempered to 18% moisture with distilled or EO water resulted in a decrease on microbial loads of both wheat and flour produced from infrared exposed wheat. Hamanaka et al. (2006) reported increased microbicidal effect of infrared radiation on wheat at higher moisture content (24% wb) and little microbicidal effect in low moisture content (14% wb) wheat. This conclusion is supported by our results that showed a slightly higher microbial reduction observed in the tempered wheat (18% moisture) compared with untempered wheat (11% moisture). Infrared radiation damages microbial cell protein and RNA, and leads to mesosome disintegration, cell wall damage, cytoplasmic membrane shrinkage and cellular content leakage, thus killing the cell.

A combination of tempering with EO water followed by infrared treatment greatly reduced the bacterial and fungal counts to 99.5% and 99.4%, respectively, in wheat. Though the wheat was tempered to 18% moisture for 24 h with EO water, the subsequent exposure to infrared radiation brought down the moisture to about 16% which is the ideal tempering moisture for wheat. Milling of these wheat samples resulted in flour with greater reduction in bacterial

counts (up to 99.9%) and fungal counts (up to 99.7%). As the majority of microbial population is on the surface of the wheat, milling process itself reduces surface microbial loads to some extent. Hence the bacterial and fungal count of flour were consistently lesser than that of whole wheat.

This study showed that EO water on wheat has a pronounced antimicrobial effect when used separately, and in combination with infrared radiation. As the conventional wheat tempering process uses chlorinated water which has residue issues, the use of EO water is recommended as an eco-friendly process for improved microbial load reduction. Subsequent use of infrared radiation not only helps in moisture removal of wheat for milling, but also in further microbial load reduction. Exposure of tempered wheat to infrared radiation had adversely affected flour functionality (Deliéphan, 2013). Therefore, for tempering wheat, it would be practicable to use just EO water. Further studies are needed to be done on the economics of using EO water for tempering wheat.

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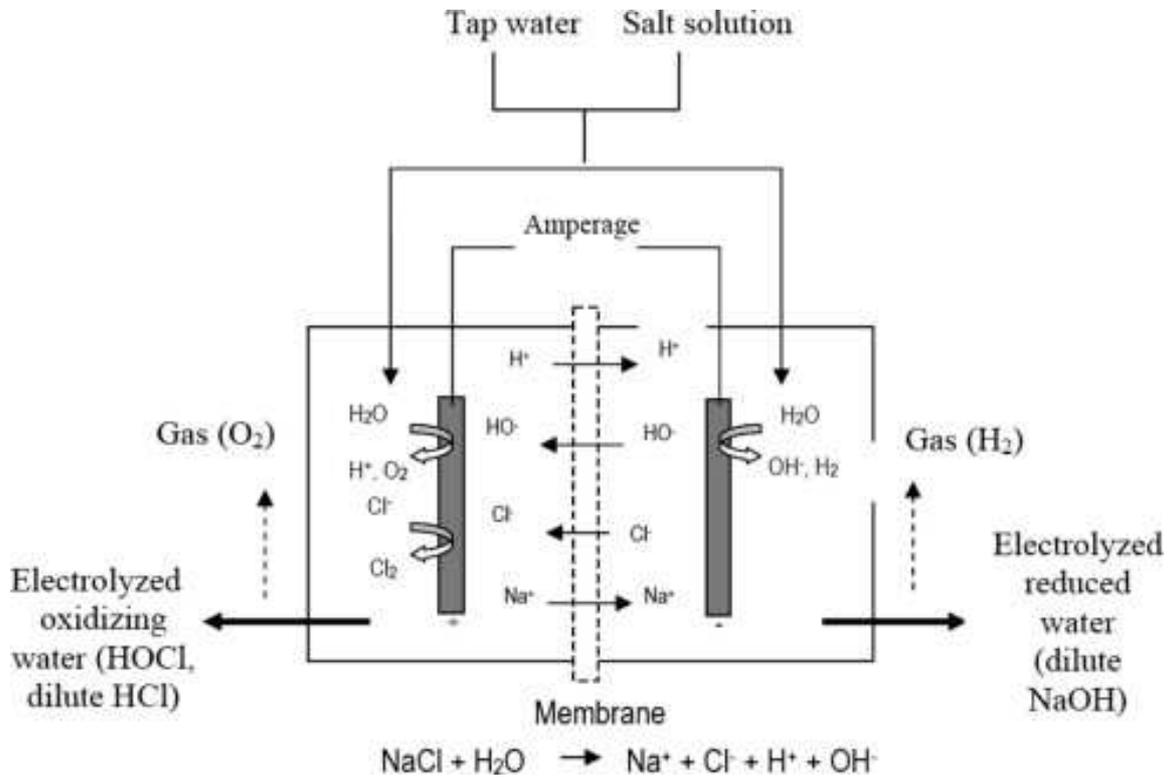
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Figure 5.1 Schematics of electrolyzed water generator and produced compounds (Source: Huang et al., 2008).



**Table 5.1 Treatment combinations used for tempering wheat with distilled water or EO water and exposing to infrared radiation to assess microbial load.**

Treatment	Factors:		
	Grain moisture (%)	Tempering time (h)	Infrared exposure time (min)
Control	11	0	0
Untempered; infrared treated	11	0	1.0
			1.5
			2.0
Distilled water-tempered; untreated	18	24	0
Distilled water-tempered; infrared treated	18	24	1.0
			1.5
			2.0
EO water-tempered; untreated	18	24	0
EO water-tempered; infrared treated	18	24	1.0
			1.5
			2.0

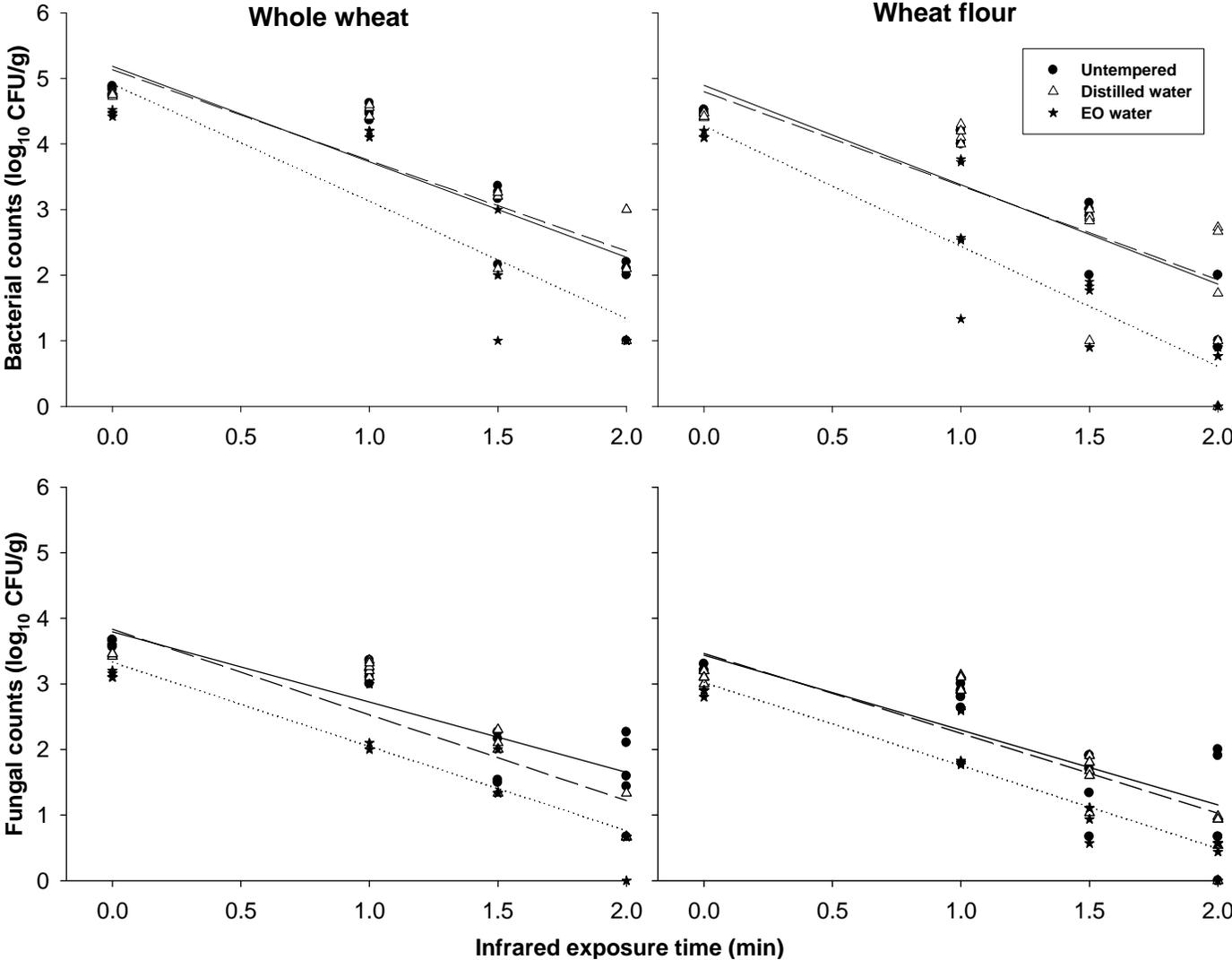
**Table 5.2 Total bacterial and fungal counts in untempered and tempered wheat exposed to infrared radiation for 0, 1, 1.5, and 2 min.**

Treatment	Infrared exposure time (min)	Mean $\pm$ SE <sup>a</sup>			
		Total bacterial counts (CFU/g)		Fungal counts (CFU/g)	
		Whole wheat	Flour	Whole wheat	Flour
Untempered <sup>b</sup>	0	$(6.67 \pm 0.38) \times 10^4$	-	$(4.33 \pm 0.21) \times 10^3$	-
	1	$(3.06 \pm 0.37) \times 10^4$	-	$(1.67 \pm 0.28) \times 10^3$	-
	1.5	$(1.87 \pm 0.17) \times 10^3$	-	$(1.67 \pm 0.15) \times 10^2$	-
	2	$(8.67 \pm 1.69) \times 10^2$	-	$(1.27 \pm 0.29) \times 10^2$	-
Distilled water	0	$(5.80 \pm 0.17) \times 10^4$	$(2.73 \pm 0.07) \times 10^4$	$(2.93 \pm 0.07) \times 10^3$	$(1.33 \pm 0.11) \times 10^3$
	1	$(3.40 \pm 0.31) \times 10^4$	$(1.53 \pm 0.17) \times 10^4$	$(1.93 \pm 0.19) \times 10^3$	$(1.20 \pm 0.26) \times 10^3$
	1.5	$(1.67 \pm 0.18) \times 10^3$	$(7.80 \pm 1.33) \times 10^2$	$(1.20 \pm 0.23) \times 10^2$	$(5.20 \pm 1.00) \times 10^1$
	2	$(7.33 \pm 1.63) \times 10^2$	$(3.33 \pm 1.01) \times 10^2$	$(4.00 \pm 0.70) \times 10^1$	$(1.30 \pm 0.30) \times 10^1$
EO water	0	$(2.93 \pm 0.12) \times 10^4$	$(1.40 \pm 0.07) \times 10^4$	$(1.47 \pm 0.08) \times 10^3$	$(8.00 \pm 0.33) \times 10^2$
	1	$(1.53 \pm 0.08) \times 10^4$	$(5.07 \pm 0.53) \times 10^3$	$(8.67 \pm 0.82) \times 10^2$	$(3.80 \pm 0.27) \times 10^2$
	1.5	$(6.67 \pm 1.05) \times 10^2$	$(3.33 \pm 0.70) \times 10^2$	$(7.30 \pm 0.70) \times 10^1$	$(2.50 \pm 0.30) \times 10^1$
	2	$(3.33 \pm 0.00) \times 10^2$	$(6.00 \pm 3.10) \times 10^1$	$(2.70 \pm 0.70) \times 10^1$	$(1.10 \pm 0.30) \times 10^1$

<sup>a</sup> Each mean is based on  $n = 5$  replications.

<sup>b</sup> Untempered wheat was not milled into flour.

**Figure 5.2** Linear regressions describing logarithmic decrease in bacterial and fungal counts of untempered wheat and wheat tempered to 18% moisture with distilled or EO water and exposed to infrared radiation for 0, 1, 1.5, and 2 min.



**Table 5.3 Linear regression parameter estimates and *D*-values of untempered and tempered wheat exposed to infrared radiation.**

Parameter	Total bacterial counts <sup>a</sup>			Fungal counts <sup>a</sup>		
	Untempered	Distilled water	EO water	Untempered	Distilled water	EO water
Intercept ( <i>a</i> )	5.01 ± 0.13	4.99 ± 0.15	4.67 ± 0.15	3.74 ± 0.11	3.70 ± 0.15	3.40 ± 0.19
Slope ( <i>b</i> )	-1.02 ± 0.09	-1.03 ± 0.11	-1.05 ± 0.11	-0.86 ± 0.08	-0.98 ± 0.11	-0.98 ± 0.14
<i>R</i> <sup>2</sup>	0.85	0.82	0.83	0.86	0.82	0.74
<i>D</i> -value (min) <sup>b</sup>	0.98	0.97	0.95	1.16	1.02	1.02

<sup>a</sup> Intercept and slope values are means ± SE (*n* = 5).

<sup>b</sup> *D*-value shows 1-log reduction in bacterial/mold count as a function of infrared exposure time in minutes.

**Table 5.4 Linear regression parameter estimates and *D*-values of untempered and tempered wheat exposed to infrared radiation and milled into flour.**

Parameter	Total bacterial counts <sup>a</sup>			Fungal counts <sup>a</sup>		
	Untempered	Distilled water	EO water	Untempered	Distilled water	EO water
Intercept ( <i>a</i> )	4.68 ± 0.13	4.73 ± 0.28	4.50 ± 0.28	3.37 ± 0.23	3.44 ± 0.21	3.11 ± 0.16
Slope ( <i>b</i> )	-1.02 ± 0.10	-1.20 ± 0.21	-1.45 ± 0.21	-0.90 ± 0.17	-1.09 ± 0.16	-1.03 ± 0.12
<i>R</i> <sup>2</sup>	0.85	0.65	0.72	0.61	0.73	0.80
<i>D</i> -value (min) <sup>b</sup>	0.98	0.83	0.69	1.11	0.92	0.97

<sup>a</sup> Intercept and slope values are means ± SE.

<sup>b</sup> *D*-value shows 1-log reduction in bacterial/mold count as a function of infrared exposure time in minutes.