USE OF OZONE AS AN ALTERNATIVE TO CHLORINE FOR TREATMENT OF SOFT WHEAT FLOURS

by

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AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

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Abstract

Ozonation was studied as an alternative to chlorination for cake flour. Ozone treatment in a wooden tumbler at room temperature was conducted. Unchlorinated flour was treated with ozone at the rate of 0.06 L/min for 10 and 36 min using 5 lb of flour. Ozonation of cake flour decreased pH and increased the lightness (L value) of flour. Baking studies using a high-ratio white layer cake formulation showed that the volume of cakes significantly increased ($p< 0.05$) as ozonation time increased and cakes were softer than those made with chlorinated or control flours. The cell brightness and number of cells measured by image analysis (C-Cell) of cakes from ozone treated flour for 36 min exhibited similar values to those from chlorinated flour. Cakes made from flours after lipid extraction and after lipid extraction plus ozonation had low volume, indicating that lipids play a role in cake performance. Identification of volatile compounds that contribute to the odor of ozone treated flour was conducted. Volatile compounds of chlorinated, unchlorinated, defatted control, and ozonated defatted flours were analyzed using a purge and trap instrument and GC-MS. Aldehydes, alcohols, hydrocarbons and other compounds were found in unchlorinated and chlorinated flours while the volatile compounds present in ozone treated flours were mainly aldehydes and ketones. A rapid reduction in volatile compounds was detected when ozone treated flours were stored uncovered in a fume hood. Ozonation of defatted soft wheat flour produced less volatile aldehydes than ozone treated whole flour.

The optimum temperature and time for ozone treatment in a metal tumbler using a response surface methodology design was studied. Time (5, 15, and 25 min) and temperature (25, 35 and 45 ºC) was used with three response parameters. The optimum ozonation time was about 8 to 11 min with the temperature range between 36 and 46 ºC. Ozone treatment for 5 to 25 min at room temperature showed an increase in unextractable polymeric protein, indicating a shift of protein to a higher molecular weight. Increase in Mixograph peak time, peak viscosity, and water retention capacity were observed as ozonation time increased. The ozone treatment did not affect the transition temperature and enthalpy change of the flour samples.

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CHAPTER 1 - Introduction

Ozone is a strong oxidizing gas produced naturally by ultraviolet irradiation and commercially generated by UV light or corona discharge. Ozone has a high oxidation potential which can be used to oxidize compounds in waste and waste water treatment. It is used as a disinfectant for drinking water and as an antimicrobial agent for controlling pathogens by the food industry, such as in meat, poultry, fruits, and vegetables (Kim *et al*., l999a). Ozone was approved in 2001 by the U.S. Food and Drug Administration as an antimicrobial agent in the form of either the gaseous or aqueous phase for the treatment, storage, and processing of foods including meat and poultry. For the applications of ozone to cereals and cereal products, published research has reported on the usage of ozone for washing and tempering of wheat, in the steeping process of corn, control of insects and fungi in stored grain, decontamination of mycotoxins and pesticides, and improvement of flour quality in grains. There has been limited study of ozonation in wheat flour. Ibanoglu (2001) studied hard and soft wheat grains tempered with ozonated water and investigated rheological properties of flour. Yvin *et al*. (2001), Dubois *et al*. (2006), and Mendez *et al*. (2003) applied ozone to wheat grain and studied resulting flour properties and baking performance. Earls (2003) studied the ozonation of cake flour and found that cakes produced from ozonated flour had higher volumes than those from the control chlorinated flour. Ozone has a unique odor. It alters the sensory attributes of food products, depending on the chemical composition of the food, ozone concentration, and operating condition. The sensory quality of some grains was decreased during ozone treatment due to lipid oxidation (Kim *et al*., 1999a). Volatile compounds mainly found in wheat flour are hexanal, nonanal, hexanol, octanol, 1-octen-3-ol, some enals, 2-methylnaphthalene, and naphthalene (Seitz, 1995 and Buttery *et al*., 1978). The objectives of the present study were:

1) To investigate the effect of ozone on the properties of soft wheat flour and to study the potential use of ozone treated flour for cake manufacture.
2) To investigate the volatile compounds in ozone treated flour and the effects of the flour lipids on the volatile compounds.
3) To study the effects of time and temperature variation during ozonation on flour properties and cake quality.

CHAPTER 2 - Literature Review

2.1 Ozonation

Ozone is a strong oxidizing gas with high oxidation potential formed in the atmospheric layers, stratosphere and troposphere. Stratospheric ozone is formed (0.05 mg/liter) by interaction of solar ultraviolet irradiation with oxygen, producing single oxygen atoms that readily react with other oxygen molecules to form ozone (O_3). "Tropospheric ozone is produced by photochemical oxidation of compounds including nitrogen oxides and volatile organic compounds emitted from automobile exhausts, industrial emissions, gasoline vapor, and natural sources" (Kim *et al.*,1999a; Graham, 1997; U.S. Environmental Protection Agency, 2007). In the upper atmosphere, high energy ozone decomposes to oxygen by UV irradiation. As a result of this reaction, the UV irradiation is absorbed before passing through to the earth's surface (Graham, 1997)

2.1.1 Decomposition of ozone

Ozone is readily decomposed in aqueous solution. It may directly react with dissolved substances or decompose via radical chain reactions, forming radicals that further react with various solutes in the solution (Staehelin and Holgne, 1985; Khadre *et al.*, 2001). Khadre *et al.* (2001) described the decomposition of ozone in water following first order kinetics. The pathways are as followed:

Direct reaction of ozone with organic compounds (M):

$O_3 + M \rightarrow M_{ox}$

Decomposition of ozone in water into free radical:

$O_3 \xrightarrow{OH^-} \cdot OH \xrightarrow{M} M_{ox}$

The half life of ozone in aqueous solution at 20 °C is 20 to 30 min (Graham, 1997). Graham (1997) stated that the half life of ozone depends on the ozone reactive materials available in aqueous solutions. "The half life can be as short as seconds in dirty water but can be about an hour in clean water". Decomposition of ozone increases at high pH and with the mechanical energy applied (Khadre et al., 2001). Moreover, ozone decomposition at specific pH values is accelerated by a radical chain reaction in the presence of organic solutes that can act as initiators, promoters, or inhibitors (Staehelin and Holgne, 1985).

Staehelin and Holgne (1985) proposed a mechanism for the kinetics of ozone decomposition in aqueous solution containing organic and inorganic solutes as impurities. The decomposition steps are as follows: initiation, propagation, and termination. For the reaction of ozone in the presence of solutes, the initiation step, the rate limiting step, occurs by reaction of ozone and OH^{-} ion producing a superoxide anion ($\cdot O_2^{-}$) and a hydroperoxyl radical ($HO_2\cdot$). Solutes in aqueous solution may react with ozone and form an ozonide ion radical ($\cdot O_3^{-}$). The propagation step includes decomposition of an ozonide ion radical ($\cdot O_3^{-}$) to a hydroxyl radical ($\cdot OH$). The hydroxyl radical further reacts with solutes to form an organic radical ($R^{\cdot}$) which then reacts with O_2 with the formation of $ROO^{\cdot}$, leading to formation of $HO_2\cdot$ and $\cdot O_2^{-}$. For the termination step, the hydroxyl radical ($\cdot OH$) reacts with organic and inorganic substrates to form secondary substrates without producing $\cdot O_2^{-}$ or $HO_2\cdot$. Therefore, these substrates terminate the chain reaction. Compounds that promote a conversion of $\cdot OH$ to $\cdot O_2^{-}$ include formic acid, primary and secondary alcohols, glyoxylic acid, and humic acids, while compounds that terminate the chain reaction consist of carbonate, aliphatic alkyl compounds, and *tert*-butyl alcohol.

The half life of ozone in air is long (12 hr) compared to that in aqueous solution (Graham, 1997; Xu, 1999). The long half life is probably due to a chain reaction initiated by hydroxide in aqueous solution that leads to the formation of free radicals (Bott, 1991). The reaction of ozone in the gas phase with various compounds produces highly active free radicals as intermediates including hydroxyl radicals and other free radicals. Many researches focus on the gas phase reaction at atmospheric pressure and low pressure of ozone with compounds such as alkenes, 2,3-dimethyl-2- butene, and 2-methyl-2-butene (Grosjean and Grosjean, 1997). The reaction of alkenes with ozone receives much attention in relation to atmospheric chemistry. The reaction can be explained by the Criegee mechanism. The mechanism of ozone and alkene reaction in the gas phase consists of two steps. The first step includes cycloaddition of ozone on the carbon-carbon double bond, producing primary oxonide which is then decomposed to produce a Criegee intermediate (carbonyl oxide) and carbonyl. For the second step, the Criegee intermediate, then isomerizes to dioxirane and dissociates to a hydroxyl radical, CO, CO_2 and other products (Martinez et al., 1981; Kroll et al., 2001a,b). Grosjean et al. (1994) stated that the subsequent reaction of the Criegee intermediate leads to the formation of different types of compounds such as dicarbonyl, hydroxycarbonyl, hydroxyl radical and other products. Grosjean and Grosjean (1997) stated that the hydroxyl radical reacts faster with alkenes than ozone does, and it can react with the carbonyl produced during reactions between alkene and ozone.

2.1.2 Reaction of ozone with organic compounds

Ozone can oxidize both inorganic and organic compounds. Ozone reacts with organic compounds using two pathways, cyclo-addition or reaction with a free radical. Organic compounds that react with ozone mainly contain multiple bonds or nucleophilic sites (O, N, S, P). Ozone can oxidize various types of organic compounds dissolved in water such as alcohols, ethers, aldehydes, carboxylic acids, aromatic compounds, phenols, amines and amine derivatives, pesticides, surfactants, and natural organic compounds such as amino acids, and carbohydrates. (Bablon *et al*., 1991).

The reaction of ozone with biological molecules has received more attention within the past few decades due to its potential hazard to humans, plants, and animals. "The geometry of biological molecules determines the accessibility of ozone, and the nature of damage of the substrates" (Pryor *et al*., 1984). Biological damage from ozone occurred by formation of free radicals from the peroxidation of polyunsaturated fatty acids, and oxidation of proteins, thiols, and amines (Mehlman and Borek, 1987). Ozone reacts with double bonds of unsaturated fatty acids by 1-3 dipolar cyclo addition and the main products formed are aldehydes, acids, and hydrogen peroxide (Bablon *et al*., 1991). Zhao and Cranston (1995) studied the effect of ozone treatment at a concentration of 6.7 mg/L on volatile oil constituents of ground black pepper by using GC and GC-MS and concluded that changes in the volatile oils occurred during ozone treatment. Some compounds disappeared while other compounds such as aldehydes occurred from oxidation of hydrocarbons.

For amino acids, ozone highly reacts at neutral and basic pH. Ozone reacts with both amine groups and R groups of amino acids. The R groups of amino acids (alkyl, sulfur, aromatic or unsaturated heterocyclic groups) produced different types of product including acids, aldehydes, nitrate ions, ammonia, aromatic aliphatic acids, and condensation products (Bablon *et al*., 1991). Pryor *et al*. (1984) studied the kinetics of ozonation of amino acids in water and drew conclusions about the effects of ozone on biological systems. For amino acids, the α-amino group mostly reacts with ozone. However, in polypeptides or proteins, the α-amino group is linked with the other carboxyl groups, resulting in the formation of a peptide bond. As a result, the side chain groups of amino acids of polypeptides and proteins such as cysteine, tryptophan, methionine, tyrosine, histidine, and cystine in proteins mainly react with ozone. Mudd *et al*. (1969) studied the reaction of gaseous ozone with amino acid solutions. Cysteine highly reacts with ozone, followed by methionine, tryptophan, tyrosine, histidine, cystine, and phenylalanine, respectively.

"The reaction of ozone with polysaccharides causes breaking of the glycosidic bonds, oxidation of free monosaccharides, and formation of aldehydes and aliphatic acid products" (Bablon *et al*., 1991). Katai and Schuerch (1966) studied the mechanism of ozone reacting with cellulose and cellulose derivatives and found that ozone initiated autooxidation processes that resulted in the formation of peroxides, carbonyls, carboxyls, and lactones. Another mechanism occurred when ozone hydrolyzed the glycosidic linkages of methyl α-D-glucoside, which produced glucose as a main product.

2.1.3 Ozone production

Ozone is an unstable gas which is normally generated and used at the production line (Rice 1997). Ozone can be produced by irradiation of oxygen with ultraviolet light at a wavelength of 185 nm (Kim *et al*., 1999a; Rich, 1994). For industrial uses, ozone is normally generated by corona discharge. This method involves passing air or oxygen across a discharge gap of two electrodes, one of which contains dielectric material. High voltage is applied to the electrodes and energetic electrons in the corona split oxygen molecules, followed by the formation of ozone by oxygen combining with split oxygen atoms (Kim *et al*., 1999a; Rice, 1997; EPA, 1999). Rich (1994) stated that the electrical energy input is mostly dissipated as heat (85 to 95 %). Ozone production from a corona discharge is affected by voltage applied, current frequency, discharge gap, dielectric properties, and feed gas flow rate (Kim *et al*., 1999a; Rice, 1997; EPA, 1999).

EPA (1999) described the ozone operating systems by corona discharge as consisting of four parts; a gas feed system, ozone generator, ozone contactor, and off-gas destruction system. A gas feed system is a source of oxygen for the ozone generator. Air or oxygen can be used as a gas feed system. The advantage of using oxygen over air is that oxygen produces higher concentrations of ozone than air (8-14 % against 3-5 %). The ozone contactor is a place either in the gas or aqueous phase where the reaction of ozone with compounds occurs and off-gas destruction systems are required for destroying ozone residues. Ozone can be destroyed by using high temperatures above 350 ºC or by a combination of temperature (above 100 º C) and catalyst. The limit of ozone concentration in the off gas destruction systems is 0.1 ppm as set by OSHA for a worker exposed for an eight hour shift. The cooling system is required to minimize thermal decomposition of ozone by heat.

2.1.4 Factors affecting the efficiency of ozone

The decomposition rate of ozone involves complex mechanisms and this depends on various factors such as pH, temperature, and concentration of compounds. The factors affecting the susceptibility of microorganisms to ozone have received much attention from researchers. Kim *et al*. (1999a) stated that environmental factors which affect microorganism inactivation by ozone include temperature, pH of the medium, relative humidity, organic compounds surrounding the cell, and other additives such as acids and sugars. Yang and Chen (1979) stated that the disinfection properties of ozone in water depend on contact time, temperature, pH, and inorganic and organic compounds present. Generally, long contact time, low pH, and low temperature increase the disinfection properties of ozone.

2.1.4.1 Temperature

As the temperature increases, the reaction rate of ozone and decomposition rate increase but its solubility and stability decreases. Rice *et al*. (1982) stated that ozone lost its antimicrobial effect when the temperature was above 10 ºC. Liew and Prange (1994) reported that temperature affected the residue concentration of ozone. Residue concentration was calculated from ozone supplied minus exhausted and reacted ozone. It increased as ozone supply increased but decreased when storage temperature increased. Achen and Yousef (2001) studied the *E. Coli* O157:H7 counts on the surface of apples after treatment with bubbling ozonated water at temperatures of 4, 22, and 45 ºC and found that *E. Coli* counts decreased as temperature increased without significant difference even when the residue ozone concentration at low temperature was higher than that at high temperature. The authors proposed that increase in activity of ozone was balanced by a decrease in its stability. Katai and Schuerch (1966) stated that temperature affects ozonization of carbohydrates. “At high temperature, the solubility of ozone decreased while the rate of decomposition increased and glucose formation from the interaction of ozone with methyl glucoside increased. This resulted from the balance between increasing ozone activity at elevated temperature and decreasing activity at lower ozone concentrations". El-Din *et al*. (2006) studied the kinetics of oxidation of resin and fatty acids by ozone and found that the oxidation rate of linoleic acid and abietic acid increased as the ozone concentration and temperature increased.

2.1.4.2 pH

Ozone is more stable in aqueous solutions at low pH. The decomposition of ozone increases at high pH due to the reaction of ozone with hydroxyl ions, resulting in the formation of free radicals (Kim *et al*., 1999a). El-Din *et al*. (2006) found that the oxidation of linoleic acid and abietic acids in the presence of ozone (7 to 11 mg/L) increased as pH increased from 8 to 11 due to the production of highly reactive intermediates such as hydroxyl radicals.

2.1.4.3 Organic compounds

Organic compounds present compete with microorganisms for reaction with ozone so they minimize the disinfection properties of ozone (Khadre *et al*., 2001).

2.1.4.4 Relative humidity

The rate of decomposition of ozone increases as the relative humidity increases. The optimum relative humidity for applying ozone in a cold storage room is 90-95 %, which effectively controls the growth of microorganisms at the surface of perishable foods (Rice *et al*., 1982)

2.1.5 Ozone measurements

Measurement of ozone in the gas phase and liquid phases is used to analyze the ozone concentration in the ozone generator output, ozone transfer efficiency in the contactor, and ozone residues (Bablon *et al*., 1999).

2.1.5.1 Gas phase ozone measurement

There are several methods for measuring ozone concentration in the gas phase, including the iodometric method, UV absorption, calorimetry, and chemiluminescence. The iodometric method and UV absorption are the methods recommended by the International Ozone

Association (IOA) for measurement of ozone concentration in the process gas from the ozone generator (Bablon *et al*., 1991; Rakness *et al*., 1996).

The UV absorption method is based on "absorption of ozone in the short UV wavelength region with a maximum absorbance at 253.7 nm. The gas phase absorption coefficient for ozone is 3000 ± 30 $M^{-1}cm^{-1}$ at 273 K and 1 atm" (Bablon *et al*., 1991). This method measures the different readings between light intensity in the presence and absence of ozone. The concentration readings can be displayed either on a volume basis (g/m^3, mg/L, and ppm by volume) or on a mass basis (% wt) (Rakness *et al*., 1996).

The iodometric method is based on oxidation of iodide ion with ozone. Ozone gas is passed through a potassium iodide solution, causing oxidation of iodide ion, resulting in formation of iodine. The pH of solution is adjusted to pH 2 and a solution containing starch as an endpoint indicator is titrated against standardized sodium thiosulfate (Bablon *et al*., 1991; Rakness *et al*., 1996). This method measures both ozone and other free radicals produced during ozone decomposition including $HO_2\cdot$, $\cdot O_3^-$, and $\cdot O_2^-$ (Khadre *et al*., 2001).

The calorimetric method measures the difference in temperature that is proportional to the concentration of ozone between the inlet gas and gas that reacts in the catalyzer (Bablon *et al*., 1991). The chemiluminescence method determines low concentrations of ozone in the ambient air at a concentration of 0.5-1.0 ppm. This method is based on the chemiluminescent reaction between ethylene and ozone (EPA, 1999).

2.1.5.2 Aqueous phase ozone measurement

The methods for measuring residual ozone consist of the indigo colorimetric method, acid chrome violet K method, the iodometric method, and UV absorption. The indigo colorimetric method is precise and more selective compared to other methods, since ozone decomposition products and other compounds such as hydrogen peroxide and chlorine do not interfere with the reaction (EPA, 1999). This procedure measures the change in absorbance of indigo trisulfonate solution. The indigo molecule containing one carbon-carbon double bond reacts with ozone causing decolorization of the solution, which can be detected at 600 nm by using a spectrophotometric method (Bader and Hoigne, 1981).

For the acid chrome violet k method, decrease of absorbance at 550 nm is detected by the reaction of ozone with chrome violet k (ACVK). The interference by manganese, chlorine, organic peroxides, and oxidation products is less for this method (EPA, 1999; Bablon *et al*., 1991).

2.1.6 Applications for ozone

The properties of ozone such as strong oxidizing power and ready decomposition to free radicals without leaving chemical residues makes it broadly applicable to the food industry, water and waste water treatment, as well as treatment of drinking water (Graham, 1997). In 1886, the ability of ozone to disinfect contaminated water was first realized by Se Meritens and the first full scale plant for water treatment with ozone was established in Germany (1902) by Siemens and Halske. In the United States, commercial scale application of ozone treated drinking water plants was established in Niagara Falls, N.Y by 1903 and there were almost 200 plants producing ozone treated drinkable water by 1987 (Langlais, *et al*., 1991; Graham, 1997).

Early applications of ozone in the United States had limited use only for removal of contaminants in water, but the usage of ozone as a disinfectant started to increase. "Ozone was approved in 1982 by the U.S. Food and Drug Administration as generally recognized as safe (GRAS) for specific use as a disinfectant in bottled water". Now, it is approved by U.S. Food and Drug Administration for use as an antimicrobial agent in the form of gaseous or aqueous phase for the treatment, storage, and processing of foods including meat and poultry (Federal Register, Food and Drug Administration, 2001) (Fan *et al*., 2007).

Ozone in both the gas and aqueous phases can be used by the food industry for many purposes. It acts as a sanitizer and antimicrobial agent that efficiently inhibit various types of microorganisms such as bacteria, molds, yeasts, and viruses (Kim *et al*., 1999a). The oxidizing power of ozone is 1.5 times stronger than chlorine, which gives it greater disinfectant properties compared to chlorine (Xu, 1999). Ozone inactivates bacteria by causing damage to their cell constituents such as unsaturated lipids in the microbial cell envelope, the lipopolysaccharide layer of gram negative bacteria, intracellular enzymes, and microbial genetic materials (Kim *et al*., 2003). Ozone affects enzymes by oxidizing double bonds of unsaturated lipids and sulfhydryl groups in cysteine residues, which disrupts their activity such as membrane permeability (Khadre *et al*., 2001).

Numerous studies have focused on antimicrobial properties of ozone in foods resulting from an increase in foodborne disease outbreaks. Gaseous ozone can be used to increase the shelf life of foods such as fruits and vegetables during storage and transportation. Aqueous ozone can be applied as an antimicrobial agent to sanitize food surfaces, packaging materials, and food equipment. Applied aqueous ozone decreases microbial spoilage in fresh and cut fruits and vegetables, meat and poultry carcasses, and in chilled water (Graham, 1997, Xu, 1999, and Kim *et al*., 1999a)

Treatment of ozonated water in fruits and vegetables can decrease microbial contamination on the surface. Cherry (1999) reported that the decrease in pathogenic microorganisms of fruits and vegetables by washing with antimicrobial agents was affected by various factors such as the attachment of microbes in the form of biofilms to the fruit and vegetable surfaces and the irregular shapes of produce. The usage of 1-4 ppm of ozone to wash fruits and vegetables decreases microbial population by 1-3 log numbers. Kim *et al*. (1999b) used ozone to disinfect spoilage bacteria found in lettuce and concluded that the disinfection properties of ozone depended on the type of microorganism, the amount of contamination, physiology of the lettuce, water quality, temperature, pH, and reactor design. Direct ozone bubbling (4.93% v/v at 0.5 L/min) into the reactor in combination with high speed stirring (300 rpm) or stomaching is more effective at inactivating microorganisms on lettuce-water mixtures than ozonated water alone. Achen and Youself (2001) observed a decrease of *E coli* during dipping of inoculated apples with *Escherchia coli* O157:H7 in bubbled ozonated water (24.5 mg/l) for 3 min.

Sheldon and Brown (1986) applied 3-4.5 ppm of aqueous ozone for 45 to 60 min to disinfect the bacterial load in poultry chill water, spent chiller water, and broiler carcasses after chilling with ozonated water and during storage of chilled carcasses for 11 days. Aqueous ozone effectively disinfected pathogenic and spoilage microorganisms in broiler carcasses after chill and during storage, and in chiller water without diminished quality of the carcass in terms of sensory attributes and surface color. The spent chilling water also showed a decrease in bacterial loads and 33 % reduction in chemical oxygen demand (COD).

Gaseous ozone can extend shelf life of foods such as strawberries, bananas, potatoes, cheeses, eggs, and meats. Rice *et al*. (1982) stated that ozone can inactivate growth of molds, fungi, and odor causing chemicals during storage and transportation under high relative humidity and near freezing point temperature. Ozone extends shelf life of ripening fruits and vegetables such as tomatoes, bananas, and strawberries by reacting with ethylene, breaking carbon-carbon double bonds leading to formation of water and carbon dioxide (Rice *et al*., 1982). Zhao and Cranston (1995) studied microbial decontamination of ground black pepper by ozonised air with an ozone concentration of 6.7 mg/L and at a flow rate of 6 L/min and concluded that ozone treatment decreased the microbial count of ground black pepper inoculated with *Salmonella* spp, *E*. coli, *Penicillium* spp and *Aspergillus spp* by 3-4 log numbers. William *et al*, (2005) studied the inactivation of *Escherichia coli* O157:H7 and *Salmonella* inoculated in apple cider and orange juice and concluded that ozone treatment for 60 min (0.9 g/h at a flow rate of 2.4 L/min) in combination with dimethyl dicarbonate (500 ppm) or hydrogen peroxide (300 ppm or 600 ppm) followed by storage at 4 ºC for 24 h decreased microbial populations more than 5 log numbers. This was comparable to thermal pasteurization standards that require a 5 log reduction in pathogen populations.

A limited amount of research has focused on usage of ozone in cereals and cereal products. The published research has included the usage of ozone for washing and tempering of wheat, the steeping process of corn, controlling insects and fungi in stored grain, decontamination of mycotoxins and pesticides, and improvement of flour quality in grain (Ibanoglu, 2001, Ibanoglu, 2002, Yvin *et al*. 2001, Dubois *et al*. 2006, and Mendez *et al*. 2003). Ibanoglu (2001) studied the effect of soft and hard wheat grains tempered with ozonated water compared to those tempered with water. Tempering wheat grains at 20 ºC for 24 hr using ozonated water (1.2 and 11.5 mg ozone/L) had no effect on the milling efficiency of hard and soft wheat kernels. The results showed that tempering with ozonated water did not change alpha amylase activity of soft and hard wheat flours as measured by Falling Number. Results from farinograph and extensograph tests on the flours did not show significant differences between tempering wheat with water or ozonated water, indicating that there was no oxidizing effect on wheat kernels during tempering. The lightness (L values) and yellowness (b values) of flours from ozonated water did not show significant differences from those tempered with water, indicating that ozonated water did not oxidize pigments in wheat flours. Reduction of bacteria, yeasts, and mold counts was observed when tempering wheat with ozonated water. Ibanoglu (2002) investigated the effect on flour properties of washing soft and hard wheat kernels with water and ozonated water. Wheat samples were washed with ozonated water at a concentration of 1.5 mg/L for 30 min followed by drying in a tray drier at 30 ºC and milling to flours. Washing hard and soft wheat kernels with ozonated water did not change the alpha amylase activity, lightness, or yellowness of flour samples as compared to those washed with water. Results from the farinograph did not show any significant differences among hard and soft wheat flours washed with either water or ozonated water. Extensograph parameters of soft wheat flour showed a decrease in dough extensibility and maximum resistance to extension of flour washed with ozonated water, compared to flour washed with water.

Yvin *et al.* (2001) patented the method and equipment for treatment of grains with aqueous and gaseous ozone, expecting to make flours with less microbial and pesticide contamination. The production schemes were as follows: storage of grain, first cleaning, grain moistened with ozonated water (18 % final moisture content) and, rested for 24 hr, Then, ozonation in the reactor for 15 to 40 min at the rate of 4 to 5 g of ozone/kg of grain for dough and bread making and 8-20 g of ozone/kg of grain for sweetened products. The ozone concentration in the carrier gas varied between 80 and 160 g/m^3 STP (standard temperature and pressure) and pressure between 200 and 500 bar. This was followed by a second cleaning, and grinding into flours. The experimental results for flour made from wheat grain showed about 99 % reduction of microbial counts including mesophilic aerobic flora, total coliforms, molds, and yeasts. The reduction of mycotoxins, particularly ochratoxine A, and pesticide were observed. Ozonated wheat flour doughs showed an increase in strength and a decrease in extensibility measured by the Chopin alveograph as ozone application increased. Sweetened products such as English and Genoese fruit cake type made from ozone treated flour did not collapse after baking. The author suggested usage of ozone at a concentration of 3g/kg of grain to decrease dough extensibility for industrial manufacture of doughs and breadmaking products.

Ozone can be used to control pests in stored grain. Treatment of maize with ozone (50 ppm) for 3 days destroyed 92-100% of insects such as adult maize weevils and adult red flour beetles in infested maize (Kells *et al.*, 2001). Kells *et al.* (2001) and Mendez *et al.* (2003) described the movement of ozone into grains which consists of two phases. “The first phase starts when ozone is passed through the column of grain and the concentration of ozone decreases rapidly due to the interaction of ozone with compounds at the grain surfaces. For the second phase, little degradation of ozone occurs due to elimination of the active sites at the surface of grains.” Mendez *et al.* (2003) reported that treatment of soy bean, paddy rice, popcorn, hard wheat, and soft wheat with gaseous ozone (50 ppm) for 30 days had no effect on nutrition and properties of kernels. The fatty acid and amino acid compositions of soy bean, hard wheat, soft wheat, and maize did not change during ozone treatment. Ozone did not change the milling efficiency or the kernel pigment. There were no significant changes in bread making properties such as mixing tolerance, flour absorption, mixing time, loaf volume and specific volume of hard wheat during treatment by ozone.

The Oxygreen ® process was developed to improve flour quality, and to decrease insects, mycotoxins, and pesticide contamination (Dubois *et al*., 2006). It can be used for cakes such as English high ratio cakes, sponge cake, and bread, to modify flour properties without the addition of ascorbic acid or alpha amylase. It was approved by The French Food Safety Authority (AFSSA) in 2004 (Gaou *et al*., 2005). Ozone was applied to the reactor after the cleaning step and before passing of grains to the first break roll. Ozone reacts with pericarp and can pass through the testa and aleurone layers (Gaou *et al*., 2005). Dubois *et al*. (2006) studied safety of Oxygreen ® process on wheat grains. The authors concluded that the Oxygreen ® process did not alter vitamins, ferulic acid, phytates, proteins, carbohydrates, or lipid contents when treated with ozone at a dose of 5g, 8g and 12g of ozone consumed /kg of grains. The lipid oxidation detected by hexanal concentration present did not show much difference between ozone treated grain and the control. The amount of malondialdehyde, an indicator of polyunsaturated fatty acid oxidation, decreased as ozone dose increased. The result indicated that "the process did not induce the radical oxidation that increases lipid oxidation". Ozone increases proteolytic activity while inhibiting other enzymes such as amylase, and lipase activities. The molecular weight distribution of gliadins was not affected by an increase in proteolytic activity. The amounts of sugars including glucose, maltose, and fructose increased significantly after ozone treatment.

Ruan *et al*. (2004) studied the utilization of ozone in corn wet milling. Normally, sulfur dioxide was used in the steeping process to help control the growth of microorganisms and break down disulfide bonds of protein, leading to easy separation of starch from protein. The corn wet milling industry has tried to find an alternative method to replace sulfur dioxide since it is harmful to the environment and human health when discharged to the air and in waste water. Ozone can be used as an alternative to sulfur dioxide because of its high oxidant power and disinfectant properties. Ozone aided the steeping (OAS) process and had a high starch yield comparable to steeping of corn with sulfur dioxide when ozone was applied in the steeping water at different times (1 hr each at 1st, 13th, and 24th hr) for 36 or 48 hr periods at 20 ºC or 50 ºC. The lower temperature of 20 ºC of the ozone-aided steeping process decreased energy consumption compared to the conventional SO_2 method that generally operated at 52 ºC.

2.1.7 Ozone and chlorine toxicity

Chlorine is commonly used in the food industry as a sanitizing agent. Aqueous chlorine is applied for sanitizing food equipment and food containers (100-200 ppm), cooling sterilized canned foods (1-2 ppm), and washing raw fruits and vegetables (1-5 ppm) (Wei *et al*., 1985). However, there are some health concerns about the disinfection by-products (DBPs) of chlorine such as trihalomethanes (THMs) and other disinfectant residues (Xu, 1999). Types of disinfection by-products produced varied, depending on disinfectant type and dose, type and concentration of organic matter present, pH, temperature, and organic nitrogen concentration (EPA, 1999). Chlorine reacts with natural organic matter to produce different types of by-products including halogenated organic by-products such as trihalomethanes (THMs) and haloacetic acids, inorganic by-products such as bromate ion, and organic oxidation by-products. Many halogenated compounds exhibit carcinogenic activity (EPA, 1999). Brominated compounds also form when free chlorine and ozone reacts with bromide ion, leading to formation of brominated DBPs such as bromoform (EPA, 1999; Wei *et al*., 1985)

Ozone can be used as an alternative to chlorine with some benefits. The oxidizing power of ozone is greater than chlorine, and by-products of ozone treatment are less harmful compared to chlorine since halogen-substituted DBPs are not formed in the absence of bromide (EPA, 1999). Gaou *et al*. (2005) studied the toxicity of the Oxygreen ® process on rats. The study was conducted by treating wheat grain with the Oxygreen ® process and studying the effects in 10 male and 10 female rats. The results observed included clinical haemotological, blood biochemical, urinary, and pathology examinations. The results did not show an adverse affect of the Oxygreen ® treatment. Changes of some parameters were observed after feeding with the Oxygreen ® treatment, including an increase in rectal temperature in female rats, slight decrease of calcium concentration in male rats, and a slight decrease in blood cell count. Therefore, the authors concluded that the Oxygreen ® treatment could be considered to be safe for consumers.

2.2 Chlorination

Chlorination of cake flour was introduced in the early 1930s to improve baking properties of high ratio cakes (high % sugar/flour) (Gough *et al*., 1978; Thomasson et at., 1995). Chlorine acts as a bleaching and improving agent. The optimum level of chlorination produced cakes that do not collapse during baking and have a fine uniform grain, white crumb color, are more tender, and have improved symmetry and volume (Sollars, 1958, Pyler *et al.*, 1988; Thomasson et at., 1995).

For reaction of chorine with flour, chlorine reacts with water present in flour to form hypochlorous acid (HOCl) and chlorine ion (Cl^-). Hypochlorous acid is a weak acid and can dissociate to hypochlorite ion (OCl^-) and hydrogen ion (H^+). Hypochlorite ion is a strong oxidizing agent which acts upon the flour pigments and hydrogen ions present, lowering the pH of flours (Wei *et al.*, 1985; Gough *et al.*, 1978).

Soft wheat flour used for cakes is produced from blends of flour streams that have low ash and protein contents. The extraction rate is in the range of 45-60 % (Pyler, 1988). Properties of cake flour are usually as follows: protein content 8.5±0.5%, ash content 0.36±0.04%, and particle size 10±0.5 μm. Cake flours have lower water absorption, finer particle size, and less damaged starch compared to bread flour (Pyler, 1988)

2.2.1 Chlorination treatment

Commercial cake flour can be treated with chlorine within the range of 5-10 oz/sack (280 lb) or 1100-2300 ppm (Gouch *et al.* 1978). In the United States, chlorine gas is applied at the rate of 0.5 to 2.5 oz per cwt of flour (Pyler, 1988). Chlorinated flour is produced continuously by agitating flour of low protein content (less than 9%) in a stream of chlorine gas. The pH decrease of flour is an indicator of the reaction between gas and flour and optimum gas addition. The amount of chlorine required varies depending on different crop years and ash content. A pH of 4.7-4.9 is normally desired for most high ratio cakes (Gouch *et al.*, 1978; Pyler, 1988; Hoseney, 1994; Stauffer, 1990).

2.2.2 Distribution of chlorine in the flour components

All flour components such as gluten, starch, lipids, pentosans, and water solubles chemically react with chlorine. Gouch *et al.* (1978) stated that one third of chlorine associates with flour lipids, one half with protein, and one fifth to one seventh with carbohydrate, including starch. Huang *et al.* (1982) reported that the protein fraction obtained from an air classifier contained a high level of chlorine and chlorine uptake increased with an increase of chlorine dose. Varriano-Marston (1985) stated that only 15-20% of chlorine reacts with starch components of chlorinated flour. Even small proportions of the starch component react with chlorine, A study by Johnson *et al.* (1979) showed that the starch fraction was responsible for baking improvement of chlorine treated flour. Sollars (1958) conducted an experiment by fractioning unbleached and bleached cake flours into four fractions, water solubles, gluten, tailings, and prime starch and then reconstituted them with interchange between the bleached and unbleached fraction. The author concluded that prime starch and gluten were responsible for cake improvement. Whistler *et al.* (1966) reported that the reaction of chlorine on semidry wheat starch is due to oxidative polymerization, leading to the cleavage of glycosidic bonds and formation of D-glucose and D-gluconic acids. Johnson *et al.* (1980) reported that the drying method of starch isolated from untreated flour affected cake baking performance. Cake baked from reconstituted flour when starch was air dried gave similar properties to cake made from starch obtained from chlorine treated flour. The authors concluded that improvement in baking properties of starch made from chlorine treatment and air drying is due to oxidative polymerization by cleavage of α-1,4 bonds between glucose residues or oxidative cleavage between C-2 and C-3 carbons in glucose residues. Varriano-Marston (1985) stated that chlorination did not affect X-ray diffraction or crystallinity of starch. Chlorine changed starch surface properties by increasing surface hydrophobicity. “The oxidative polymerization of starch interrupted the chains connecting crystallites and the amorphous region made starch structure more relaxed and open and resulted in increase of surface porosity which can bind tightly with water and can bind more oil”. Seguchi (1984) and Seguchi (1987) reported that increase in hydrophobicity on the surface of wheat starch treated with chlorine was observed, suggesting that chlorinated surface proteins may cause the hydrophobic character of chlorinated starch granules, which helps to improve bubble stability by associating in the bubble (air-water) interface. In addition, the surface proteins extracted from wheat starch granules showed more hydrophobicity and slight polymerization of some large molecular weight proteins at the surface when the chlorination level increased (Seguchi, 1990).

Gluten proteins are also affected by chlorination. Chlorine cleaves peptide bonds, which causes an increase of protein solubility in water. Degradation of aromatic amino acids and oxidation of SH groups was also observed during chlorine treatment. “The oxidation of SH groups indicated the maturity effect of chlorine in dough” (Tsen and Kulp, 1971; Kulp, 1972). Kulp (1972) stated that chlorine could cleave high molecular weight proteins through oxidative and hydrolytic action. Daviau *et al*. (1996) studied the extractability of wheat flour proteins from chlorinated and unchlorinated flour. Changes in protein extractability were observed when flour was chlorinated and SH groups decreased in treated flour while no change in SS- bonds was detected. Tsen and Kulp (1971) stated that the disruption of inter- and intra- molecular noncovalent bonds and breaking of peptide linkages may increase protein extractability.

The reaction of chlorine with flour lipids involves different reaction mechanisms. Daniels (1960) stated that chlorine treatment caused a decrease in linoleic acid contents mainly from the addition of chlorine at double bonds rather than oxidation. A further study by Daniels (1963) indicated a decrease of about 60.6 % of unsaturated fatty acid content such as oleic, linoleic, and linolenic acids and an increase of about 16.3 % of new material in the chromatogram measured by Gas liquid chromatography. The identification of compounds showed the presence of compounds derived from dichlorostearic forming by direct addition of chlorine to the unsaturated bonds of oleic acid.

Numerous studies focused on the effects of lipids from no treatment and chlorine treatment on the properties of cakes. These have shown inconsistent results. Johnson *et al*. (1979) conducted a study to investigate the effect of chlorine treated and untreated lipids on the properties of cakes. Cake baked from untreated and chlorine treated flour defatted with petroleum ether had poor baking properties in terms of grain structure and volume of cakes. However, they regained their original properties when extracted lipid was reconstituted. There was no quality difference between cake baked from chlorine treated nondefatted flour and flours reconstituted with lipids from untreated and chlorine treated flours. This indicated that chlorine improves baking properties of other components of flours rather than lipid components. However, Kissell *et al*. (1979) reported that lipids extracted from chlorinated flour by hexane improved the volume of cake and internal scores compared to lipids from untreated flour when interchange of lipids and extracted residues were carried out. Donelson *et al*. (1984) agreed with the results from Kissell *et al*. (1979). The results showed that the reconstitution of lipids extracted by hexane from chlorinated flour significantly improved volume of cake and crumb score compared to those from unbleached flour.

2.2.3 Mechanisms during mixing and baking

Cake batter is a complex mixture composed of dispersed phases in an aqueous medium (Hoseney, 1994). Air bubbles are incorporated into the batter during mixing and the batter becomes aerated and more viscous as more air bubbles are incorporated. Air bubbles move slowly due to high batter viscosity, reducing the rate of flotation of small air bubbles which then merge into larger bubbles. The production of small and uniform air bubble sizes is important since they are less buoyant than large bubbles and the driving force for bubble coalescence is reduced (Hoseney, 1994; Payne, 1995).

"During baking, leavening gas CO_2 and water vapor migrate from the aqueous phase into bubble nuclei, the pressure inside the entrapped bubbles increases, and the volume of cake increases due to the expansion of gas in the bubbles" (Payne, 1995). The viscosity of batter drops during baking, allowing air bubbles to move freely. At this point, the viscosity of the batter should be sufficient to minimize air bubble coalescence and flotation to the surface, and keep the dense starch granules suspended before gelatinization of starch occurs (Hoseney, 1994; Payne, 1995). During late stages of baking, starch granules gelatinize to set the cake structure. The starch granules absorb water and swell, resulting in an increase in viscosity of the batter. Starch gelatinization is affected by sugar. High sugar levels in cakes tend to delay onset of gelatinization to 85-90 ^{o}C. Flour treated with chlorine can swell more than untreated flour, making the batter more viscous than untreated flour and providing support to prevent collapse of cake during cooling. Flour protein is part of the skeletal network for cakes. Some fraction of proteins stabilizes gas cell walls and the air/water interfaces (Hoseney, 1994; Payne, 1995). Gouch *et al*. (1978) stated that a cake's structure must have sufficient strength to resist the stresses that occur during cooling to prevent collapse of cakes. Chlorine treatment improves the strength of the cake crumb structure as indicated by an increase in cake firmness.

2.2.4 Flour, Batter, and Cake properties

Chlorine treatment affects flour properties. The pH of flour decreases when chlorine level increases. The carotenoid pigments in flour are reduced due to the bleaching effect of chlorine (Kulp, 1972). Kulp and Tsen (1972) reported that flour pasting properties are affected by chlorination. The viscosity of chlorine treated flours as measured by the Brabender Viscograph increased faster than untreated flour at temperatures of 50 ºC-75 ºC. The increase in viscosity improved gas retention and increased batter stability in the early stages of baking. Gaines and Donelson (1982) stated that cake batter made from chlorine bleached flour with optimum liquid levels caused high increases in pasting viscosity.

Gouch *et al*. (1987) stated that chlorine treatment does not affect starch pasting properties and gelatinization temperature. Kulp (1972) stated that viscoamylograph properties of starch isolated from unbleached and bleached flour indicated no change in pasting behavior. High sugar concentration in cake batter delays the onset of gelatinization of starch to 90 ºC (Bean *et al*, 1978). The reasons for the delay of starch gelatinization have been suggested by some research groups. Sugar may limit the water available for starch, causing delay of gelatinization (Hoseney *et al*. 1977). Sugar reduces the water activity of the starch water system. "Sugar may interact with starch chains in the amorphous region, limiting the flexibility of chains and requiring more energy to pull crystals apart, resulting in higher gelatinization temperature" (Spies and Hoseney, 1982).

Flour absorption increases with increasing chlorine level, indicating an increase in the hydration capacity of the starch component. Dough stability as measured by the Farinograph increased up to 2 oz of chlorine per cwt and then decreased, indicating oxidative and hydrolytic changes to proteins (Kulp 1972). It was suggested that the increase in water absorption is due to increased permeability of the starch granule surface (Conforti and Johnson, 1992). Huang *et al*. (1982) stated that chlorine treatment increased swelling power and solubility of the starch fraction obtained from the air classifier at temperatures of 90 ºC and above. Studies of heating characteristics by DSC of untreated and chlorine treated flour in simple flour (flour to water ratio= 1:1) and batter systems (flour-sugar-shortening-water = 1:1.3:0.279:1) did not show noticeable differences. The transition temperature (T_i) of untreated and chlorine treated flour in the simple systems was 64 ºC while the transition temperature in the batter system increased to 76 ºC. The starch granules from chlorinated flour batter showed high swelling and loss of birefringence compared to untreated batter at 90 ºC. Russo and Doe (1970) also stated that the difference between treated and untreated flour with chlorine was not observed until the final few minutes of baking (temperature reaching 90 ºC).

An increase in protein extractability by water and acetic acid was observed by Tsen and Kulp (1971). Changes in surface hydrophobicity of gliadins from unchlorinated and chlorinated flour was observed by Sinha *et al*.(1997). The difference between three dimensional plots of retention time, wavelength, and absorbance plotted from RP-HPLC of unchlorinated and chlorine treated flours may suggest conformational changes. Decrease in enthalpy (ΔH) and slight increase in denaturation transition temperature (T_d) of gliadins measured by DSC from chlorinated flour may indicate partial folding and increased hydrophobicities.

Chlorinated flour produced cake crumb that was drier and less sticky than untreated flour, which is gummy in texture (Donelson *et al*., 2000). Seguchi and Matsuki (1977) stated that chlorination improved springiness and volume of pancakes. Reconstitution studies showed that improvement of springiness and gumminess of pancakes were caused by the prime starch fraction and volume of pancakes was influenced by the gluten fraction.

2.2.5 Alternative treatment to chlorination

Chlorine treatment of cake flour improves the baking quality of high ratio cakes. The advantage of chlorinated flour over unchlorinated flour was that chlorination altered flour lipid, resulting in increasing batter expansion and chlorination also changed starch properties, leading to a rapid increase in the viscosity of the batter (Donelson *et al*., 2000; Clements and Donelson, 1982 a,b; Gains and Donelson,1982). Gough *et al*. (1978) stated that an alternative treatment should overcome the collapse of high ratio cakes after cooling. Development of the treatment process or modification of the formulation can be used to solve the problem. Russo and Doe (1970) showed that heat treatment of flour at a temperature of 120 ^{o}C improves baking performance of high layer sponge cakes. Donelson *et al*. (2000) developed the formula for production of cake without using chlorine treated flour. The formula, consisting of a blend of non chlorinated flour and wheat starch (60:40), 18 % egg albumin, 0.15 % xanthan gum, and 0.1 % lecithin, produced good quality cakes in terms of visual characteristics, size, and contour. Textural properties of the cakes were comparable to cakes made from chlorine treated flour. Thomasson *et al*. (1995) developed a method to replace chlorine treatment for cake flour by using heat treated flour. Unchlorinated flour treated at 125 ^{o}C for 30 min with addition of 0.12 % (fwb) xanthan gum produced higher volume cakes than did chlorinated flour, and gave a similar crumb structure.

2.2.6 Volatile compounds of grains and flours

Seitz (1995) reported that more than one hundred volatile compounds in wheat cultivars from several locations in Kansas harvested in 1992 and 1993 were detected by using the dynamic headspace sampling method and Gas chromatography-Fourier transform infrared spectroscopy-Mass spectrometry (GC-IR-MS). Volatile compounds mainly found were alcohols followed by aldehydes, alkanes, alkylbenzenes, ketones, methyl esters, naphthalenes, terpenes, and other compounds. Compounds associated with off-odor in grains were detected, including 1-octen-3-ol, 2-pentanol, and 3-methyl-1-butanol (Seitz, 1995). Volatile compounds mainly found in wheat flour are hexanal, nonanal, hexanol, octanol, 1-octen-3-ol, some enals, naphthalene, and 2-methylnaphthalene (Seitz, 1995; Buttery *et al*., 1978). Lipids associated with starch are probably accountable for off-odors and off-flavors in starches. The main surface lipids in cereal starches are polyunsaturated fatty acids which account for 50 % (Sayaslan *et al*., 2000; Morrison, 1988). Sayaslan *et al*. (2000) reported that volatile compounds in commercial and laboratory prepared wheat starches measured by a purge and trap concentrator interfaced to a gas chromatograph equipped with a Fourier transform infrared detector (FTIRD) and a mass selective detector (MSD), were mainly aldehydes followed by alcohols, ketones, benzenes, esters, and terpenes. The major compounds detected were the degradation products of lipid oxidation including hexanal, heptanal, octanal, nonanal, decanal, benzaldehyde, 2-propanone, 2-propanol, 1-butanol, 2-ethyl-1-hexanol, methyl benzene, and tetradecane

CHAPTER 3 - Materials and Methods

Part 1 Ozone treatment using wooden lab scale chlorination box blender

3.1.1 Materials

Unchlorinated and chlorinated soft wheat flours from the same wheat were provided by ConAgra Foods (Alton, IL). All vegetable shortening was donated by ADM packaged oils (Decatur, IL). Non fat dried milk was provided by Ryt-way Foods Inc (Northfield, MW). Double acting baking powder was obtained from Clabber Girl (Terre Haute, IN). Fine granulated sugar was obtained from Domino Foods Inc. (Baltimore, MD).

3.1.2 Analytical determination

Protein contents of flour samples were analyzed using a LECO FP-2000 Nitrogen/Protein Analyzer. A factor of 5.7 was used to convert nitrogen (N) to protein. Moisture content was determined according to AACC method 44-15A (AACC 2000).

3.1.3 Ozone production

Ozone gas was generated by a pilot scale ozone generator (Clear Water Tech, Inc., San Luis Obispo, CA., USA) using oxygen produced by an oxygen generator (Dwyer Instruments, Inc., San Luis Obispo, CA., USA). The rate of ozone production as a function of time (10, 20, 30, 36 and 40 min) was measured by an iodometric method for determination of ozone in a process gas. Ozone gas was passed through three sets of glass wash bottles containing 250 ml distilled water, 4 g of potassium iodide, and 15 ml of 0.3 M sulfuric acid for a period of time(10 to 40 min) and then the solution was titrated against 0.01 N sodium thiosulfate containing starch indicator solution. Ozone concentration was calculated from ml of sodium thiosulfate usage.

3.1.4 Production of ozone treated flour

The ozone gas was tumbled in the wooden lab scale chlorination box blender filled with 5 lbs of non chlorinated soft wheat flour. The ozone was introduced at the rate of 0.06 L/min for times of 10, 20, 30, 36, and 40 min.

3.1.5 Properties of flour

3.1.5.1 pH measurement

The pH of flour samples was measured using AACC method 02-52 (AACC 2000). Ten grams of flour were added to 100 ml of distilled water. The flour suspension was placed on a stirring plate and was stirred for 15 min. After that, flour samples were allowed to stand for 10 min and then the supernatant liquid was decanted and used for pH measurement.

3.1.5.2 Color measurement

The color of flour samples was measured using a Minolta colorimeter. The L*, a*, b* values were recorded.

3.1.6 Dough mixing properties

Dough mixing properties of flour samples were measured using a 10 g Mixograph (National Manufacturing Co., Lincoln, NE) according to AACC method 54-40A (AACC 2000). Two percent sodium chloride was added instead of distilled water. Mixing parameters including peak development time, midline peak height, and width at peak were measured.

3.1.7 Baking tests

Baking tests of flour samples were conducted to study cake baking potential. All cakes were baked according to AACC method 10-90 (AACC 2000).The optimum level of water and double acting baking powder used was determined. The white layer cake formula is shown in Table 3.1. The procedure for cake baking was as follows: all dry ingredients were sifted and transferred to the mixing bowl, shortening and 60 % of distilled water was added. The ingredients were mixed at a low speed using a Hobart mixer for 30 sec, then scraped down and mixed at medium speed for 4 min. One half of the remaining water (20 %) was added to the batter and mixed at a low speed for 30 sec then mixed at medium speed for 2 min. The remaining water (20 %) was added to the batter and mixed at low speed for 30 sec and then again mixed at medium speed for 2 min. 425 g of cake batter was transferred into two greased pans and baked at 190 ^{o}C (375 ^{o}F) for 22 min.

Table 3.1 Formula for high ratio white layer cakes

Ingredients	Flour Basis (%)
Flour (14% mb)	100.0
Sugar	140.0
Shortening(emulsified)	50.0
Non fat dried milk	12.0
Dried egg white	9.0
Salt	3.0
Baking powder	5.5
Distilled water	135.0

3.1.8 Baking quality of cakes

3.1.8.1 Volume of cakes

Volumes of cakes were measured with a plastic measurement template according to AACC method 10-91 (AACC 2000). Volume index was calculated.

3.1.8.2 Textural properties

A texture analyzer (TA.XT2, Stable Micro System, Ltd.) was used to measure hardness, springiness and cohesiveness of cakes. Texture profile analysis (TPA) was performed using a 1 inch diameter cylinder probe. The TPA test setting was set as follows: pretest speed, test speed and post test speeds were 2.0 mm/sec, a distance of 10 mm was used to compress the sample, time between each stroke was 3.0 seconds, auto trigger feature was set at 5 grams, and data was collected at 200 pps. For sample preparation, each cake was cut into 4 pieces with dimensions of 2 inch wide, 2 inch deep, and 1 inch tall.

3.1.8.3 Color of cakes

The color of the cake crumb was measured with a Minolta colorimeter (CR-300). The L*, a*, b* values were recorded.

3.1.8.4 Gas cell structure

Gas cell structure of cakes was performed with an image analysis instrument (C-Cell imaging system, Calibre Control International Ltd., UK). Cakes were sliced with an electric knife and central slices of 15 mm thickness were used. Image analysis parameters including slice brightness, number of cells, and cell diameter were used to compare different treatments.

Part 2 Volatile compounds of ozone treated flour measured by GC-MS

3.2.1 Materials

Two types of flour, unchlorinated soft wheat flour provided by ConAgra Foods (Alton, IL) and defatted soft wheat flour from the same wheat, were used in this experiment.

3.2.2 Lipid extraction of unchlorinated flour

Unchlorinated flour was extracted with chloroform according to MacRitchie and Gras (1973). Three extractions were performed using 200 g of flour and 400 ml of chloroform for each extraction. The flour suspension was filtered through a Whatman No. 1 filter paper and then the defatted flour was placed in a fume hood for 12 hours for chloroform evaporation at room temperature.

3.2.3 Ozone treatment

Unchlorinated flour was treated with ozone gas for 10, 20, 30, 36 and 40 min according to the procedure mentioned in Part 1. Defatted flour was treated with ozone for 10 and 36 min. Ozone treated flours with ozonation times 10 and 36 min were kept in a fume hood overnight. Analysis of volatile compounds was performed using Gas chromatography and Mass Spectrometry (GC-MS).

3.2.4 Analysis of volatile compounds

3.2.4.1 Dynamic headspace sampling

Volatile compounds of flour samples were collected using a Tekmar purge and trap instrument as described by Seitz *et al*.(1999). Two gram of sample was placed in a U-shaped sparge tube connected to a Hewlett-Packard purge-and-trap instrument (model G1901A-60500) equipped with a sample pocket heater (model 14-5737-020) and a capillary interface module (model G1908-60500). The sample was preheated to 70 ºC for 3 min, and then the volatiles from the heated samples were purged with helium onto a Tenax trap. After 2 min purge time, a 5 min dry purge was performed to remove excess moisture from the Tenex trap, and the collected volatiles were preheated at 200 ºC and then at 225 ºC for 5 min to desorb the volatiles. With the capillary interface module, the desorbed volatiles were cryofocused at -140 ºC by liquid nitrogen at the top of the gas chromatography (GC) column and released into the GC column by rapid heating of the cryofocused zone at 225 ºC for 0.85 min.

3.2.4.2 Analysis of Volatiles by Gas chromatography and Mass Spectrometry (GC-MS)

A model 5890 series II gas chromatograph (GC) coupled with a model 5971 series mass selective detector (MSD), from Hewlett Packard Co (Palo Alto, CA) were used to analyze volatile compounds of flour samples. A BPX35 column (50 m x 0.32 mm i.d. x 0.25 µm film thickness) from Scientific Glass Engineering Inc. (Austin, TX) was used for separation. Column head pressure was 31 kPa (4.5 psi) at 50 ºC. Carrier gas was helium at a constant flow rate of 1.2 mL/min. Oven temperature was set at 40 ºC, increased to 140 ºC at a rate of 7.0 ºC/ min and then to 260 ºC at a rate of 17.5 ºC/ minute. Effluent from the column was passed through a MS detector. MSD conditions were as follows: direct transfer line temperature, 280 ºC ; ion source temperature, 280 ºC ; ionization voltage, 70 eV; mass range, 33-300 amu; scan rate, 1.91 scans/s; and electron multiplier voltage, 1800 V.

3.2.4.3 Identification of Volatile compounds

Identification of volatiles was based on computer matching of the observed mass spectral data with the standard mass spectra in the Wiley Registry of Mass Spectral Data, 6th ed. (Palisade Corp., Newfield, NY). The total ion chromatogram (TIC) peak areas estimated the relative abundances of volatile compounds and their levels in the samples.

3.2.5 Flour properties

3.2.5.1 Size Exclusion High Performance Liquid Chromatography (SE-HPLC)

SE-HPLC was used to determine protein molecular size and molecular size distribution of samples. Total protein, extractable, and unextractable protein were analyzed. SE-HPLC was performed by using a Hewlett Packard 1100 system with UV detector at 214 nm. A BioSep-Sec-S4000 size exclusion column (Phenomenex, Torrance, CA) was used for protein fractionation. The elution solvent was deionized water and acetonitrile (1:1), both containing 0.05% trifluoroacetic acid (TFA). The flow rate of the elution solvent was set at 0.5 ml/min. For total protein analysis, 10 mg of sample was weighed and suspended in 1 ml of 0.05 % SDS and 0.05 mM dibasic sodium phosphate buffer, pH 6.9. Samples were vortexted for 5 min and were sonicated at an output of 6 watts for 15 sec. The samples were then centrifuged at 12000xg for 20 min. The supernatants were decanted, filtered and then transferred to HPLC glass vials. For extractable protein, the same procedure was followed without sonication. The unextractable protein sample was obtained from the residue of extractable protein. The same procedure as total protein was followed except sonication for 25 sec instead of 15 sec.

3.2.6 Baking tests

Cake baking tests were performed to study baking performance of the flours obtained by different treatments. Baking tests were performed according to AACC method 10-90 (AACC 2000). Volume of cakes was measured with a plastic measurement template AACC method 10-91 (AACC 2000). A texture analyzer (TA-XT2, Stable Micro Systems, Ltd.) was used to measure hardness, springiness and cohesiveness of cakes. Gas cell structure of cakes was performed with an image analysis instrument (C-Cell imaging system, Calibre Control International Ltd., UK).

Part 3 Ozonation treatment with time and temperature control

3.3.1 Materials

Unchlorinated soft wheat flour provided by ConAgra Foods (Alton, IL) was used in this experiment.

3.3.2 Ozonation treatment with variation in time and temperature

Response surface methodology was conducted to study the optimized time and temperature for ozone treatment. Unchlorinated soft wheat flour was tumbled in a metal drum (Miac Braunschweig) where ozone gas generated by an ozone generator with the rate of ozone production 2.54 g/ hr was passed through for 5 to 25 min and with temperature controlled at 25 to 45 ^oC. A central composite design with the coded values and the actual values is given in Table 3.2. The coded values equal to (actual values-midpoint)/ (range/2). The center point (35 ^oC and 15 min) was replicated 5 times while other treatments were replicated once.

Table 3.2 Temperature and time combinations tested in response surface methodology experimental design.

Treatments	Coded values		Actual values	
	Temperature (^oC)	Time (min)	Temperature (^oC)	Time (min)
1	-1.00000	-1.00000	25	5
2	-1.00000	1.00000	25	25
3	1.00000	-1.00000	45	5
4	1.00000	1.00000	45	25
5	-1.41421	0.00000	21	15
6	1.41421	0.00000	49	15
7	0.00000	-1.41421	35	1
8	0.00000	1.41421	35	29
9	0.00000	0.00000	35	15
10	0.00000	0.00000	35	15
11	0.00000	0.00000	35	15
12	0.00000	0.00000	35	15
13	0.00000	0.00000	35	15

3.3.2.1 Baking quality of cakes

The flour samples were baked and cake volumes were measured by a plastic measurement template AACC method 10-91 (AACC 2000). A texture analyzer (TA-XT2, Stable Micro Systems, Ltd.) was used to measure firmness of cakes. Gas cell structure of cakes was performed with an image analysis instrument (C-Cell imaging system, Calibre Control International Ltd., UK).

3.3.2.2 Cake batter properties

3.3.2.2.1 Specific gravity of cake batter

Batter specific gravity was determined by measuring the weight of a cup filled with water and the weight of cake batter of the same volume.

3.3.2.2.2 Viscosity of cake batter

Cake batter viscosity was determined using a Brookfield Synchro-lectric viscometer model LVt (VS-4) (Brookfield Engineering Laboratories, Inc., Stoughton, MA). Spindle number 4 was used with spindle speed at 6 rpm. The viscosity was measured in centipoises.

3.3.3. Heat treatment of flours

The effect of heat treatment on the properties of flour was studied by tumbling unchlorinated flour in the metal tumbler with temperature controlled at 49 ^{o}C for 15 min. Specific gravity and viscosity of cake batter were measured. The cake baking test was performed according to AACC method 10-90 (AACC 2000). Volumes of cakes were measured by a plastic measurement template. Gas cell structure was measured with an image analysis instrument (C-Cell imaging system, Calibre Control International Ltd., UK).

3.3.4 Ozone treatment

The effect of ozone treatment was measured by applying ozone gas to the unchlorinated flour in the metal tumbler at room temperature (21° C) for 5, 10, 15, 20, 25, and 40 min.

3.3.4.1 pH measurement

The pH of flour samples was measured using AACC method 02-52 (AACC 2000).

3.3.4.2 Gelatinization and pasting properties

The pasting properties of flour were determined using a Rapid Visco Analyser (Newport Scientific) according to AACC method 76-21 (AACC, 2000). 3.5 g of flour (14 % moisture basis) and 25 ml of distilled water were used. The parameters: peak viscosity, breakdown, final viscosity, set back, peak time, and pasting temperature were analyzed.

3.3.4.3 Water retention capacity

Water retention capacity was determined according to AACC methods 56-11 (AACC 2000). 0.2 g of flour was weighed into 2.0 ml microfuge tubes and 1 ml of deionized water was added. The samples were vortexed for 5 sec and allowed to swell for 20 min and then vortexed again at time intervals of 5, 10, 15, and 20 min, followed by centrifugation at 3000 rpm for 15 min. Supernatants were discarded and tubes were drained for 10 min before weighing the tube and pellet.

3.3.4.4 Thermal properties

Thermal properties were determined by using a differential scanning calorimeter (Perkin-Elmer Corporation, Norwalk, CT). Flour samples were weighed into DSC aluminum pans and water was added in the ratio of water to flour, 3:1. The heating was carried out between 25 ^{o}C to 180 ^{o}C with a heating rate of 10^{o}C/min. Gelatinized onset temperature, peak temperature, ending temperature, and enthalpy change (delta H) were determined.

3.3.4.1 Size Exclusion High Performance Liquid Chromatography (SE-HPLC)

SE-HPLC was performed using a Hewlett Packard 1100 system with UV detector at 214 nm according to the method mentioned in Part 2. Total protein, extractable protein, and unextractable polymeric protein were determined.

3.3.4.2 Dough mixing properties

A 10 g Mixograph (National Manufacturing Co., Lincoln, NE) was used to determine dough mixing properties of flour samples according to AACC method 54-40A(AACC 2000). Mixing parameters were analyzed including peak development time, midline peak height, and width at peak

3.3.4.3 Cake batter properties

Batter specific gravity and cake batter viscosity were determined according to the methods described above.

3.3.6.3 Statistical analysis

Data were analyzed using SAS (version 9.1, SAS Institute Inc., Cary, NC). Analysis of results was performed using analysis of variance (ANOVA). Differences among means were compared using least significance difference ($\alpha = 0.05$). For the response surface methodology experiment, data were analyzed by the response surface regression procedure using SAS. Surface response plots were plotted using SAS and RSM plus (AEW Consulting, Lincoln, NE, 1992).

CHAPTER 4 - Results and Discussion

Part 1 Ozone treatment using wooden lab scale chlorination box blender

Experiments were conducted to study the effect of ozone treatment on the properties of soft wheat flour and the cake baking potential of ozonated flour. Three types of flour were used in this experiment; unchlorinated control flour, chlorinated flour and flour treated with ozone gas for 10 to 40 min. Proximate analyses for moisture content (%), protein (%), and total lipid (%) of unchlorinated control flour and chlorinated flour are given in Table 4.1.

Rate of ozone production (mg/kg of flour) as a function of time (10, 20, 30, 36, and 40 min) as measured by the iodometric method is given in Figure 4.1. The result showed an increase in ozone concentration as ozonation time increased. Figure 4.2 shows the volume of cakes made from flour treated with ozone for 10, 20, 30, 36, and 40 min. The volume of cakes increased as the ozonation time increased up to 36 min and then decreased. This result was in agreement with the result of Earls (2003) who treated flour with ozone for 10 to 50 min and concluded that flour treated with ozone for 36 min gave the largest volume compared to all ozonated cake flours. Flour treated with ozone for 36 min had higher volume than control untreated flour and chlorinated flour, and flour treated with ozone gas for 10 min gave volumes of cakes comparable to chlorinated flour. From the results, a further study was conducted to compare the properties of flours and cakes from untreated flour, chlorinated flour and flour treated with ozone for 10 and 36 min.

Table 4.1 Proximate analysis of untreated soft wheat flour and chlorinated flour,

Flours	Moisture content (%)	Protein (%)	Lipid (%)
Control (unchlorinated)	12.48	7.53	0.56
Chlorinated flour	12.43	7.53	0.45

Figure 4.1 Rate of ozone production as a function of time

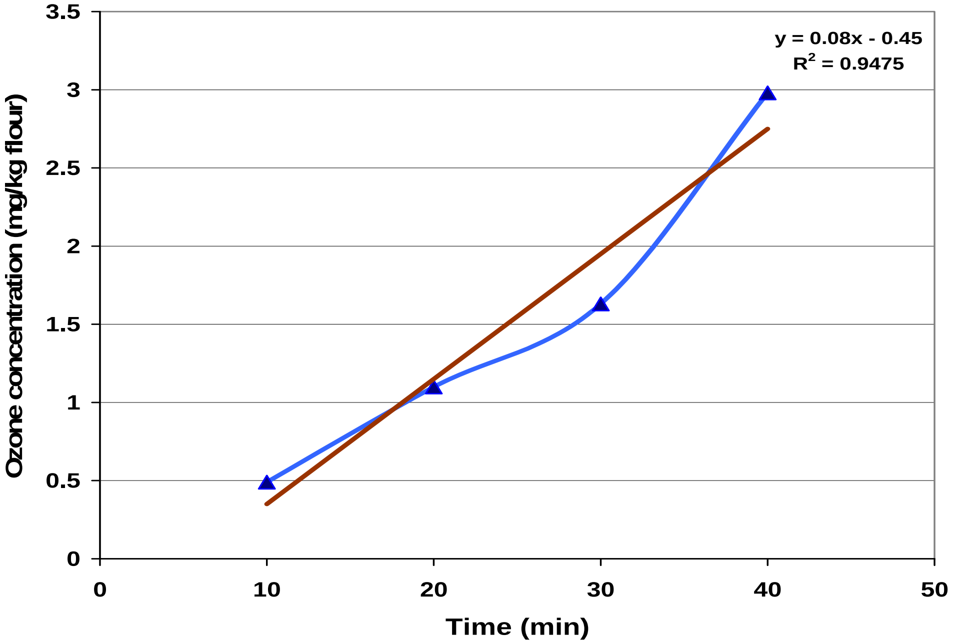


Figure 4.2 Volume of cakes made from flour treated with ozone gas for 10, 20, 30, 36, and 40 min.

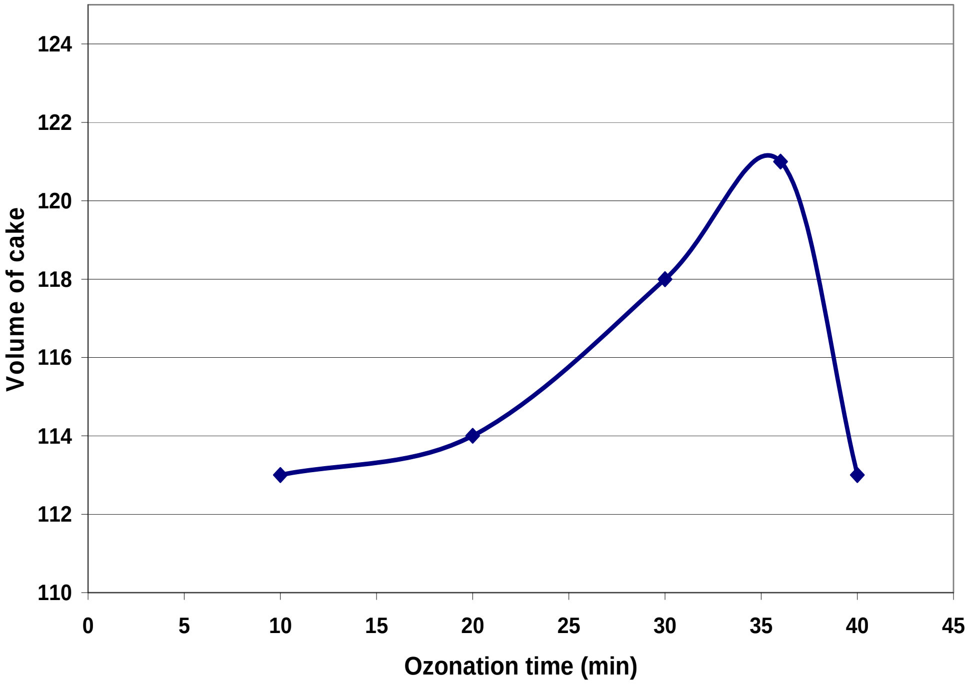


Table 4.2 pH and color of untreated soft wheat flour, chlorinated flour and flour treated with ozone gas at 10 and 36 min.

Flours	pH	Color of flour [a]		
		L*	a*	b*
Control (unchlorinated)	6.07	91.26a	-2.59a	8.10a
Chlorinated flour	4.49	91.36a	-1.78c	5.03b
10 min ozonated	5.96	92.24a	-1.94b	5.09b
36 min ozonated	5.66	91.80a	-1.88bc	5.26b

[a] Means with the same letter within columns are not significantly different ($p>0.05$)

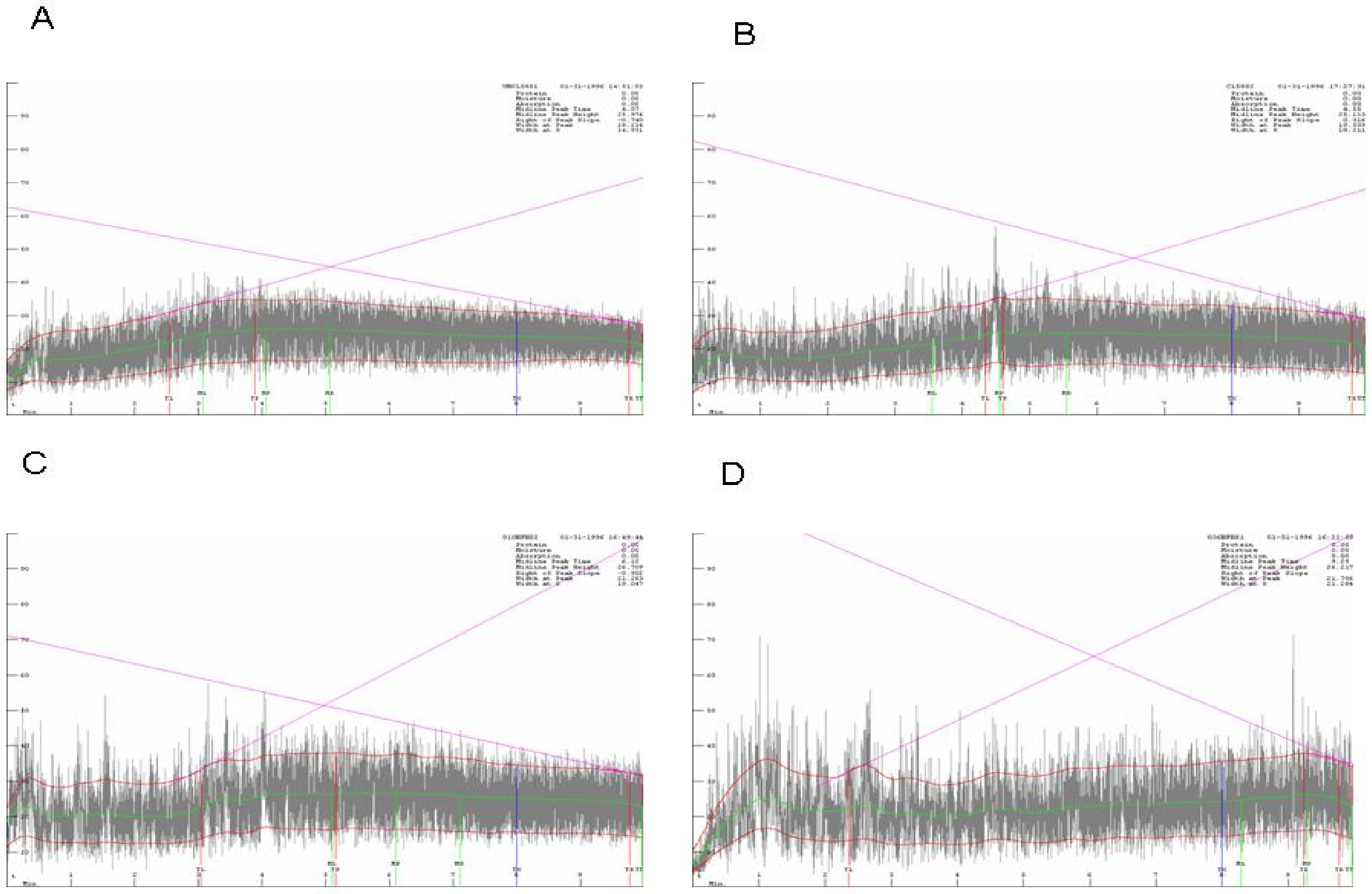
4.1.1 Properties of flour

The pH of unchlorinated flour was 6.07 and pH of chlorinated flour was 4.49. The pH of 10 and 36 min ozonated flour decreased to 5.96 and 5.66 respectively (Table 4.2). The pH of flour treated with chlorine decreased due to formation of hydrochloric acid during the reaction of chlorine with flour components (Hoseney, 1994; Stauffer, 1990). A decreasing pH of ozone treated flour was detected and it might occur from the oxidation of flour components by ozone leading to the formation of acid products. Langlais *et al*. (1991) stated that ozone can oxidize carbohydrate, amino acid, and unsaturated fatty acid compounds, leading to the formation of acid products.

The lightness (L*) of both ozonated flours (Table 4.2) were not significantly different from unchlorinated and chlorinated flour ($p>0.05$). The b values, indicating yellowness of flour of unchlorinated control flour was significantly higher than chlorinated flour and flour treated with ozone for 10 and 36 min ($p<0.05$). The results indicate that ozone has an ability to oxidize the yellow flour pigments such as the carotenoids. Liew and Prange (1994) reported the bleaching effect of ozone on ozone-treated carrots. Ozone treated carrots were high in L* value (lighter color) and low chroma values (less intense in color) compared to control carrots without ozone treatment.

Mixing properties of flours measured by the Mixograph are given in Figure 4.3. Flour treated with ozone for 36 min gave the longest mixing time (8.51 min) followed by flour treated with ozone for 10 min (5.47 min), chlorinated flour (4.90 min), and untreated control flour (4.62 min), respectively. The result showed that ozone treated flour was relatively stronger than unchlorinated control and chlorinated flour and ozone affected the mixing properties of flour by increasing mixing time and mixing tolerance (slow break down of dough). High mixing time indicates increase of dough stability, elasticity, and mixing tolerance and decrease of dough extensibility (Finney and Shogren, 1972). The oxidative action of ozone with flour components may explain these results. The viscoelastic properties occurring during the development stage in dough mixing involve the large molecular size glutenins and there is no need to form chemical bonds such as disulfide links. The properties can be modified if additives such as oxidizing or reducing agents are added in the formula (MacRitchie, 2003). Ozone may act like other oxidants by oxidizing the protein components. Nakamura and Kurata (1997) stated that oxidants can oxidize SH groups of cysteine residues in protein molecules, leading to the formation of inter- and intramolecular SS bonds. The oxidants such as potassium bromate and potassium iodate oxidize SH groups, resulting in the formation of inter and intra molecular disulfide bonds, causing an increase of polymerized protein. Both low molecular weight and high molecular weight glutenin subunits were polymerized when they were treated with oxidants. (Schropp *et al.*,1995 and Veraverbeke *et al.*, 2000a,b). However, oxidants used in bread making did not affect mixing time as measured by the Mixograph but the fast acting oxidants such as potassium iodate and azodicarbonamide exhibited a rapid break down of dough and relatively narrow mixogram tail (Weak *et al.*,1977). Yvin *et al.* (2001) reported that ozonated wheat flour doughs produced by treatment of ozone with wheat grains showed an increase in strength and decrease in extensibility as measured by the Chopin Alveograph.

Figure 4.3 Mixograms of (A) Control (unchlorinated) (B) Chlorinated flour (C) Flour treated with ozone gas for 10 min and (D) Flour treated with ozone gas for 36 min.



4.1.2 Baking tests

Table 4.3 Volume and color of cake from untreated soft wheat flour, chlorinated flour, and flour treated with ozone gas for 10 or 36 min.

Flours	Volume of cakes[a]	Color of cake [a]		
		L*	a*	b*
Control (unchlorinated)	93c	83.28c	-3.14a	21.75a
Chlorinated flour	114b	86.66b	-2.49c	15.48c
10 min ozonated	111b	89.03a	-2.88b	16.51b
36 min ozonated	123a	87.63b	-2.51c	16.65b

[a] Means with the same letter within columns are not significantly different ($p>0.05$)

Table 4.4 Textural properties of cakes measured with TA XT2.

Cakes	Textural properties [a]		
	Hardness (g)	Springiness (%)	Cohesiveness (%)
Control (unchlorinated)	544.3a	89.6a	69.6a
Chlorinated	602.7a	92.3a	75.0a
10 min ozonated	461.1b	91.3a	72.7a
36 min ozonated	446.0b	94.1a	73.2a

[a] Means with the same letter within columns are not significantly different ($p>0.05$)

4.1.2.1 Volume of cakes

Results for volumes of cakes measured with a plastic measurement template and cake color measured by a Minolta colorimeter are presented in Table 4.3. Volume of cakes made from 36 min ozone treated flour was significantly higher than flour treated with ozone for 10 min, chlorinated flour, and unchlorinated control flour ($p<0.05$). The cake volume from flour treated with ozone for 10 min was comparable to chlorine treated flour. From the results, it was found that ozone can be used to produce cakes with volumes similar to or higher than cakes made from chlorinated flour. Ozone treated flour had sufficient strength to support cake structure without collapse during cooling similar to chlorine treated flour. It was probably because ozone can oxidize flour components such as starch, protein, and lipids and modify their properties which help to improve the final quality of cakes.

4.1.2.2 Color of cakes

The lightness of cakes made with 10 min ozone treated flour was significantly higher than for cakes made from other types of flour ($p<0.05$). Chlorinated cake flour gave the lowest value of yellowness, significantly different from other types of cakes ($p<0.05$). The results indicate that cake crumb of ozone treated flour had a whiter color. As a result, ozone treated flour can be applied to different varieties of cakes, especially in white cake formulas.

4.1.2.3 Texture properties

Textural properties of cakes measured by the Texture analyzer are given in Table 4.4. Ozone treated flours were softer than chlorinated cake flour and unchlorinated control flour. Increase of ozonation time decreased the hardness of cakes. The springiness and cohesiveness of all cakes were not significantly different ($p>0.05$). Chlorine treated flour had drier cake crumb and greater resistance to compression and sprang back almost to its original height after compression, as compared with non chlorine treated flour. In agreement with Donelson, (2000), the result of this experiment showed that chlorine treated and ozone treated flours had higher springiness than unchlorinated control flour, but were not significantly different.

4.1.2.4 Gas cell structure

The slice brightness of cake made from flour treated with ozone for 10 min was higher than cake made from other types of flour (Figure 4.4). The number of cells of flour treated with ozone for 36 min gave the highest values (Figure 4.5). Cakes made from unchlorinated control flour had the lowest cell diameters (mm) while cakes made with flour treated with ozone for 10 min gave the highest values (Figure 4.6). The increase in ozonation time from 10 min to 36 min reduced cell diameter and increased the number of cells. Cakes made from flour treated with ozone for 36 min had slice brightness and numbers of cells comparable to cakes made with chlorine treated flour, and cell diameters were higher than for chlorine treated flour cakes. The size and number of air bubbles present in the cake batters are important for crumb grain structure and final cake volume. The small cell diameters in cake crumb made from unchlorinated flour might indicate less expansion of air bubbles during baking, which is reflected in low cake volume. For 10 min ozonated flour, the large cell diameter of cake crumb might indicate a high expansion of air bubbles, which helps to improve the volume of cakes. However, the numbers of cells for 10 min ozonated flour cakes indicated that less air bubbles were incorporated into the cake batter. Cake batters from flour ozonated for 10 min were less viscous. It might imply that the cake batters can not hold gas cells very well and the gas cells tended to coalesce into larger cells as indicated by the C Cell results. For 36 min ozonated flour, the smaller cell diameters and greater numbers of gas cells compared with 10 min ozonated flour indicated less gas cell coalescence and more air bubbles were incorporated in the cakes, which helps to improve cake volumes.

Figure 4.4 The slice brightness of cakes made from unchlorinated, chlorinated, 10 min ozonated, and 36 min ozonated flours measured by the C Cell.

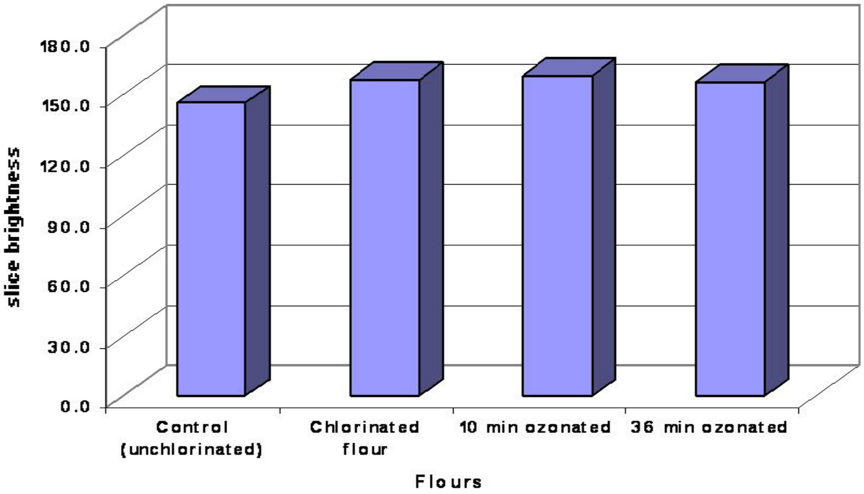


Figure 4.5 The number of cells of cakes made from unchlorinated, chlorinated, 10 min ozonated, and 36 min ozonated flours measured by the C Cell.

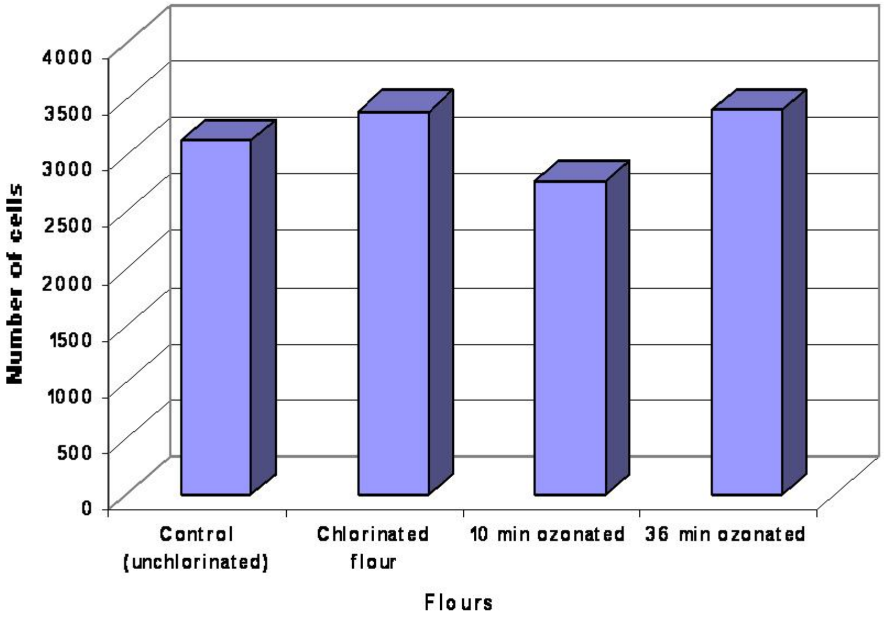


Figure 4.6 The cell diameter of cakes made from unchlorinated, chlorinated, 10 min ozonated, and 36 min ozonated flours measured by the C Cell.

Part 2 Volatile compounds of ozone treated flour measured by GC-MS

Unchlorinated flour, after treatment with ozone, had a strong odor that affects the odor and flavor in the cakes. Identification of volatile compounds that contribute to the odor of ozone treated flour was conducted. The volatile compounds from control unchlorinated flour, chlorinated flour, defatted unchlorinated flour, ozonated flour at 10, 20, 30, 36, and 40 min of ozonation, and ozonated defatted flour at 10 and 36 min of ozonation were studied. Flour volatile compounds were collected using a Tekmar purge and trap instrument and analyzed by Gas chromatography and Mass spectrometry (GC-MS). For ozonated flours, they were tumbled in the chlorination box with the same procedure as in the experiment of Part 1. Flour samples were also baked to study cake baking potential. Flour protein properties were studied using Size Exclusion High Performance Liquid Chromatography (SE-HPLC).

4.2.1 Analysis of volatile compounds

The aldehyde, ketone, benzene, alcohol, terpene, hydrocarbon and other compounds found in unchlorinated, chlorinated, ozonated flour (10 to 40 min), and ozonated flour (10 and 36 min) kept in the fume hood overnight are presented in Tables 4.5, 4.6, 4.7, 4.8, and 4.9, and 4.10, respectively. The hydrocarbons and other compounds were the most abundant compounds found in the control and chlorinated flours followed by aldehydes, alcohols, benzenes, and ketones, respectively. For ozonated flours, the most abundant compounds were aldehydes, ketones, alcohols, hydrocarbons and other compounds, and benzenes respectively.

The aldehyde compounds mostly found in unchlorinated control flour and chlorinated flour were hexanal and nonanal. The results for the control flour were in agreement with Seitz (1995) and Buttery *et al*. (1978) who reported that volatile compounds mainly found in wheat flour are hexanal, nonanal, hexanol, octanol, 1-octen-3-ol, some enals, naphthalene, and 2-methylnaphthalene.

Ozonated flours contained higher levels of hexanal, nonanal, E-2-nonenal, decanal, and hepanal compared to control and chlorinated flour. Ozone can react with the double bonds of unsaturated fatty acids by 1-3 dipolar addition leading to formation of aldehydes, acids, and hydrogen peroxide (Bablon *et al*., 1991). Flour lipids contain lipids associated with starch granules and nonstarch lipids. Nonstarch lipid contains mostly nonpolar lipids (60%) which are mainly linoleic acid, followed by oleic acid and palmitic acid, respectively. Starch lipids contain mostly phospholipids (86%). The nonpolar lipids mainly found in starch lipids are linoleic and palmitic acids (Hoseney, 1994). Hexanal was the most abundant compound detected in all flour samples. Hexanal is the major product of lipid oxidation of linoleic acid. Volatile compounds mostly found from the oxidation of linoleic acid are hexanal, (Z)-2-octenal, (E)-2-heptenal, (E)-2-octenal, (E,Z)-2,4-decadienal, (E,E)-2,4-decadienal, pentanal, heptanal, octanal, and (E,E)-2,4 nonadienal (Sayaslan *et al*, 2000; Belitz *et al*., 2004).

The ketone compound mostly found in all flour samples was 2-propanone. Benzene compounds were mainly found in control and chlorinated flour rather than ozonated flour. Various alcohol compounds were found in control, unchlorinated and ozonated flours. The most abundant alcohol compound found in ozonated flour was 1-octen-3-ol while 2-propanol was mostly found in control and chlorinated flour. Furan compounds, mainly 2-pentyl furan, were found in ozanated flour samples. Sayaslan *et al*. (2000) stated that ketones and alcohols are common products from lipid oxidation. Seitz (1995) reported that compounds associated with off-odor in grains were 1-octen-3-ol, 2-pentanol, and 3-methyl-1-butanol. The presence of 2-pentanol and 2-pentanone indicated slight damage of grain from the lesser grain borer.

Increasing time of ozonation tended to increase the relative abundance of volatile compounds. Rapid decrease of volatile compounds was detected when ozone treated flours (10 and 36 min) were kept in the fume hood overnight.

Table 4.5 Aldehyde compounds found in unchlorinated, chlorinated, ozonated flour, and ozonated flour kept in fume hood overnight.

Compound	Retention time (min)	Relative abundance of volatiles (x10^{6})[a]								
		Control	Chlorinated	Ozonated					Ozonated (fume hood)[b]	
				10 min	20 min	30 min	36 min	40 min	10 min	36 min
Acetaldehyde	3.06	0.07±0.03	0.05±0.01	1.71±0.73	3.13±1.30	2.87±1.06	2.87±0.56	2.69±0.28	-	-
Butanal	3.85	0.20±0.08	0.27±0.06	0.65±0.07	1.20±0.39	1.16±0.44	1.34±0.25	1.15±0.04	-	-
Pentanal	4.91	-	-	6.09±1.75	8.92±3.72	13.80±3.58	14.31±2.34	11.41±4.19	0.43	0.88
Hexanal	6.40	4.56±2.27	6.16±2.53	1736.36±196.9	2881.63±576.1	2554.13±523.6	2938.95±335.7	3433.94±781.5	27.47	86.70
E-2-Hexenal	7.80	-	-	11.24±1.14	21.05±5.12	20.11±3.58	21.66±4.64	17.37±0.66	0.56	0.72
Heptanal	8.34	0.22±0.13	0.27±0.03	25.92±3.29	52.41±7.45	49.14±8.32	55.65±5.97	49.77±9.57	1.05	2.51
E-2-Heptenal	9.92	-	-	0.63±0.42	1.03±0.30	1.09±0.33	0.91±0.17	0.79±0.13	-	-
Octanal	10.45	0.14±0.04	0.18±0.08	7.37±1.38	15.75±5.40	15.96±4.20	16.98±2.73	15.13±1.46	0.29	0.78
Benzaldehyde	10.96	0.19±0.07	0.38±0.06	1.09±0.43	1.34±0.42	1.30±0.53	1.17±0.21	0.98±0.03	0.30	0.69
5-Ethylcyclopent-1-ene caboxaldehyde	11.80	-	-	1.58±0.35	3.97±1.14	4.14±1.22	3.98±0.61	3.22±0.31	-	-
E-2-Octenal	12.03	-	-	7.35±1.05	20.65±4.60	22.74±3.62	25.05±5.94	24.01±7.08	-	-
Nonanal	12.59	1.09±0.20	1.99±1.47	353.14±38.87	952.26±44.81	1033.24±57.67	1122.81±27.08	1120.53±75.42	10.55	18.37
Z-2-Nonenal	13.76	-	-	3.44±0.71	7.47±1.13	8.05±1.45	9.44±2.15	9.41±2.64	-	-
E-2-Nonenal	14.12	0.60±0.12	1.11±0.04	59.91±1.55	162.05±19.96	175.08±19.71	222.32±39.95	229.71±29.09	7.01	9.00
E-Z-2,6-Nonadienal	14.25	-	-	2.43±0.29	6.38±0.57	7.01±0.50	8.34±2.59	6.57±1.93	-	-
Decanal	14.66	0.42±0.07	0.65±0.12	40.93±3.07	67.29±13.70	61.23±16.28	78.15±17.79	69.44±12.46	1.96	3.43
2,4-Nonadienal	15.53	-	-	0.75±0.37	1.26±0.35	1.26±0.28	1.42±0.26	1.61±0.58	-	-
Undecanal	16.30	-	-	0.51±0.34	0.90±0.30	0.83±0.30	0.86±0.12	0.86±0.21	-	-
2-Butyl-2-octenal	17.11	-	-	0.41±0.07	1.23±0.32	1.21±0.24	1.21±0.14	1.14±0.54	-	-

[a] Peak area from mass spectrometry; [b] Samples from one replication.

Table 4.6 Ketone compounds found in unchlorinated, chlorinated, ozonated flour, and ozonated flour kept in fume hood overnight.

Compound	Retention time (min)	Relative abundance of volatiles (x10^6)[a]								
		Control	Chlorinated	Ozonated					Ozonated (fume hood) [b]	
				10 min	20 min	30 min	36 min	40 min	10 min	36 min
2-Propanone	3.34	1.57±0.53	2.07±0.33	35.32±4.27	74.85±14.9	72.00±0.83	88.67±0.86	63.08±5.54	-	3.09
2-Butanone	3.90	0.85±0.33	0.65±0.14	1.38±0.33	1.16±0.47	1.70±1.01	1.27±0.41	-	0.41	0.40
2-Pentanone	4.76	0.10±0.05	-	-	-	-	-	-	-	-
2-Hexanone	5.31	0.12±0.01	-	-	-	-	-	-	-	-
Cyclohexanone	9.25	-	0.25±0.05	-	-	-	-	-	-	-
1-Octen-3-one	10.01	-	-	0.77±0.17	1.79±0.53	1.98±0.12	1.86±0.33	1.82±0.46	-	-
6-Methyl-5-hepten-2-one	10.27	-	-	11.74±0.68	15.33±2.38	9.93±2.14	10.55±2.59	5.30±1.06	-	-
3-Octen-2-one	11.59	-	0.10±0.01	-	-	-	-	-	-	-
1-Phenyl ethanone	13.30	0.05±0.01	-	-	-	-	-	-	-	-
2-Methyl 5-(1-methyl) 2-cyclohexen-1-one	16.29	-	0.07±0.03	-	-	-	-	-	-	-
(E)-6,10-Dimethyl-5,9 undecadiene-2-One	18.23	-	0.06±0.01	0.65±0.22	1.19±0.28	0.75±0.20	0.84±0.21	0.48±0.33	-	-

[a] Peak area from mass spectrometry; [b] Samples from one replication.

Table 4.7 Benzene compounds found in unchlorinated, chlorinated, ozonated flour, and ozonated flour kept in fume hood overnight.

Compound	Retention time (min)	Relative abundance of volatiles (x10^6)[a]								
		Control	Chlorinated	Ozonated flour					Ozonated (fume hood)[b]	
				10 min	20 min	30 min	36 min	40 min	10 min	36 min
Benzene	4.30	0.19±0.11	-	-	-	-	-	-	-	-
Methyl benzene	5.66	1.29±0.44	1.16±0.18	1.06±0.13	-	-	-	-	0.65	1.00
Ethyl benzene	7.33	0.30±0.18	-	-	-	-	-	-	-	-
Dimethyl benzene	7.47	0.90±0.37	0.69±0.33	-	-	-	-	-	-	-
1,2 Dimethyl benzene	8.09	0.33±0.18	0.14±0.01	-	-	-	-	-	-	-
Ethyl methyl benzene	9.37	0.21±0.12	-	-	-	-	-	-	-	-
Ethyl methyl benzene	9.53	0.27±0.12	-	-	-	-	-	-	-	-
Ethyl methyl benzene	9.88	0.10±0.02	-	-	-	-	-	-	-	-
1,2,4-Trimethyl benzene	10.17	0.24±0.08	0.28±0.03	0.82±0.46	0.70±0.35	0.65±0.03	0.46±0.13	0.35±0.03	-	-
1,4 Dichlorobenzene	11.36	0.71±0.13	0.66±0.15	-	-	-	-	-	0.22	-
1-Methyl 2- propyl benzene	11.68	0.09±0.02	-	-	-	-	-	-	-	-
2-Ethyl 1,3 dimethyl benzene	12.12	0.14±0.04	0.16±0.02	-	-	-	-	-	-	-
1-Methyl 2-(1-methyl ethyl) benzene	13.01	0.08±0.05	-	-	-	-	-	-	-	-

[a] Peak area from mass spectrometry; [b] Samples from one replication.

Table 4.8 Alcohol compounds found in unchlorinated, chlorinated, ozonated flour, and ozonated flour kept in fume hood overnight.

Compound	Retention time (min)	Relative abundance of volatiles (x10^6)[a]								
		Control	Chlorinated	Ozonated					Ozonated (fume hood)[b]	
				10 min	20 min	30 min	36 min	40 min	10 min	36 min
Ethanol	3.28	1.76±0.17	0.45±0.06	1.20±0.73	1.42±0.72	1.29±0.37	1.30±0.79	1.16±0.91	-	0.34
2-Propanol	3.37	1.94±0.83	2.49±1.13	-	-	-	-	-	-	-
1-Butanol	4.38	0.20±0.03	0.17±0.11	-	-	-	-	-	-	-
1-Pentanol	5.78	0.16±0.04	-	5.34±1.69	12.29±2.81	13.01±3.62	12.97±2.19	11.86±0.37	-	-
1-Octen-3-ol	9.62	0.13±0.03	0.17±0.13	6.31±2.84	20.28±6.47	20.26±5.49	21.79±3.32	18.37±3.63	-	0.91
1-Octanol	11.65	-	0.18±0.08	0.67±0.17	1.17±0.21	1.53±0.25	1.35±0.14	1.07±0.10	-	-

[a] Peak area from mass spectrometry; [b] Samples from one replication.

Table 4.9 Terpene compounds found in unchlorinated, chlorinated, ozonated flour, and ozonated flour kept in fume hood overnight.

Compound	Retention time (min)	Relative abundance of volatiles (x10^6)[a]								
		Control	Chlorinated	Ozonated					Ozonated (fume hood)[b]	
				10 min	20 min	30 min	36 min	40 min	10 min	36 min
Limonene	10.32	3.68±0.73	-	3.22±0.59	-	2.91±0.90	2.08±0.94	1.33±0.95	-	-

[a] Peak area from mass spectrometry; [b] Samples from one replication.

Table 4.10 Hydrocarbon and other compounds found in unchlorinated, chlorinated, ozonated flour, and ozonated flour kept in fume hood overnight.

Compound	Retention time (min)	Relative abundance of volatiles (x10^6)[a]								
		Control	Chlorinated	Ozonated					Ozonated (fume hood) [b]	
				10 min	20 min	30 min	36 min	40 min	10 min	36 min
Hexane	3.39	4.07±0.01	2.01±1.17	-	-	-	-	-	-	-
Acetonitrile	3.51	2.80±0.55	3.11±0.73	3.25±0.86	2.56±0.73	2.37±1.31	2.65±0.83	3.37±0.53	1.74	4.50
Chloroform	3.98	6.71±0.75	5.58±1.29	6.69±0.73	6.47±2.12	5.21±1.33	5.34±1.76	6.05±1.87	3.10	3.17
Tetrahydrofuran	4.11	0.18±0.09	-	-	-	-	-	-	-	-
Decane	8.57	0.40±0.23	0.44±0.17	-	-	-	-	-	-	-
Methyl nonane	8.87	0.15±0.01	-	-	-	-	-	-	-	-
2-Pentyl furan	9.67	0.36±0.05	0.41±0.04	10.45±5.05	27.04±6.91	22.62±3.02	23.11±3.78	13.79±1.43	1.74	2.10
Undecane	10.68	0.36±0.10	0.41±0.06	-	0.87±0.42	-	-	0.65±0.12	-	-
1-Ethyl 1-methyl cyclopentane	11.70	-	-	0.49±0.22	1.72±0.56	2.51±0.89	2.04±0.40	3.77±1.46	-	-
Hexadecane	12.78	0.19±0.09	0.16±0.05	-	-	-	-	-	-	-
Tridecane	14.80	0.13±0.03	0.11±0.02	-	-	-	-	-	0.34	-
3,4-dihydro 2H-thiopyran	16.42	-	-	0.62±0.27	1.16±0.30	1.11±0.14	0.87±0.03	5.25±1.01	-	-

[a] Peak area from mass spectrometry; [b] Samples from one replication.

To determine the effect of lipid in flour samples on the volatile compounds present in the ozonated flour, another experiment was performed by extracting lipid with chloroform from unchlorinated control flour before ozonation for 10 and 36 min. The lipid contents of control and chlorinated flour were 0.56 % and 0.45 % respectively. Unchlorinated flour after treatment with chloroform contains 0 % non-starch lipid. The aldehydes, ketones, benzenes, alcohols, terpenes, and hydrocarbons and other compounds found in unchlorinated flour, defatted unchlorinated flour, and flour defatted with chloroform and then treated with ozone gas for 10 and 36 min are presented in Tables 4.11, 4.12, 4.13, 4.14, 4.15, and 4.16, respectively.

The volatile compounds found in defatted flour were mainly chloroform followed by aldehyde compounds such as hexanal, nonanal, E-2-nonenal, and decanal. Alcohol compounds such as ethanol and 1-pentanol were present in defatted flour. All benzene compounds disappeared after defatting with chloroform. The volatile compounds present in ozonated defatted flour at 10 min and 36 min were mainly chloroform followed by aldehydes, including hexanal, nonanal, E-2-octenal, and heptanal. A decrease in abundance of volatile compounds especially aldehyde compounds of flours after extracting lipid and treating with ozone was detected in both 10 min and 36 min of ozonation compared to ozone treated whole flour, suggesting that some of these compounds originated from the lipid.

Table 4.11 Aldehyde compounds found in control (unchlorinated), defatted control, and defatted ozonated flours.

Compound	Retention time (min)	Relative abundance of volatiles (x10^6)[a]			
		Control	Defatted control	Ozonated defatted flour[b]	
				10 min	36 min
Acetaldehyde	3.06	0.07±0.03	-	-	-
Butanal	3.85	0.20±0.08	-	-	-
Pentanal	4.91	-	0.73±0.02	4.43	7.96
Hexanal	6.40	4.56±2.27	4.08±0.24	595.92	189.76
E-2-Hexenal	7.80	-	-	1.32	-
Heptanal	8.34	0.22±0.13	0.22±0.03	8.90	8.05
E-2-Heptenal	9.92	-	-	-	0.15
Octanal	10.45	0.14±0.04	0.13±0.00	5.66	4.77
Benzaldehyde	10.96	0.19±0.07	0.30±0.10	0.46	0.65
5-Ethylcyclopent-1-ene caboxaldehyde	11.80	-	-	0.33	0.08
E-2-Octenal	12.03	-	-	10.74	0.98
Nonanal	12.59	1.09±0.20	1.50±0.36	80.95	49.06
Z-2-Nonenal	13.76	-	-	-	-
E-2-Nonenal	14.12	0.60±0.12	0.89±0.56	5.66	1.68
E-Z-2,6-Nonadienal	14.25	-	0.05±0.00	0.11	-
Decanal	14.66	0.42±0.07	0.89±0.14	39.79	30.26
2,4-Nonadienal	15.53	-	0.09±0.01	0.09	0.06
Undecanal	16.30	-	0.02±0.01	0.22	0.38
2-Butyl-2-octenal	17.11	-	0.04±0.01	0.29	0.30

[a] Peak area from mass spectrometry; [b] Samples from one replication.

Table 4.12 Ketone compounds found in control (unchlorinated), defatted control, and defatted ozonated flours.

Compound	Retention time (min)	Relative abundance of volatiles (x10^6)[a]			
		Control	Defatted control	Ozonated defatted[b]	
				10 min	36 min
2-Propanone	3.34	1.57±0.53	0.66±0.45	0.97	4.85
2-Butanone	3.90	0.85±0.33	-	-	-
2-Hexanone	5.31	0.10±0.05	-	-	-
Cyclohexanone	9.25	0.12±0.01	-	-	-
1-Octen-3-one	10.01	-	-	-	0.21
6-Methyl-5-hepten-2-one	10.27	-	0.25±0.20	0.18	0.16
3-Octen-2-one	11.59	-	-	-	-
1-Phenyl ethanone	13.30		-	-	-
2-Methyl 5-(1-methyl) 2-cyclohexen-1-one	16.29	-	-	-	-
(E)-6,10-Dimethyl-5,9 undecadiene-2-One	18.23	0.05±0.01	0.05±0.03	0.02	-

[a] Peak area from mass spectrometry; [b] Samples from one replication.

Table 4.13 Benzene compounds found in control (unchlorinated), defatted control, and ozonated defatted flours.

Compound	Retention time (min)	Relative abundance of volatiles (x10^6)[a]			
		Control	Defatted control	Ozonated defatted [b]	
				10 min	36 min
Benzene	4.30	0.19±0.11	-	-	-
Methyl benzene	5.66	1.29±0.44	-	-	-
Ethyl benzene	7.33	0.30±0.18	-	-	-
Dimethyl benzene	7.47	0.90±0.37	-	-	-
1,2 Dimethyl benzene	8.09	0.33±0.18	-	-	-
Ethyl methyl benzene	9.37	0.21±0.12	-	-	-
Ethyl methyl benzene	9.53	0.27±0.12	-	-	-
Ethyl methyl benzene	9.88	0.10±0.02	-	-	-
1,2,4-Trimethyl benzene	10.17	0.24±0.08	-	-	-
1,4 Dichlorobenzene	11.36	0.71±0.13	-	-	-
1-Methyl 2- propyl benzene	11.68	0.09±0.02	-	-	-
2-Ethyl 1,3 dimethyl benzene	12.12	0.14±0.04	-	-	-
1-Methyl 2-(1-methyl ethyl) benzene	13.01	0.08±0.05	-	-	-

[a] Peak area from mass spectrometry; [b] Samples from one replication.

Table 4.14 Alcohol compounds found in control (unchlorinated), defatted control, and ozonated defatted flours.

Compound	Retention time (min)	Relative abundance of volatiles (x10^6)[a]			
		Control	Defatted control	Ozonated defatted [b]	
				10 min	36 min
Ethanol	3.28	1.76±0.17	1.19±0.14	1.15	2.25
2-Propanol	3.37	1.94±0.83	-	-	-
1-Butanol	4.38	0.20±0.03	-	-	-
1-Pentanol	5.78	0.16±0.04	1.27±0.15	4.65	3.46
1-Octen-3-ol	9.62	0.13±0.03	0.10±0.04	2.13	0.14
1-Octanol	11.65	-	-	-	-

[a] Peak area from mass spectrometry; [b] Samples from one replication.

Table 4.15 Terpene compounds found in control (unchlorinated), defatted control, and ozonated defatted flours.

Compound	Retention time (min)	Relative abundance of volatiles (x10^6)[a]			
		Control	Defatted control	Ozonated defatted [b]	
				10 min	36 min
Limonene	10.32	3.68±0.73	-	-	-

[a] Peak area from mass spectrometry; [b] Samples from one replication.

Table 4.16 Hydrocarbons and other compounds found in control (unchlorinated), defatted control, chlorinated, ozonated flour, and ozonated defatted flours.

Compound	Retention time (min)	Relative abundance of volatiles (x10^6)[a]			
		Control	Defatted control	Ozonated defatted flour	
				10 min	36 min
Hexane	3.39	4.07±0.01	-	-	-
Acetonitrile	3.51	2.80±0.55	3.83±1.01	5.37	2.92
Chloroform	3.98	6.71±0.75	438.92±213.0	658.04	440.99
Tetrahydrofuran	4.11	0.18±0.09	-	-	-
Decane	8.57	0.40±0.23	-	-	-
Methyl nonane	8.87	0.15±0.01	-	-	-
2-Pentyl furan	9.67	0.36±0.05	0.15±0.03	1.95	0.58
Undecane	10.68	0.36±0.10	-	-	-
1-Ethyl 1-methyl cyclopentane	11.70	-	-	0.70	0.04
Hexadecane	12.78	0.19±0.09	-	-	-
Tridecane	14.80	0.13±0.03	-	-	-
3,4-dihydro 2H-thiopyran	16.42	-	0.06±0.01	0.08	0.12

[a] Peak area from mass spectrometry; [b] Samples from one replication.

4.2.2. Baking tests

Volumes of cakes made from unchlorinated, chlorinated, defatted unchlorinated and ozonated unchlorinated flours are presented in Table 4.17. The pictures of cake samples are shown in Figure 4.7. The volume of cakes made from all defatted flours with or without further ozonation had low volumes. Cake made from the control with lipid extraction and ozonation for 36 min gave the lowest volumes with significant difference from other types of cakes ($p<0.05$). The result indicated that lipid in flour affected volume of cakes. The batters made from defatted flour had a grainy texture compared to a smooth texture from control, chlorinated, and ozonated flour. Johnson *et al*. (1979) reported that cake made from petroleum ether defatted flour had poor grain quality and a flat contour. Spies and Kirleis *et al*. (1978) stated that free lipids are required for cake structure formation. Kissell *et al*. (1979) studied the volumes of cakes made from unchlorinated patent flour extracted with hexane to remove free lipids before and after chlorine treatment at different levels. It was found that cakes made from defatted flour before chlorination had low volume. Cakes made from defatted flour after chlorination had higher volume than those made from defatted flour before chlorination. The authors concluded that the baking potential of defatted base flour was better when free lipids were present at the time of chlorination.

4.2.3 Flour properties

Size exclusion high performance liquid chromatography (SE-HPLC) was used to analyze flour proteins. It separates protein based on molecular size. SE- HPLC results of unchlorinated, chlorinated, defatted unchlorinated, and ozonated defatted flours at times of 10 and 36 min, and ozonated flours at times of ozonation of 10 and 36 min are shown in Figure 4.8 and Table 4.18. Areas under the chromatograms were integrated in three parts. The first peak with elution time of 0-15.9 min was mainly glutenins (polymeric proteins). The second peak contained mostly gliadins (monomeric proteins) at the elution time of 15.9-19.7 min. The third peak was albumins and globulins at the elution time of 19.7-22.0 min. The percentage of total polymeric protein, mainly glutenins, (peak 1) did not show much difference among flour samples (Figure 4.9 and

Table 4.18). The gliadins (peak 2) also did not show differences among flour samples. The results for percentage of unextractable polymeric protein (UPP) showed that control flour after lipid extraction, control flour with lipid extraction and ozonation for 10 min, control flour with lipid extraction and ozonation for 36 min, control flour treated with ozone for 10 min, and control flour treated with ozone for 36 min had UPP greater than control unchlorinated flour and chlorinated flour. The increase in percentage of UPP from ozonated flour might result from the oxidation of SH groups in polymeric protein resulting in an increase in polymerized protein. Tsen and Kulp (1971) stated that the degradation of aromatic amino acids and oxidation of SH groups was observed during chlorine treatment. Kulp (1972) stated that "the oxidation of SH groups indicated the maturing effect of chlorine in the dough."

Table 4.17 Volumes of cakes made from unchlorinated, chlorinated, defatted unchlorinated and ozonated defatted, and ozonated flours.

Flours	Volume of cakes
Control (unchlorinated)	101c
Chlorinated	112b
Defatted unchlorinated	66d
Control with lipid extraction and ozonation for 10 min	60de
Control with lipid extraction and ozonation for 36 min	54e
10 min ozonated	108bc
36 min ozonated	127a

[a] Means with the same letter within columns are not significantly different ($p>0.05$)

Figure 4.7 Pictures of cakes made from (1) Control (unchlorinated) (2) Chlorinated (3) Control with lipid extraction (4)Control with lipid extraction and ozonation for 10 min (5) Control with lipid extraction and ozonation for 36 min (6) ozonation for 10 min (7) ozonation for 36 min .

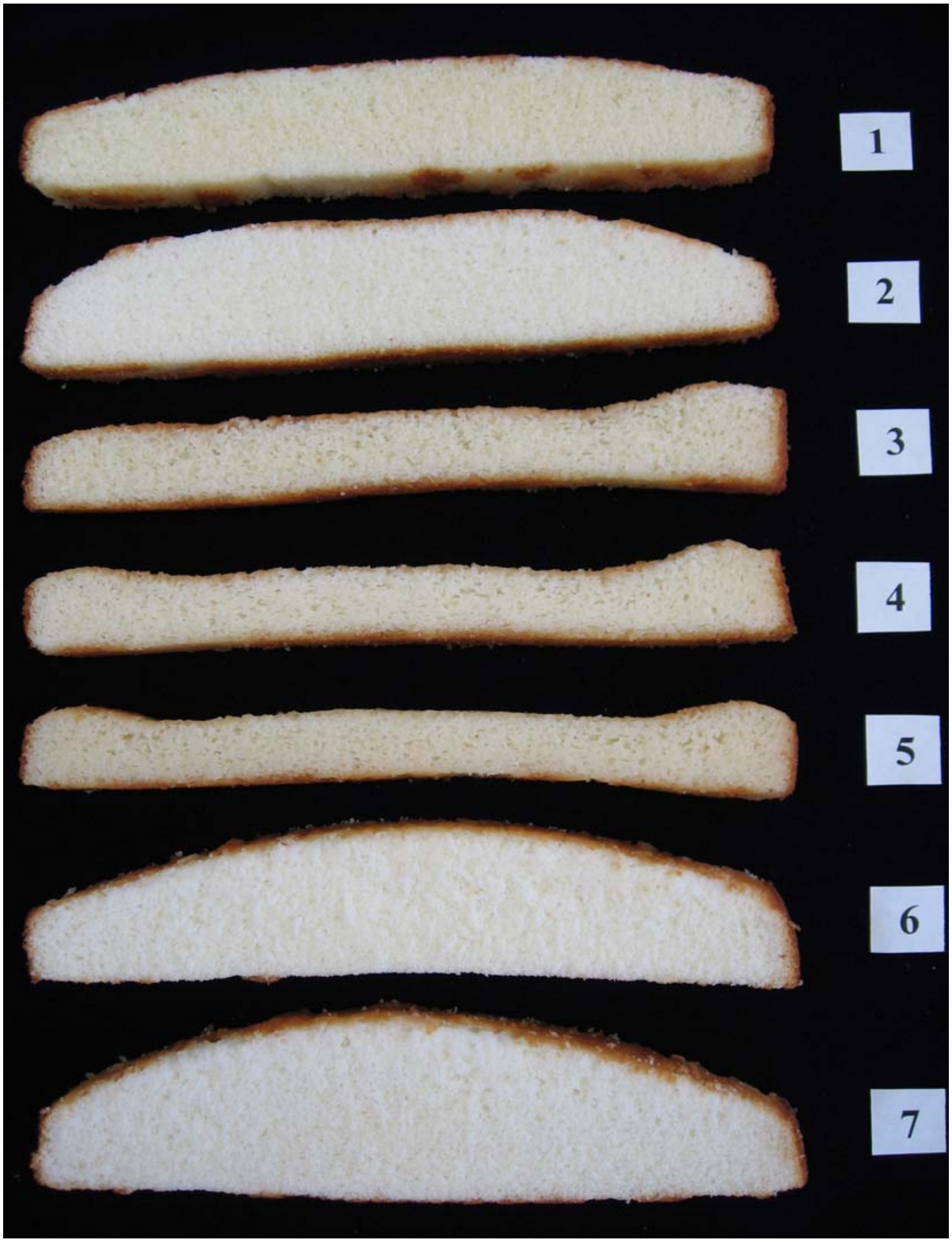


Figure 4.8 SE-HPLC chromatogram of total protein of unchlorinated, chlorinated, defatted unchlorinated and defatted ozonated, and ozonated flours.

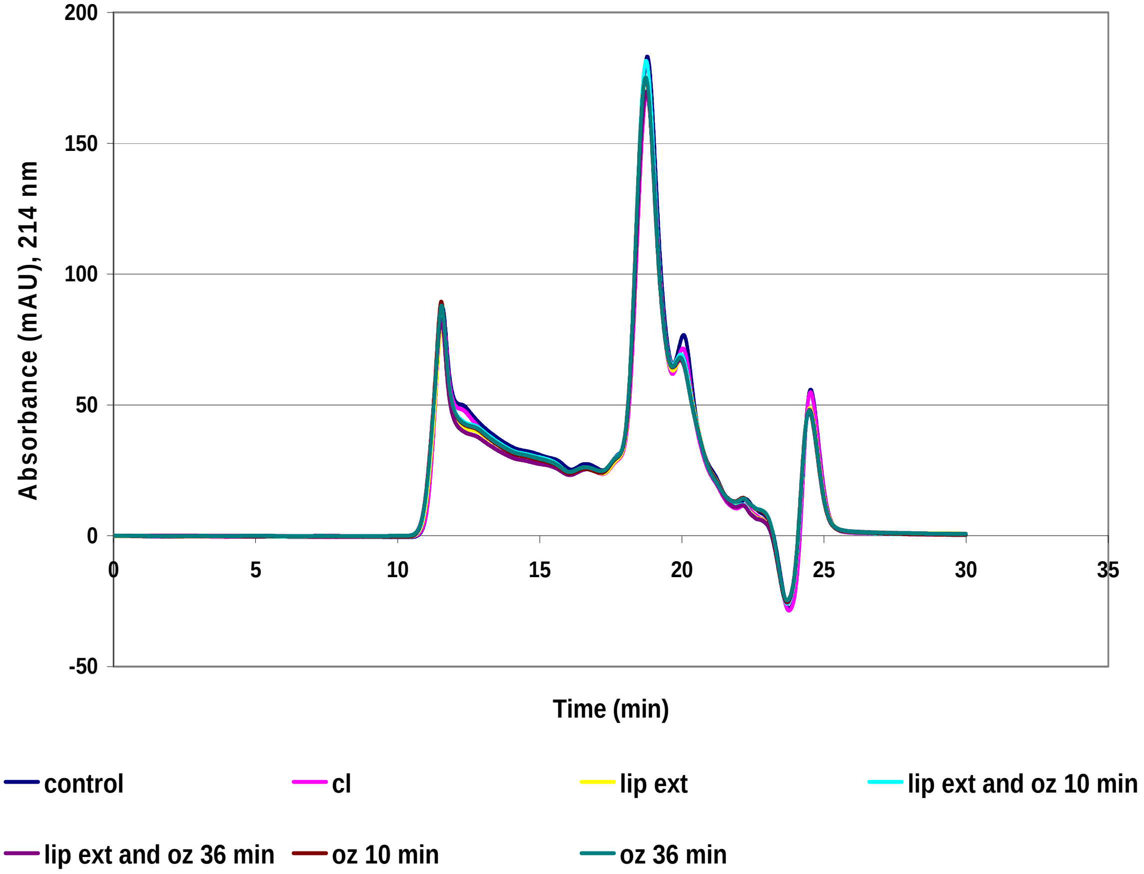


Table 4.18 SE-HPLC relative composition (percentage) of polymeric protein in unchlorinated, chlorinated, defatted unchlorinated and ozonated defatted, and ozonated flours.

Flours	TPP[a]	EPP[a]	UPP[a]
Control(unchlorinated)	38.26±0.28	62.68±0.41	37.32±1.43
Chlorinated flour	38.55±0.33	62.17±0.19	37.83±1.01
Control after lipid extraction	37.98±1.00	58.96±0.44	41.04±1.83
Control with lipid extraction and ozonation for 10 min	38.49±0.06	56.80±0.24	43.20±2.05
Control with lipid extraction and ozonation for 36 min	37.84±0.09	51.45±0.32	48.55±1.85
10 min ozonated	38.80±0.28	56.57±0.11	43.43±1.39
36 min ozonated	38.99±0.59	54.30±0.09	45.70±2.00

[a] Values represent mean± standard deviation; TPP = total polymeric protein; EPP = extractable polymeric protein; UPP = unextractable polymeric protein

Part 3 Ozonation treatment with time and temperature control

4.3.1 Ozonation treatment with variation in time and temperature

A response surface methodology design was conducted to determine the optimum condition for ozone treatment. Two parameters, temperature and time, were used in this experiment. Temperature was varied from 25, 35, and 45 ^{o}C and time was varied from 5, 15, and 25 min. Flour samples were tumbled in the metal drum equipped with temperature control and ozone gas was passed through the box. Three measured responses were volume of cakes, firmness of cakes, and brightness of cakes. The ANOVA results for three responses as a function of temperature and time are shown in Table 4.19 and the response surface graphs and contour plots are shown in Figure 4.9, 4.10, 4.11, 4.12, 4.13, and 4.14.

The response surface graph plot and contour plots of the effects of time and temperature on the volume index of cakes are shown in Figures 4.9 and 4.10. Analysis of variance indicated that linear and quadratic models were highly significant ($p<0.0001$) but the model showed a significant lack of fit which indicated the lack of model adequacy (Table 4.19). The graph plot, at short ozonation time and low temperatures, indicates low volume indices and, as time of ozonation and temperature treatment increased, the volume index tended first to increase and then increased as time and temperature progressed. The optimum time and temperature suggested for ozone treatment from the model was about 11.3 min at a temperature of 39.8 ^{o}C which gave a value of cake volume of about 113. Analysis of variance of independent variables, time and temperature, indicated that time was significantly different ($p<0.0001$) and temperature was not significantly different ($p=0.0796$). The results indicated a pronounced effect of time for ozonation compared to temperature.

Figure 4.11 and 4.12 show the effect of time and temperature on the firmness of cakes. Analysis of variance indicated that linear and quadratic models were highly significant ($p<0.0001$) and the lack of fit was significant ($p=0.0956$). Therefore, the model was adequate to represent the relationships. The graph plots showed that short ozonation times and high temperatures increased the cake firmness and the trend was the same when ozone was applied for long periods of time at high temperature. The low firmness of cakes occurred at the mid range of time and temperature. The optimum time and temperature from the model indicated about 8.4 min and 36.8 ^{o}C, which gave a firmness of 385.3. Analysis of variance of time and temperature indicated the same result as from the volume index. Time was highly significant ($p<0.0001$) and temperature was not significant ($P=0.4272$).

The effect of time and temperature on the slice brightness is shown in Figure 4.13 and Figure 4.14. Analysis of variance indicated that linear and quadratic models were also highly significant ($p<0.0001$) and the lack of fit was significant ($p=0.3360$). The graph of slice brightness followed the same trend as the graph for volume index. The optimum time and temperature for ozone treatment was 10.3 min and 46.9 ^{o}C and the value of slice brightness was 158.8. Analysis of variance of time and temperature indicated the same result as for the volume index and firmness which showed that ozonation time was highly significant ($p<0.0001$) and temperature was not significant ($P=0.8914$).

The results of the experiment showed a high effect of ozone treatment time on the response parameters. The results of experiments in Part 1 showed that the increase in time of ozone treatment increased ozone concentration. The increase of ozone concentration might increase the oxidizing effect of ozone on flour components, resulting in improvement of the quality of cakes. For the effect of temperature, reaction rate of ozone and decomposition rate increased as temperature increased, but stability decreased. The effect of temperature from the result of the experiment showed little improvement in cake quality at high temperature. This might be due to the decomposition of ozone at high temperature, even though the rate of reaction increases. Han *et al*. (2002) studied the response surface modeling for the inactivation of *E. coli* O157:H7 by ozone gas and found that ozone concentration (2 to 8 mg/l), ozonation time (10 to 40 min), and relative humidity (60 to 90%) affected the rate of log reduction of *E. coli* O157:H7 on the surface of green peppers. The ozone gas concentration was the most effective in the inactivation of *E. coli* O157:H7 followed by time and relative humidity. Liew and Prange (1994) stated that the residue ozone concentration increased when ozone supply concentration increased but decreased at high storage temperature.

Table 4.19 ANOVA summary of three responses of cake as a function of temperature and time

Parameters/Responses	Volume index	Firmness	Brightness
R^2	0.8639	0.8903	0.6670
Regression[a]			
Linear	<0.0001	<0.0001	<0.0001
Quadratic	<0.0001	<0.0001	<0.0001
Cross product	0.4453	0.8575	0.5063
Lack of fit[b]	<0.0001	0.0956	0.3360
Factor[c]			
Time	<0.0001	<0.0001	<0.0001
Temperature	0.0796	0.4271	0.8914

[a] F-statistic for regression terms with statistical significance at $p<0.05$

[b] lack of fit without statistical significance indicated model adequacy ($p>0.05$)

[c] F-statistic for factors with statistical significance at $p<0.05$

Figure 4.9 Response surface graph of volume of cake as a function of treatment, temperature and time.

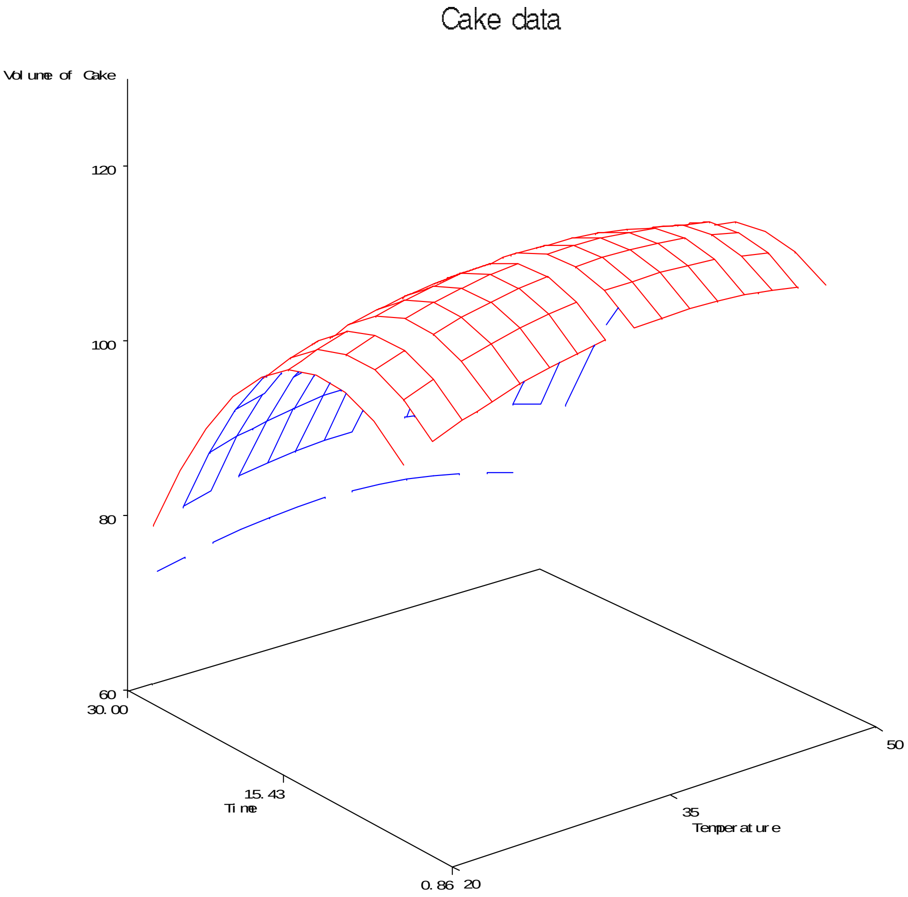


Figure 4.10 Contour plot of volume of cake as a function of treatment, temperature and time.

```
    +21.000 |      GGGGGGGGG                  HHHH                   GGGG
    +20.500 |   GGGGGGG           HHHHHHHHHHHHHHHHHHHHHHHHHHHHH
    +20.000 |GGGGGGG        HHHHHHHHHHHHHHHHHHH   HHHHHHHHHHHHHHHHHHH
    +19.500 |GGGG       HHHHHHHHHHH                            HHHHHHHHHH
    +19.000 |G       HHHHHHHHH                                      HHHHH
    +18.500 |     HHHHHHHH            IIIIIIIIIIIIIIIIIIIIII            H
    +18.000 |   HHHHHHH          IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII
    +17.500 | HHHHHHH        IIIIIIIIIIIIIIIIII      IIIIIIIIIIIIIIIII
    +17.000 |HHHHHH       IIIIIIIIIIII                        IIIIIIIIIII
    +16.500 |HHHH       IIIIIIIIII                                 IIIIII
    +16.000 |HHH      IIIIIIIII                                       III
    +15.500 |HH      IIIIIIII              JJJJJJJJJJJJJJJJ
    +15.000 |H      IIIIIII            JJJJJJJJJJJJJJJJJJJJJJJJ
    +14.500 |      IIIIIII          JJJJJJJJJJJJJJJJJJJJJJJJJJJJJJ
    +14.000 |     IIIIIII         JJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJ
    +13.500 |    IIIIIII         JJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJ
    +13.000 |    IIIIIII        JJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJ
    +12.500 |    IIIIII         JJJJJJJJJJJJJJJJJ       JJJJJJJJJJJJJJJJJ
T   +12.000 |    IIIIII        JJJJJJJJJJJJJJJJ           JJJJJJJJJJJJJJJ
I   +11.500 |    IIIIII        JJJJJJJJJJJJJJJ              JJJJJJJJJJJJJ
M   +11.000 |    IIIIII         JJJJJJJJJJJJJJ              JJJJJJJJJJJJJ
E   +10.500 |     IIIIII        JJJJJJJJJJJJJJJ            JJJJJJJJJJJJJJ
-   +10.000 |     IIIIIII        JJJJJJJJJJJJJJJJJ       JJJJJJJJJJJJJJJJ
M    +9.500 |      IIIIII         JJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJ
I    +9.000 |H      IIIIII         JJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJ
N    +8.500 |HH      IIIIIII         JJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJ
     +8.000 |HHH      IIIIIII           JJJJJJJJJJJJJJJJJJJJJJJJJJJJJJ
     +7.500 |HHHHH      IIIIIII            JJJJJJJJJJJJJJJJJJJJJJJJ
     +7.000 | HHHHH      IIIIIIII               JJJJJJJJJJJJJJJ
     +6.500 |  HHHHHH      IIIIIIIII
     +6.000 |    HHHHHH       IIIIIIIII                                 I
     +5.500 |      HHHHHH       IIIIIIIIIIII                       IIIIII
     +5.000 |GG      HHHHHH         IIIIIIIIIIIIIIIIIII  IIIIIIIIIIIIIIII
     +4.500 |GGGG      HHHHHHH          IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII
     +4.000 | GGGGGG      HHHHHHHH            IIIIIIIIIIIIIIIIIIIII
     +3.500 |    GGGGG       HHHHHHHHH
     +3.000 |F     GGGGGG        HHHHHHHHHH                            HH
     +2.500 |FFFF     GGGGGGG        HHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHH
     +2.000 | FFFFF      GGGGGGGG          HHHHHHHHHHHHHHHHHHHHHHHHHHHHH
     +1.500 |    FFFFFF      GGGGGGGGG
     +1.000 |EE     FFFFFF        GGGGGGGGGG                            G
            \+---------+---------+---------+---------+---------+---------+
          +20.000   +25.000   +30.000   +35.000   +40.000   +45.000   +50.000

                                        TEMP-C

    Legend:    +75.00=A    +79.00=B    +83.00=C    +87.00=D    +91.00=E
               +95.00=F    +99.00=G   +103.00=H   +107.00=I   +111.00=J
              +115.00=K
```

Figure 4.11 Response surface graph of firmness of cake as a function of treatment, temperature and time.

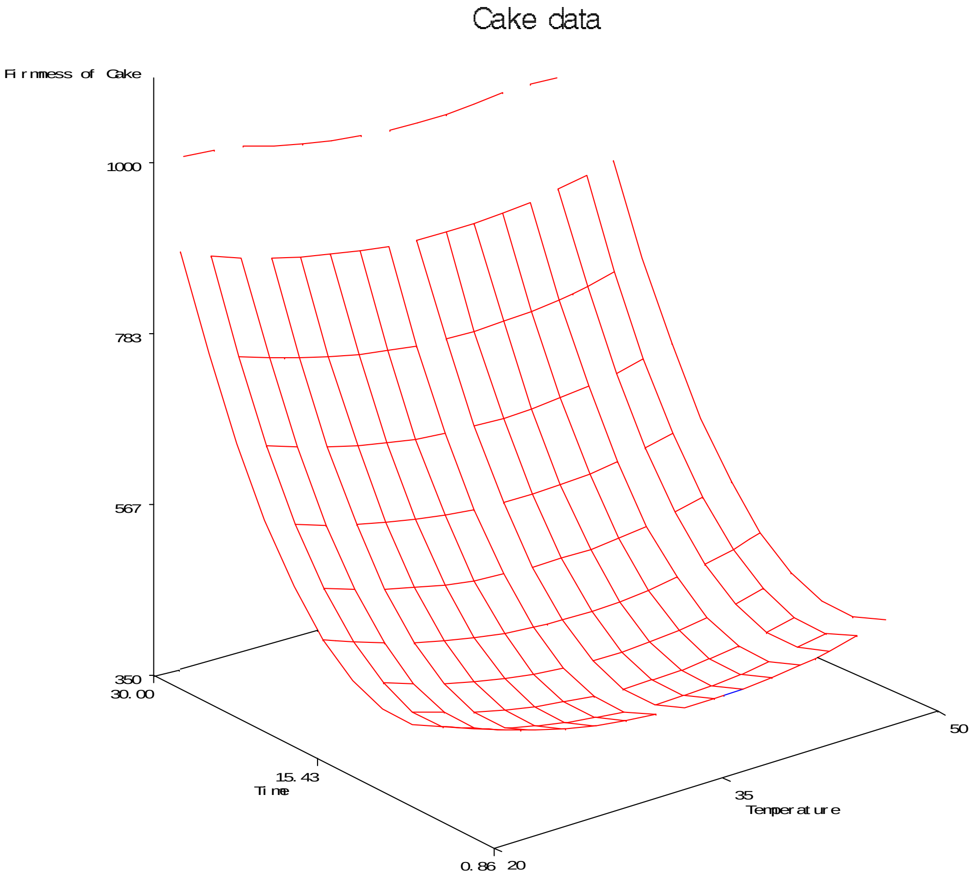


Figure 4.12 Contour plots of firmness of cake as a function of treatment, temperature and time.

```
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    +20.500 |KKKKKKKKKK     JJJJJJJJJJ               JJJJJJJJJ      KKKKK
    +20.000 |KKKK     JJJJJ        IIIIIIIIIIIIIIIIIIIII        JJJJJ
    +19.500 |    JJJJ     IIIIIII                        IIIIIIII     JJJ
    +19.000 |JJJ    IIIII       HHHHHHHHHHHHHHHHHHHHHHHHHHH      IIIIII
    +18.500 |   IIII     HHHHHHH                           HHHHHHH     II
    +18.000 |III    HHHHH       GGGGGGGGGGGGGGGGGGGGGGGGGGG       HHHHH
    +17.500 |   HHHH      GGGGGG                           GGGGGGG     HH
    +17.000 |HHHH    GGGGG       FFFFFFFFFFFFFFFFFFFFFFFFF       GGGGGG
    +16.500 |H   GGGGG     FFFFFFFF                      FFFFFFFF     GGG
    +16.000 | GGGG     FFFFF         EEEEEEEEEEEEEEEEEE        FFFFFF
    +15.500 |GG    FFFFF      EEEEEEEEEEE          EEEEEEEEEEE      FFFFF
    +15.000 |    FFFF     EEEEEEE                           EEEEEE     FF
    +14.500 | FFFF    EEEEEE        DDDDDDDDDDDDDDDDDDDD        EEEEEE
    +14.000 |FFF    EEEEE      DDDDDDDDDDDD      DDDDDDDDDDDDD      EEEEE
    +13.500 |F    EEEE     DDDDDDDD                       DDDDDDD      EE
    +13.000 |   EEEE     DDDDDD           CCCCCCCCC           DDDDDD
    +12.500 | EEEE    DDDDDD       CCCCCCCCCCCCCCCCCCCCCC        DDDDD
T   +12.000 |EEEE    DDDDD      CCCCCCCCCCCCCCCCCCCCCCCCCCCCC      DDDDD
I   +11.500 |EE    DDDDD      CCCCCCCCC               CCCCCCCCCC     DDDD
M   +11.000 |E    DDDDD     CCCCCCC                       CCCCCCC      DD
E   +10.500 |E   DDDD      CCCCCC                           CCCCCCC     D
-   +10.000 |    DDDD     CCCCCC                              CCCCCC
M    +9.500 |   DDDD     CCCCCC           BBBBBBBBBB           CCCCCC
I    +9.000 |   DDDD     CCCCC          BBBBBBBBBBBBB          CCCCCC
N    +8.500 |   DDDD    CCCCCC          BBBBBBBBBBBBBB          CCCCCC
     +8.000 |   DDDD     CCCCCC         BBBBBBBBBBBBBB          CCCCCC
     +7.500 |   DDDD     CCCCCC           BBBBBBBBBBB          CCCCCC
     +7.000 |    DDDD     CCCCCC               B              CCCCCCC
     +6.500 |E    DDDD     CCCCCCC                           CCCCCCC
     +6.000 |EE    DDDD     CCCCCCCC                       CCCCCCCC     D
     +5.500 |EEE    DDDD      CCCCCCCCC                 CCCCCCCCC      DD
     +5.000 | EEE    DDDDD      CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC      DDDD
     +4.500 |  EEEE    DDDDD        CCCCCCCCCCCCCCCCCCCCCCC        DDDDD
     +4.000 |    EEEE    DDDDDD          CCCCCCCCCCCCC          DDDDDD
     +3.500 |FF    EEEE     DDDDDDD                         DDDDDDD
     +3.000 |FFFF    EEEEE      DDDDDDDDDD            DDDDDDDDDD      EEE
     +2.500 |   FFF     EEEEE       DDDDDDDDDDDDDDDDDDDDDDD        EEEEE
     +2.000 |G    FFFF     EEEEEE                              EEEEEE
     +1.500 |GGGG    FFFF      EEEEEEEEEE              EEEEEEEEEE      FF
     +1.000 |   GGGG    FFFFFF       EEEEEEEEEEEEEEEEEEEEEE        FFFFF
            \+---------+---------+---------+---------+---------+---------+
          +20.000   +25.000   +30.000   +35.000   +40.000   +45.000   +50.000

                                      TEMP-C

    Legend:   +350.00=A   +375.00=B   +400.00=C   +425.00=D   +450.00=E
              +475.00=F   +500.00=G   +525.00=H   +550.00=I   +575.00=J
              +600.00=K
```

Figure 4.13 Response surface graph of brightness of cake as a function of treatment, temperature and time.

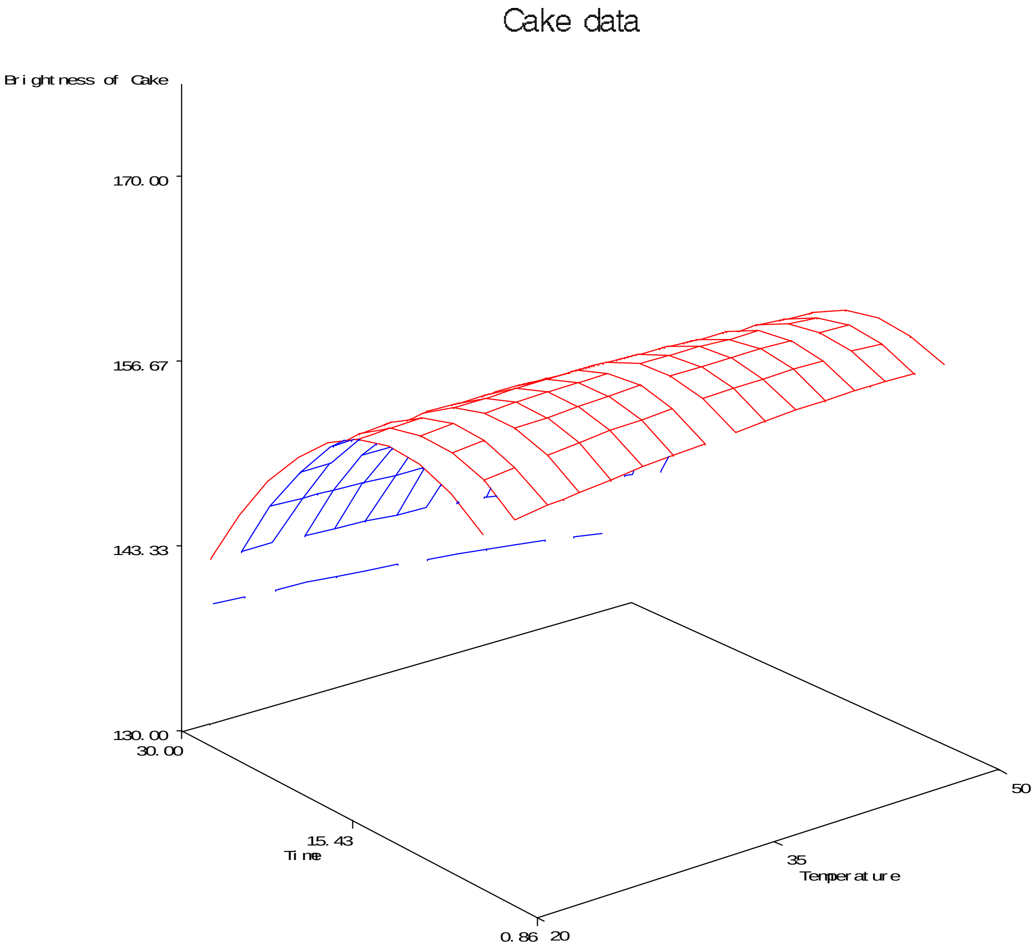
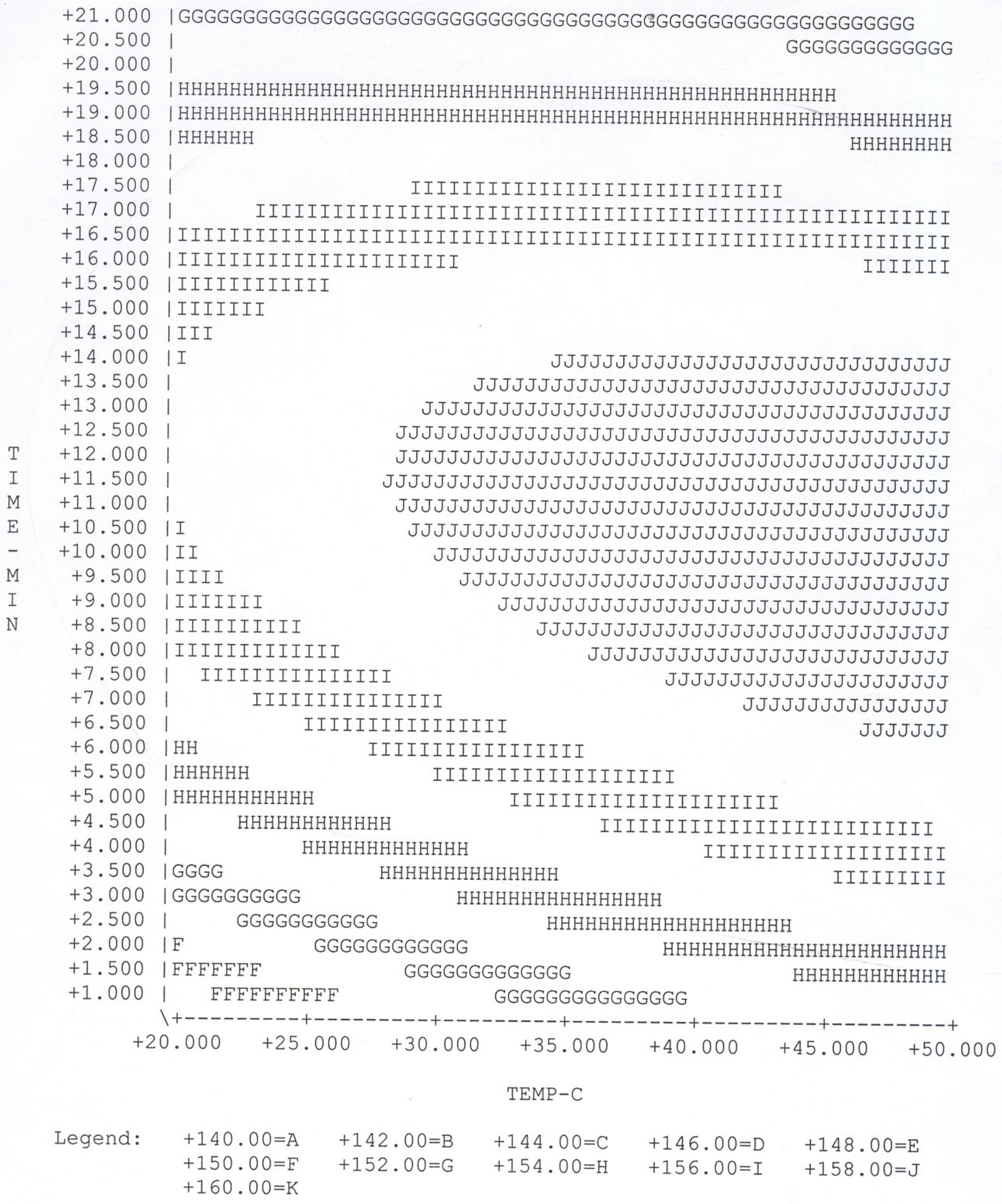


Figure 4.14 Contour plots of brightness of cake as a function of treatment, temperature and time.



```
    +21.000 |GGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG
    +20.500 |                                               GGGGGGGGGGGGG
    +20.000 |
    +19.500 |HHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHH
    +19.000 |HHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHH
    +18.500 |HHHHHH                                              HHHHHHHH
    +18.000 |
    +17.500 |                  IIIIIIIIIIIIIIIIIIIIIIIIIIIII
    +17.000 |      IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII
    +16.500 |IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII
    +16.000 |IIIIIIIIIIIIIIIIIIIIII                               IIIIIII
    +15.500 |IIIIIIIIIIII
    +15.000 |IIIIIII
    +14.500 |III
    +14.000 |I                            JJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJ
    +13.500 |                       JJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJ
    +13.000 |                   JJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJ
    +12.500 |                 JJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJ
T   +12.000 |                 JJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJ
I   +11.500 |                JJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJ
M   +11.000 |                 JJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJ
E   +10.500 |I                 JJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJ
-   +10.000 |II                  JJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJ
M    +9.500 |IIII                  JJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJ
I    +9.000 |IIIIIII                  JJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJ
N    +8.500 |IIIIIIIIII                  JJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJ
     +8.000 |IIIIIIIIIIIII                   JJJJJJJJJJJJJJJJJJJJJJJJJJJJ
     +7.500 |  IIIIIIIIIIIIIII                     JJJJJJJJJJJJJJJJJJJJJJ
     +7.000 |      IIIIIIIIIIIIIII                       JJJJJJJJJJJJJJJJ
     +6.500 |          IIIIIIIIIIIIIIII                           JJJJJJJ
     +6.000 |HH             IIIIIIIIIIIIIIIII
     +5.500 |HHHHHH              IIIIIIIIIIIIIIIIIII
     +5.000 |HHHHHHHHHHH               IIIIIIIIIIIIIIIIIIIII
     +4.500 |     HHHHHHHHHHHH                IIIIIIIIIIIIIIIIIIIIIIIIII
     +4.000 |          HHHHHHHHHHHHH                  IIIIIIIIIIIIIIIIIII
     +3.500 |GGGG            HHHHHHHHHHHHHH                     IIIIIIIII
     +3.000 |GGGGGGGGGG            HHHHHHHHHHHHHHHH
     +2.500 |     GGGGGGGGGGG             HHHHHHHHHHHHHHHHHHH
     +2.000 |F          GGGGGGGGGGGG               HHHHHHHHHHHHHHHHHHHHHH
     +1.500 |FFFFFFF           GGGGGGGGGGGGG                 HHHHHHHHHHHH
     +1.000 |   FFFFFFFFFF            GGGGGGGGGGGGGGG
            \+---------+---------+---------+---------+---------+---------+
          +20.000   +25.000   +30.000   +35.000   +40.000   +45.000   +50.000

                                        TEMP-C

    Legend:    +140.00=A   +142.00=B   +144.00=C   +146.00=D   +148.00=E
               +150.00=F   +152.00=G   +154.00=H   +156.00=I   +158.00=J
               +160.00=K
```

Table 4.20 Viscosity and specific gravity of cake batter of all ozone treatments with varied time and temperature.

Treatment	Parameters[a]		Specific gravity [a]	Viscosity [a] (x1000 cps)
	Temperature (ºC)	Time (min)		
1	25	5	1.0040±0.006	8.50±0.71
2	25	25	0.8493±0.001	24.25±1.00
3	45	5	0.9905±0.001	8.88±0.25
4	45	25	0.8697±0.006	23.75±0.50
5	21	15	0.8918±0.001	21.25±0.50
6	49	15	0.9549±0.002	14.50±0.58
7	35	1	1.0382±0.005	7.50±0.41
8	35	29	0.8417±0.006	28.25±1.15
9-13	35	15	0.8995±0.004	20.40±1.11

[a] Values represent mean± standard deviation

4.3.1.1. Cake batter properties

Specific gravity and viscosity of cake batter from flours of different treatments as a function of time and temperature is presented in Table 4.20. During mixing, air incorporates into cake batter and the viscosity of batter increased. Since no new air cells are formed after mixing, air bubbles can be lost by coalescence or floatation to the top (Hoseney, 1994; Payne, 1995). A high batter viscosity prevents air bubbles from rising to the top, resulting in increase of batter stability at room temperature. Specific gravity is related to batter viscosity (Kim and Walker, 1992). Generally, a specific gravity close to 1.0 indicates a low number of air cells incorporated into batter while lower specific gravity indicates more air cells incorporated (Payne, 1995). Pyler (1988) stated that batter specific gravity related to the volume, tenderness, and final grain and texture. The optimum specific gravity of batter prepared with emulsified plastic shortening in white layer cake with 140% sugar level with a mixing time of 10 min was about 0.925. The high specific gravity of cake batter gave cake with low volumes and dense grain, whereas low specific gravity of cake batter provided cake with dips on top and with brittle crumb.

The result of this experiment showed that batter specific gravity was related to viscosity of the batter. As the specific gravity of cake batter made from ozonated flours increased, the viscosity decreased. The increase in time of ozonation at a specific temperature increased batter viscosity and decreased batter specific gravity. Temperature did not show a pronounced effect compared to ozonation time. Compared to the results between cake volume index, batter specific gravity, and batter viscosity, the short ozonation time (5 min) gave high batter specific gravity, low batter viscosity and low cake volume. This indicated that less air cells were incorporated into the batter and the viscosity of the batter might not be sufficient to prevent the gas cells from rising to the top of the batter and coalescing to larger air cells. In contrast, long times of ozonation (25 and 29 min) gave lower specific volumes, higher batter viscosities and lower volumes of cakes, indicating that large numbers of air cells were incorporated but the viscosity of the batter might be too high to prevent gas cell expansion during baking, or it might be from other effects.

4.3.2 Heat treatment of flours

Another experiment was conducted to check on the effects of temperature and time, by studying the effect of heat treatment temperature and time of ozonation on the properties of cakes. Heat treatment was conducted at a temperature of 49 ^{o}C for 15 min and the effect of ozonation time was conducted at a temperature of 49 ^{o}C for a time of ozonation of 15 min.

4.3.2.1 Cake batter properties

The viscosity and specific gravity of cake batters from unchlorinated, chlorinated, heat treated, and heat treated plus ozonated flours are given in Table 4.21. The pictures of the cake samples are presented in Figure 4.15. The results showed that the ozonated flour gave higher batter viscosity and lower specific gravity compared to batter from heat treated flour, unchlorinatd flour, and chlorinated flour, indicating a larger number of gas cells were incorporated into the cake batter. Heat treated batter had a lower viscosity and higher specific gravity than other batters. Thomasson *et al*, (1995) reported that viscosity of cake batter at room temperature of heat treated flour (125 ºC, 30 min) was much lower than untreated and chlorine treated flour. Fustier and Gelinas (1998) studied cake batter viscosity using a Brookfield viscometer with a standard high sugar ratio white layer cake method and found that chlorinated flour increased batter viscosity. Cake batter from untreated flour had a viscosity of 8225 cp while chlorinated flour had a viscosity of 14,700 cp.

4.3.2.2 Baking tests

Table 4.22 shows volume index and gas cell structure measured by the C Cell from unchlorinated, chlorinated, heat treated (49 °C, 15 min), and heat treated plus ozonated flours (49 °C,15 min). The volume of cake from heat treatment was less than cake made from ozonated flour treated at the same temperature and time. This result showed a large effect of ozonation on cake performance. The slice brightness and number of cells in cakes made from heat treatment was also lower than those of cakes made from other types of flours. The low number of cells indicated a lower number of gas cells incorporated into the batter. This result was in agreement with the results from Table 4.21 which showed a low specific gravity of the cake batter.

Table 4.21 Viscosity and specific gravity of cake batter from unchlorinated, chlorinated, heat treated, and heat plus ozonated flours.

Flours	Specific gravity [a]	Viscosity [a] (x1000 cps)
Control (unchlorinated)	1.0414±0.008	8.38±0.48
Chlorinated flour	0.9909±0.004	9.63±0.75
Heat treatment 49 °C, 15 min	1.0530±0.002	6.92±0.42
Ozonated 49 °C, 15 min	0.9549±0.002	14.5±0.58

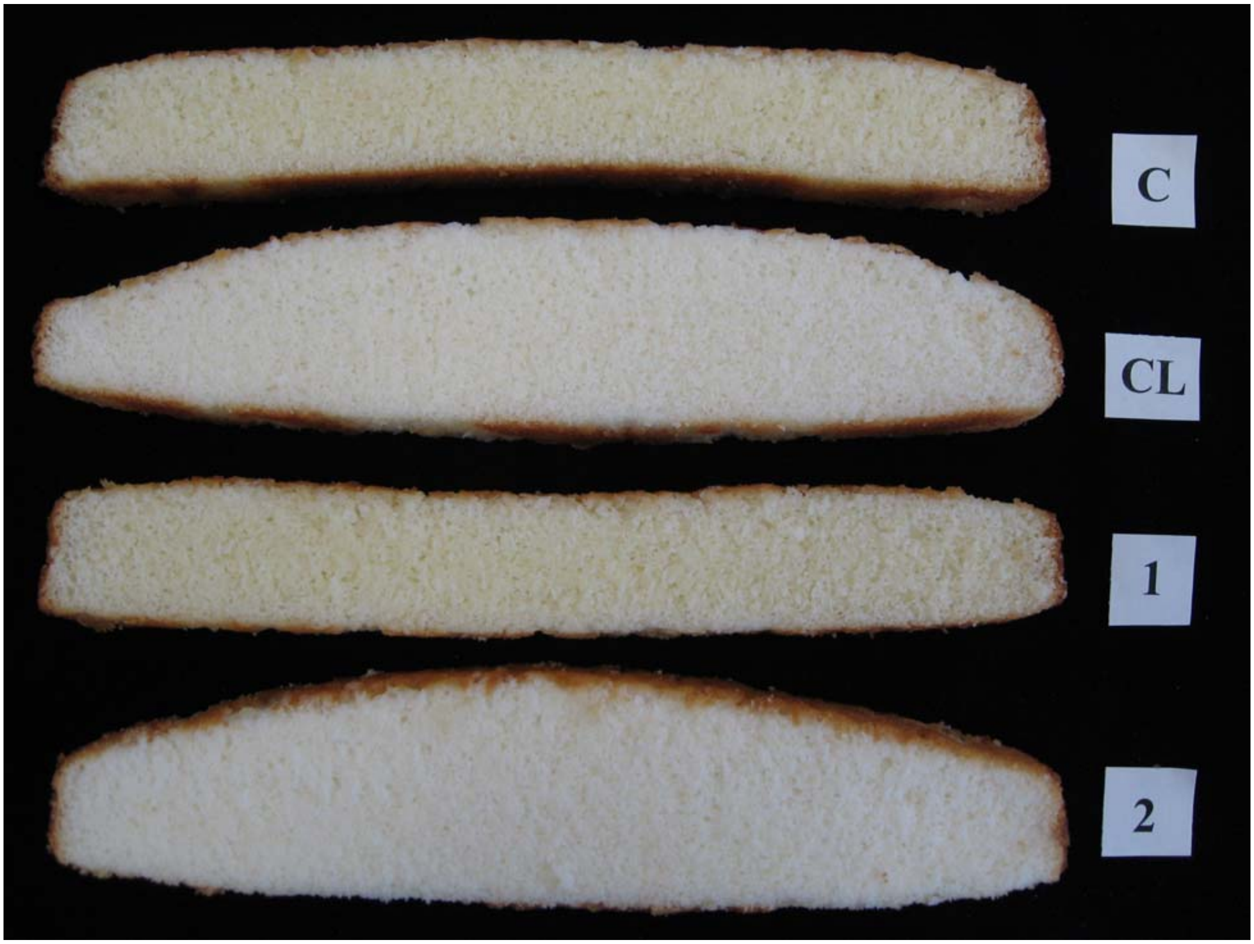
[a] Values represent mean± standard deviation

Table 4.22 Volume and cake cell structure measured by C Cell from unchlorinated, chlorinated, heat treated, and ozonated flours.

Flours	Volume[a]	Slice brightness[a]	Number of cells[a]	Cell diameter[a] (mm)
Control (unchlorinated)	85c	143.5b	2903a	2.07b
Chlorinated flour	110b	159.6a	3151a	2.45ab
Heat treatment 49 °C, 15 min	85c	139.5b	2682b	2.31b
Ozonated 49 °C, 15 min	120a	158.1a	3501a	2.94a

[a] Means with the same letter within columns are not significantly different ($p>0.05$).

Figure 4.15 Pictures of cakes made from (C) Control (unchlorinated flour (CL) Chlorinated flour (1) Flour heat treated at 49 °C for 15 min (2) Heat treatment plus ozonated 49 °C for 15 min flour.



4.3.3. Ozone treatment

The effect of ozone treatment on flour properties and cake batter properties was studied. Unchlorinated flour was treated with ozone in the metal tumbler at room temperature for 5 to 40 min.

4.3.3.1 Flour properties

The pH of flour from unchlorinated, chlorinated, heat treated, and ozonated flours are given in Table 4.23. The results showed that, as time of ozonation progressed, the pH of flour decreased.

4.3.3.1.1 Gelatinization and pasting properties

Gelatinization, pasting, and set back profiles of unchlorinated control, chlorinated, and ozonated flours are shown in Table 4.24. Pasting temperature, defined by the initial rapid increase in paste viscosity of the ozonated flour, decreased as ozonation time increased. However, pasting temperature of control, chlorinated, and ozonated flours were not much different. The pasting temperature of unchlorinated flour was about 1 ºC higher than chlorinated flour and ozonated flour at long treatment times. Peak times for all flours occurred at about 5 to 6 min.

The difference in pasting viscosity of flour may result from the degree of alpha amylase activity and treatment of the flour. Peak viscosity, the highest viscosity during the heating cycle, of ozonated flour, increased as time of ozonation increased and ozone treatments for 20 and 25 min gave values of peak viscosity that were significantly different from other treatments ($P<0.05$). Chlorine treatment gave high pasting viscosity. Whistler *et al*. (1966) reported that the reaction of chlorine on starch components was due to oxidative polymerization leading to the cleavage of glycosidic bonds and formation of D-glucose and D-gluconic acids. Varriano-Marston (1985) stated that chlorine changed starch surface properties by increasing surface hydrophobicity. "The oxidative polymerization of starch broke chains connecting crystallites and the amorphous region made starch structure more relaxed and open, resulting in an increase of surface porosity. The starch then binds tightly with water and can bind more oil". Hoseney (1994) and Payne (1995) stated that chlorine modified properties of starch by oxidation, resulting in greater swelling of starch compared with normal untreated starch and gave a batter that was more viscous than the batter of unoxidized flour at the same temperature. The increase in swelling of starch prevents cakes from collapsing during cooling by occupying the void space that will be left when gas cell pressure decreases as cake temperature is lowered. The increase in peak viscosity of ozone treated flour might be explained by oxidization of starch by ozone, leading to increasing swelling of starch granules during heating.

Break down illustrates the stability of the paste during heating. It is measured as the difference between the peak viscosity and that observed after stirring the hot paste at 95 ^{o}C for a specific time. The decrease in viscosity is caused by the alignment of polymer molecules with the shear field. The flour with a sharp drop in viscosity indicates weakening by mechanical disruption. Chlorinated flour had the highest break down followed by ozonated flour and unchlorinated flour, respectively. Increasing ozonation time enhances the break down of flour samples. The flour samples did not show significant differences in break down values ($P>0.05$).

During cooling, paste consistency increased due to the interaction between molecules, especially amylose molecules, that tend to organize with each other in the continuous phase. The longer ozonation time increased set back and ozonation times greater than 5 min gave set back values comparable to chlorine treated flour ($p>0.05$).

4.3.3.1.2 Water retention capacity

The water retention capacity of flour samples is presented in Table 4.25. The solvent retention capacity was designed to evaluate specific flour qualities. Gaines (2000) stated that water SRC is associated with flour components such as damaged starch, pentosan, gliadins and glutenins. The results showed increased water retention capacity for chlorinated flour and ozone treated flours compared to unchlorinated flour. The ozonation time affected water retention. Increasing ozonation tended to increase water retention capacity. Kulp (1972) stated that chlorination increased the water holding capacity of flour. The increase in water holding capacity was due to intergranular binding at low level of chlorine treatment (lower than 4.0 oz per cwt). The flour absorption as measured by the Farinograph increased, suggesting increased hydration capacity of starch components. The dough stability of chlorine treated flour increased up to 2.0 oz per cwt and then decreased, indicating a change in protein from oxidation and hydrolytic actions. Conforti and Johnson (1992) stated that chlorination increased flour absorption by

Table 4.23 pH of flours from unchlorinated, chlorinated, ozonated flours.

Flours	pH
Control (unchlorinated)	6.13
Chlorinated flour	4.53
5 min ozonated	6.00
10 min ozonated	5.72
15 min ozonated	5.57
20 min ozonated	5.34
25 min ozonated	4.88

Table 4.24 Gelatinization and pasting properties of flour from unchlorinated, chlorinated, and ozonated flours.

Flours	Peak Viscosity (RVU) [a]	Breakdown (RVU) [a]	Final viscosity (RVU) [a]	Set back (RVU) [a]	Peak time (min)	Pasting temperature (^{o}C)
Control	240.79c	79.42a	281.96cd	120.58ab	6.24	66.83
Chlorinated flour	262.03ab	102.97a	295.19bc	136.14ab	5.91	65.75
5 min ozonated	236.38c	86.31a	268.92d	118.86b	6.15	66.43
10 min ozonated	243.71c	88.90a	279.86cd	125.04ab	6.11	66.04
15 min ozonated	249.92bc	91.02a	290.02cd	131.13ab	6.06	65.61
20 min ozonated	265.48ab	95.33a	315.46ab	145.31a	5.98	65.43
25 min ozonated	269.96a	97.50a	321.09a	148.63a	5.96	65.33

[a] Means with the same letter within columns are not significantly different ($p>0.05$)

increasing the permeability on the surface of starch granules. Increased hydration capacity allows starch to interact more with water during baking.

4.3.3.1.3 Thermal properties

The gelatinization temperatures and enthalpies measured by differential scanning calorimetry (DSC) of samples from unchlorinated flour, chlorinated flour, and flours treated with ozone for 10 and 25 min are shown in Table 4.26. The transition temperature of all flour samples did not show a great difference. The enthalpy change (ΔH) indicating the energy required to dissociate or disrupt the ordered structure of granules during gelatinization did not show differences among flour samples. The result was in agreement with Allen *et al*. (1982) who reported that chlorine treatment did not affect the transition temperatures and enthalpies of flour and starch samples as measured by differential scanning calorimetry. Jacobsberg and Daniels (1974) also found insignificant differences in gelatinization temperatures as measured by DSC of batters prepared from treated and untreated flours with water and sugar. However, gelatinization temperature was greatly affected by sugar concentration. Huang *et al.*(1982) studied the gelatinization phase transition using DSC and found that the gelatinization phase transition of untreated and chlorine treated flour did not show differences in simple flour- water and batter containing flour-sugar-shortening-water at the ratio of 1:1.3:0.279:1. For the flour water system (1:1), the transition temperature was 51 ^{o}C, peak temperature was 64 ^{o}C, and termination temperature was 89 ^{o}C. In the batter system, the transition temperature increased to 76 ^{o}C due to the delay of gelatinization by sugar.

Table 4.25 Water retention capacity of flour samples.

Flours	WRC (%)[a]
Control	65.27d
Chlorinated flour	77.03c
5 min ozonated	68.96d
10 min ozonated	80.45bc
15 min ozonated	83.17b
20 min ozonated	92.01a
25 min ozonated	94.40a

[a] Means with the same letter within columns are not significantly different ($p>0.05$)

Table 4.26 Gelatinization temperature and enthalpy of samples from unchlorinated, chlorinated, and ozonated flours.

Flours	Transition temperature (°C)[a]			Δ H (J/g)[a]
	Onset temp	Peak temp	End temp	
Control	59.48a	65.08a	71.09a	8.43a
Chlorinated flour	59.79a	65.51a	71.49a	8.99a
10 min ozonated	58.97a	64.74a	70.62a	9.32a
25 min ozonated	58.45a	64.75a	70.45a	8.60a

[a] Means with the same letter within columns are not significantly different ($p>0.05$).

4.3.3.1.4 Size Exclusion High Performance Liquid Chromatography (SE-HPLC)

Size exclusion high performance liquid chromatography (SE-HPLC) was used to analyze flour proteins. SE- HPLC results of unchlorinated, chlorinated, and ozonated flours at different times (5-40 min) are shown in Figure 4.16, Figure 4.17, and Table 4.27. Areas under the chromatograms were integrated in three parts. The first peak with elution time of 0-15.9 min was mainly glutenins (polymeric proteins). The second peak contained mostly gliadins (monomeric proteins) at the elution time of 15.9-19.7 min. The third peak was albumins and globulins at the elution time of 19.7-22.0 min. The results showed that the percentage of total polymeric protein, mainly glutenins represented by peak 1, did not show differences among the flour samples (Figure 4.14). The gliadins represented in peak 2 and albumins and globulins represented in peak 3 did not show differences for all flour samples. However, the unextractable polymeric protein (UPP) results (Figure 4.17 and Table 4.27) showed an increase in unextractable polymeric protein when the time of ozonation increased. The UPP indicated large-sized polymeric protein (above a certain size). This result indicated that ozone might oxidize SH groups of cysteine residues in protein molecules leading to the formation of intermolecular SS bonds, resulting in increasing the molecular weight of polymeric protein. Schropp *et al*. (1995) found that the thiol groups of HMW glutenin subunits were oxidized by potassium bromate or potassium iodate, leading to an increase in polymerized protein and average molecular weight as measured by gel permeation chromatography. Veraverbeke *et al*. (2000a,b) studied the polymerization of wheat glutenin subunits with oxidizing agents, measured by size exclusion HPLC, multilayer SDS-PAGE, and flow-field flow fractionation, and found that SH groups of high and low molecular weight glutenin subunits were oxidized by oxidizing agents such as potassium bromate, potassium iodate, and hydrogen peroxide. Increase in average molecular size was detected, and the polymerization efficiency of the low molecular weight glutenin subunits was higher than for high molecular weight glutenin subunits.

4.3.3.1.4 Mixing properties

Mixing properties of flours measured by the Mixograph are given in Figure 4.18. The midline peak times of unchlorinated, chlorinated, 5 min ozonated, 10 min ozonated, and 15 min ozonated flours were 5.03, 7.14, 6.85, 8.00, and 9.10 min, respectively. Ozone treated flours had longer mixing times than unchlorinated and chlorinated flours. The longer ozonation times increased the peak time. Gupta *et al*. (1993) stated that unextractable polymeric protein (UPP) positively correlated with dough strength. With increasing UPP, the dough development time increased. The increase in peak time of ozonated flour might occur from the shift of the MWD to higher values as ozonation time increased.

4.3.3.1.5 Cake batter properties

Specific gravity and viscosity of cake batters from ozonated flour at times of ozonation of 5, 10, 15, 20, and 25 min are presented in Figure 4.19 and Figure 4.20, respectively. The specific gravity of cake batter decreased as time of ozonation increased and the batter viscosity increased as time of ozonation increased. The increase of viscosity and decrease of specific gravity at long treatment time indicated large numbers of gas cells incorporated into the cake batter.

Figure 4.16 SE-HPLC chromatograms of total polymeric protein (TPP) of unchlorinated flour, chlorinated flour, and flour treated with ozone for 5 to 40 min.

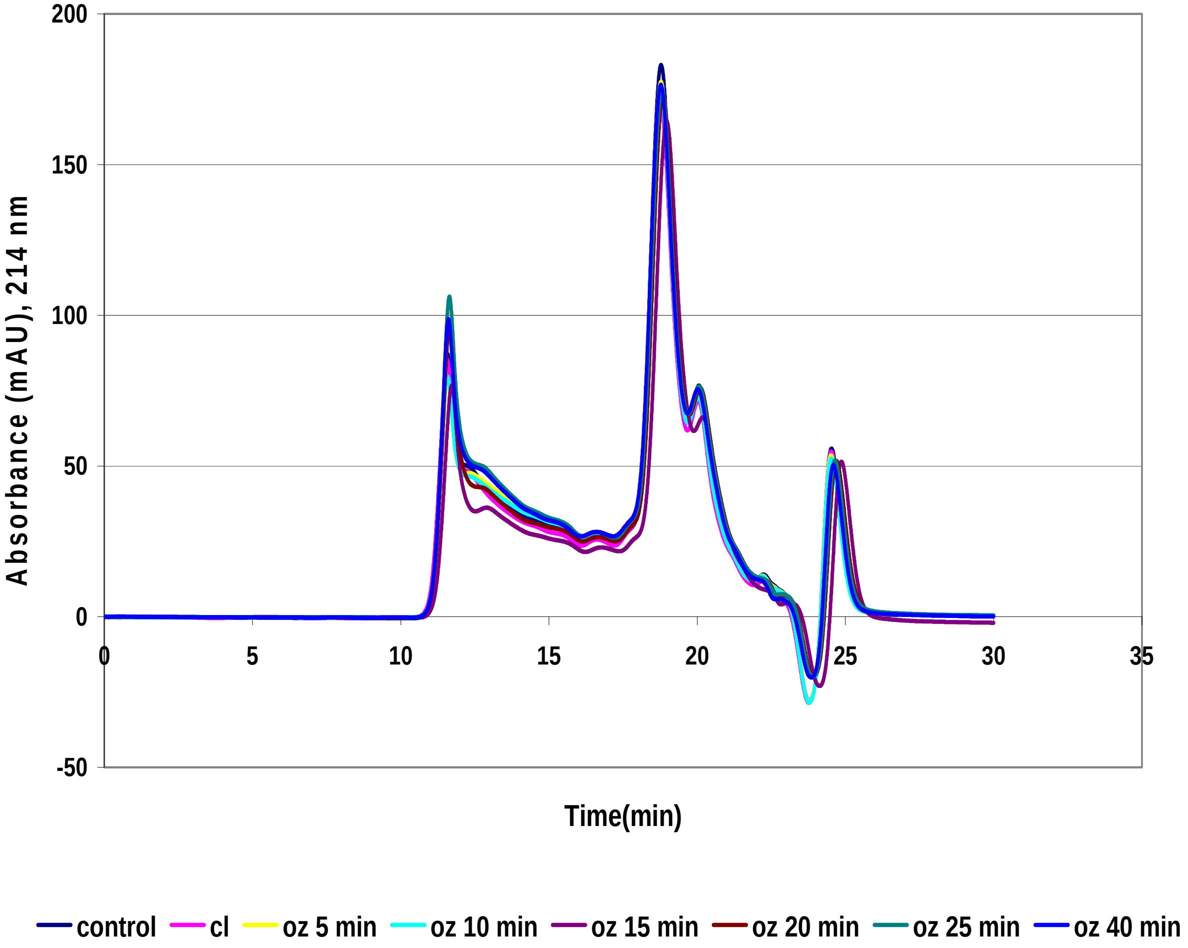


Figure 4.17 SE-HPLC chromatograms of unextractable polymeric protein (UPP) of unchlorinated flour, chlorinated flour, and flour treated with ozone for 5 to 40 min.

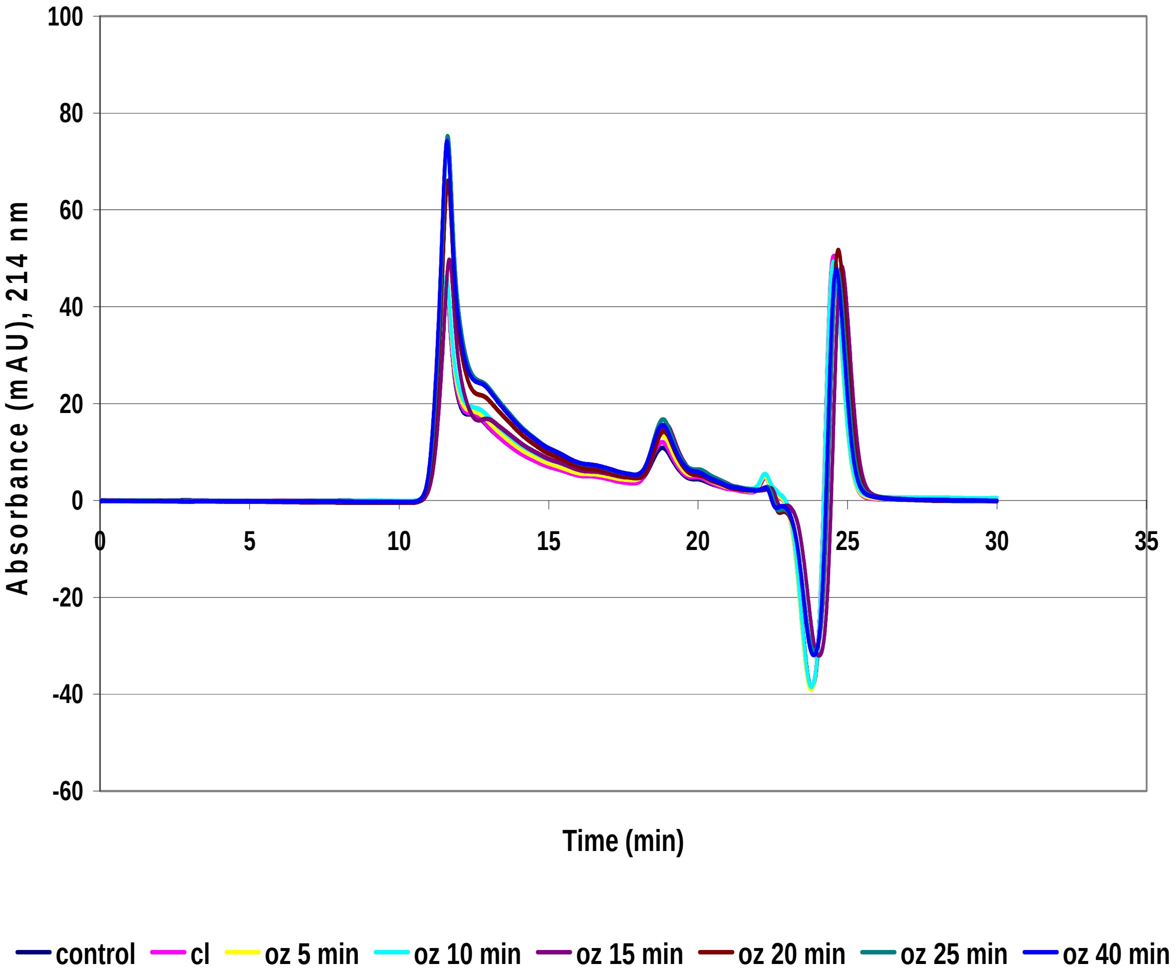


Table 4.27 SE-HPLC relative composition (percentage) of polymeric protein in flour samples.

Flours	TPP[a]	EPP[a]	UPP[a]
Control	38.26±0.28	62.68±0.41	37.32±1.43
Chlorinated flour	38.55±0.33	62.17±0.19	37.83±1.01
5 min ozonated	38.54±0.43	60.65±0.79	39.35±0.98
10 min ozonated	38.05±0.04	59.30±0.04	40.70±1.33
15 min ozonated	36.44±0.29	58.82±0.32	41.18±2.57
20 min ozonated	37.30±0.69	55.71±1.00	44.29±0.50
25 min ozonated	39.53±0.97	52.32±0.58	47.68±0.05
40 min ozonated	38.47±0.88	52.27±0.21	47.73±1.01

[a] TPP = total polymeric protein; EPP = extractable polymeric protein; UPP = unextractable polymeric protein

Figure 4.18 Mixographs of (A) Unchlorinated flour (B) Chlorinated flour (C) Flour treated with ozone for 5 min (D) Flour treated with ozone for 10 min (E) Flour treated with ozone for 15 min.

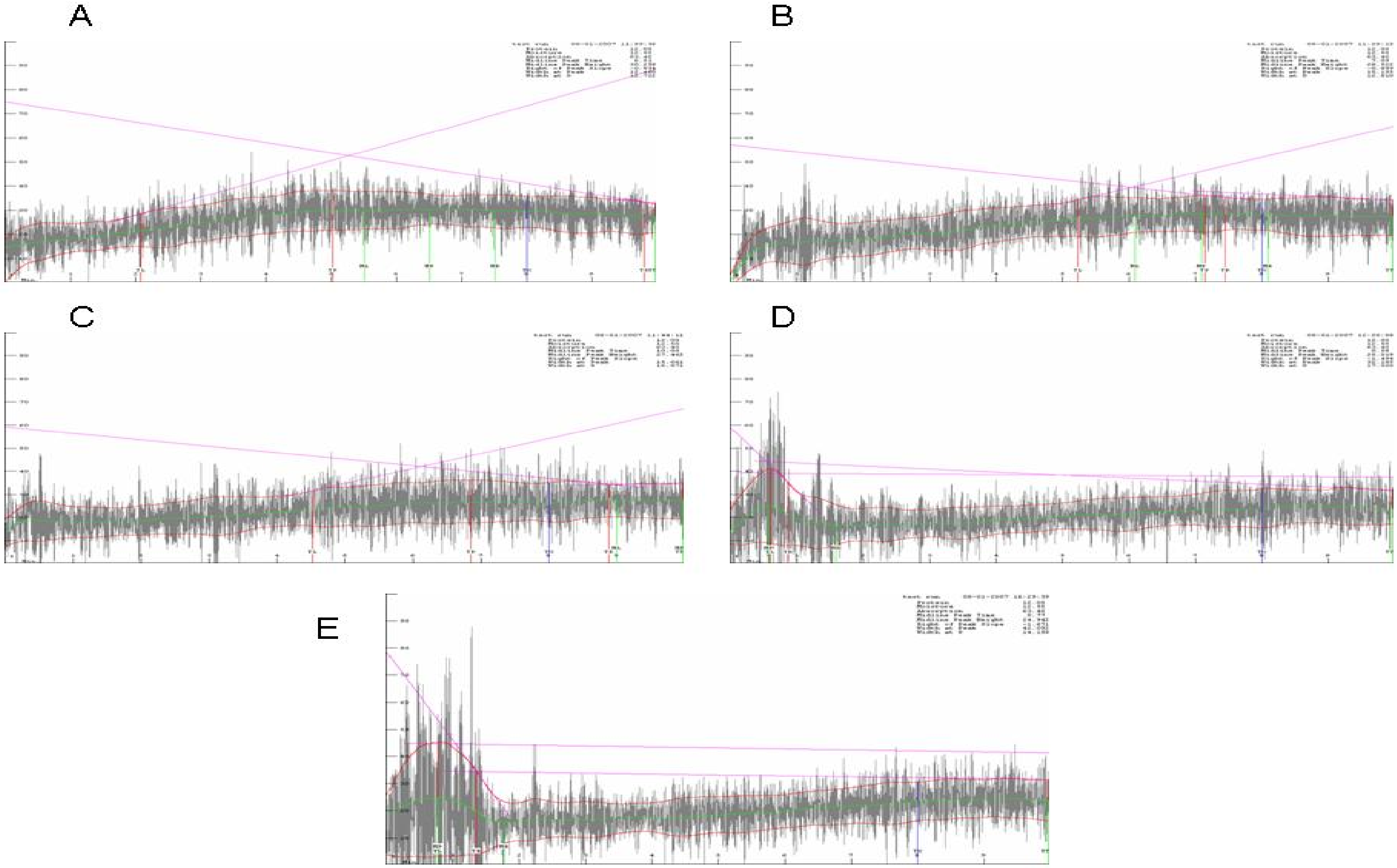


Figure 4.19 Specific gravity of cake batters made from unchlorinated flour, and ozonated flour at different times of ozonation.

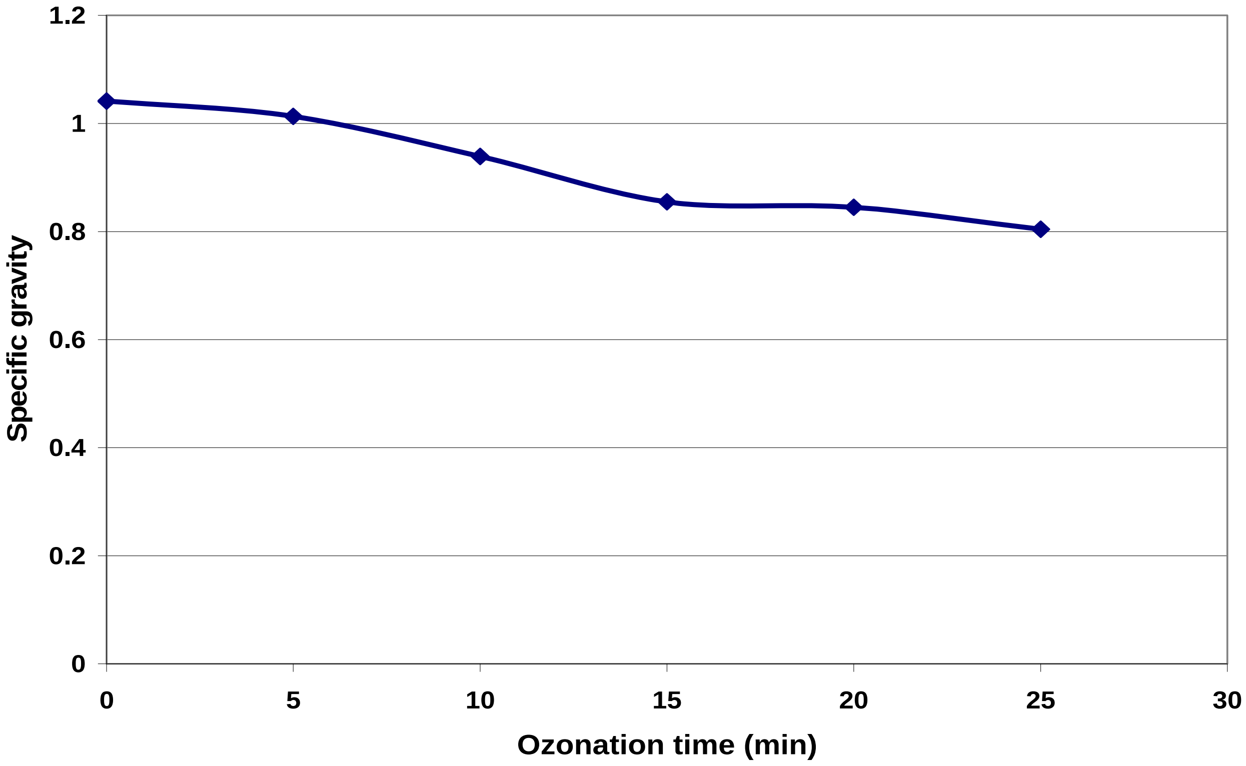
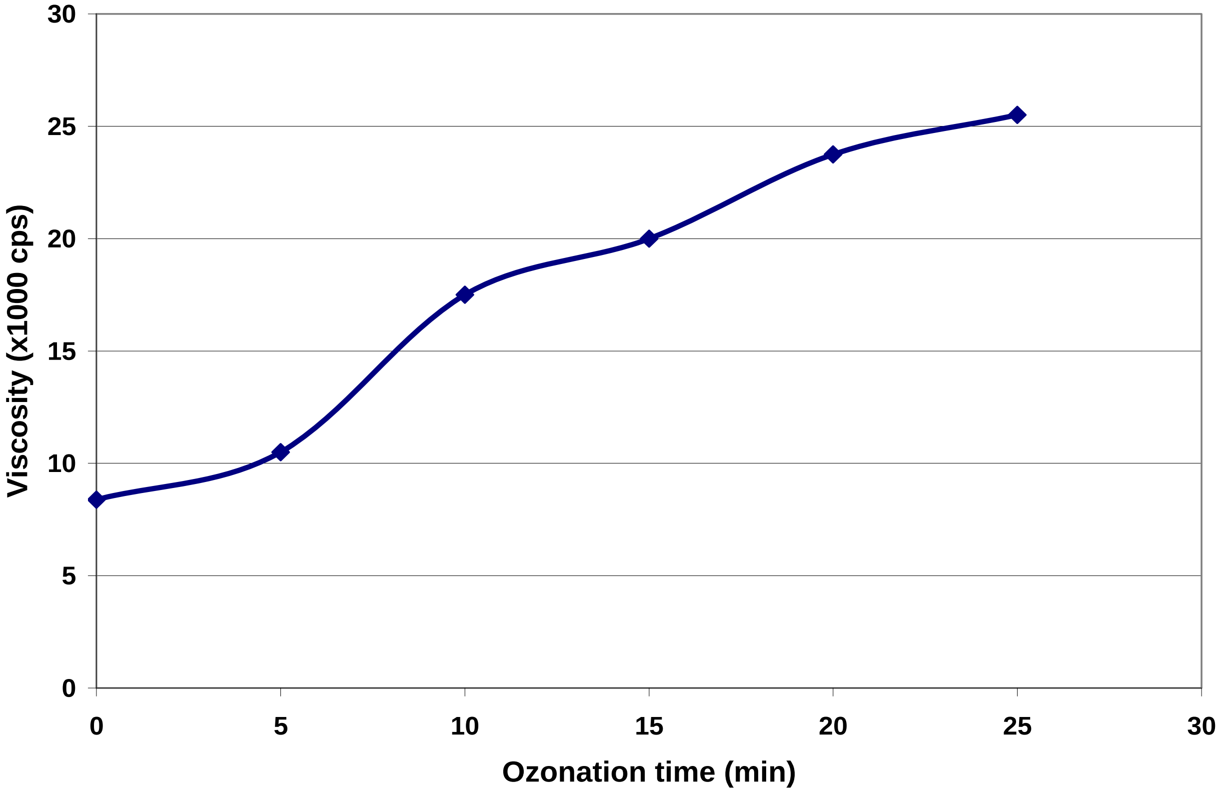


Figure 4.20 Viscosity of cake batters made from unchlorinated flour, and ozonated flour at different times of ozonation.



CHAPTER 5 - Conclusions and Future studies

5.1 Conclusion

A decrease in pH and a slight increase in lightness (L) of flour occurred when flour was treated with ozone in the wooden tumbler. Ozonation of soft wheat flour improved cake volume, brightness, and increased softness of cakes. As the ozonation time increased, the volume of cakes increased and at 10 min ozonation time, cakes had similar volumes to chlorinated cake flour. The springiness and cohesiveness of cakes from ozonated flours were similar to cakes produced from chlorinated flour. The study of cake cell structure showed that cell brightness and number of cells of cake from 36 min ozonation time gave similar values to chlorinated cake flour.

Different types of volatile compounds were detected in flour samples. Hydrocarbon and other compounds such as benzene, and acetonitrile were mainly found in unchlorinated control flour, followed by aldehydes including hexanal and nonanal, alcohols, benzenes, and ketones. The volatile compounds present in ozone treated flours were mainly aldehydes and ketones including hexanal, nonanal, E-2-nonenal decanal, heptanal, octanal, E-2 octenal, and 2-propanone. Increasing ozonation times tended to increase the levels of those volatile compounds. A rapid decrease in volatile compounds was detected when ozone treated flours were kept in the fume hood. Ozonation of defatted soft wheat flour produced less volatile aldehydes than ozone treated non-defatted flour, suggesting that some of these compounds originated from the lipid. Cakes made from flours after lipid extraction and after lipid extraction plus ozonation had low volumes compared to control, chlorinated and ozone treated whole flours, indicating that lipids play a role in cake performance.

The optimum temperature and time for ozone treatment using response surface methodology design with three response parameters (volume index, firmness, and slice brightness) was about 8 to 11 min with the temperature range between 36-46 ^{o}C. The effect of ozone treatment time on the response parameter was highly significant compared to the temperature. Ozone treatment for 5 to 25 min at room temperature showed a decrease in pH of flour when time of ozonation increased. Increases in peak viscosity as measured by the Rapid Visco Analyzer, water retention capacity, Mixograph peak time, and unextractable polymeric protein (UPP) were observed when ozonation time was increased. The ozone treatment did not affect the transition temperature and enthalpy change of flour samples. Decrease in batter specific gravity and increase in batter viscosity were observed when ozonation time was increased.

5.2 Future Studies

Further research should be conducted to study the shelf life of flours and cakes and application of ozonated flour to other types of products such as cookies. Since ozonated flour had a strong odor that affects the odor and flavor in the cakes, research needs to be focused on how to decrease the strong odor in flours by using processing techniques or other methods.

References

AACC. 2000. Approved Methods of the AACC, Tenth Edition. American Association of Cereal Chemists, St. Paul, Minnesota, USA.

Achen, M., and Yousef, A.E. 2001. Efficacy of ozone against *Escherichia coli* O157:H7 on apples. Journal of Food Science. 66(9):1380-1384.

Allen, J.E., Sherbon, J.W., Lewis, B.A., and Hood, L.F. 1982. Effect of chlorine treatment on wheat flour and starch: measurement of thermal properties by different scanning calorimetry. Journal of Food Science. 47: 1508-1511.

Bablon, G., Bellamy, W.D., Bourbigot, M., Daniel, F.B., Dore, M., Erb, F., Gordon, G., Langlias, B., Laplanche, A., Legube, A., Martin, G., Masschelein, W.J., Pacey, G., Reckhow, D.A., and Ventresque. 1991. Fundamental aspects. In: Langlias, B., Reckhow, D.A., and Brink, D.R. (Eds.). Ozone in Water Treatment Application and Engineering. Lewis Publishers, Inc. Michigan, USA.

Bader, H., and Hoigne, J. 1981. Determination of ozone in water by the indigo method. Water Research. 15:449-456.

Bean, M.M., Yamazaki, W.T., and Donelson, D.H. 1978. Wheat starch gelatinization in sugar solutions. II. Fructose, glucose, and sucrose: cake performance. Cereal Chemistry. 55(6):945-952.

Belitz, H.D., and Grosch, W. and Schieberle, P. 2004. Food Chemistry. Springer-Verlag Berlin Heidelberg, New York.

Bott, T.R. 1991. Ozone as a disinfecting in a process plant. Food Control. 2(1):44-49.

Buttery, R.G., Ling, L.C., and Bean, M.M. 1978. Coumarin off-odor in wheat flour. Journal of Agriculture and Food Chemistry. 26(1):179-180.

Cherry, J.P. 1999. Improving the safety of fresh produce with antimicrobials. Food Technology. 53(11): 54-57.

Clements, R.L. and Donelson, J.R. 1982a. Role of free flour lipids in batter expansion in layer cakes. I. Effects of aging. Cereal Chemistry. 59(2):121-124.

Clements, R.L. and Donelson, J.R. 1982b. Role of free flour lipids in batter expansion in layer cakes. II. Effects of heating. Cereal Chemistry. 59(2):125-128.

Conforti, F.D., and Johnson, J.M. 1992. Use of the farinograph in predicting baking quality of unchlorinated and chlorinated flours. Journal of Food Quality. 15:333- 347.

Daniels, D.G.H. 1960. Changes in the lipid of flour induced by treatment with chlorine dioxide or chlorine, and on storage. Journal of the Science of Food and Agriculture. 11:664-670.

Daniels, N.W.R., Frape, D.L., Russell Eggitt, P.W., and Coppock, J.B.M. 1963. Studies on the lipids of flour. II. Chemical and toxicological studies on the lipid of chlorine-treated cake flour. Journal of the Science of Food and Agriculture. 11:883-893.

Donelson, J.R., Gaines, C.S., and Finney, P.L. 2000. Baking formula innovation to eliminate chlorine treatment of cake flour. Cereal Chemistry. 77(1):53-57.

Donelson, J.R., Yamazaki, W.T., and Kissell, L.T. 1984. Functionality in white layer cake of lipids from untreated and chlorinated patent flours. II. Flour fraction interchange studies. Cereal Chemistry. 61(2):88-91.

Dubois, M., Coste, C., Despres, A.-G., Efstathiou, T., Nio, C., Dumont, E., and Parent-Massin, D. 2006. Safety of Oxygreen®, an ozone treatment on wheat grains. Part 2. Is there a substantial equivalence between Oxygreen-treated wheat grains and untreated wheat grains. Food Additives and Contaminants. 23(1): 1-15.

Duviau, M.P., Yamamoto, H., Ng, P.K.W., and Kobrehel, K. 1996. Modifications of wheat proteins due to flour chlorination. Cereal Chemistry. 73(4):490-494.

Earls, D. 2003. Alternative to chlorination of cake flour. Ph.D. thesis. Kansas State University, Manhattan.

El-Din, M.G., Smith, D.W., Momani, F.A., and Wang, W. 2006. Oxidation of resin and fatty acids by ozone: kinetics and toxicity study. Water Research. 40:392-400.

EPA, United States Enviromental Protection Agency. 1999. Alternative disinfectants and oxidants guidance manual, Publication: 815 R 99014.

Fan, L., Song, J., McRae, K.B., Walker, B.A., and Sharpe, D. 2007. Gaseous ozone treatment inactivates *Listeria innocua* in vitro. Journal of Applied Microbiology. June: 1-7.

Finney, K.F., and Shogren, M.D. 1972. A Ten-gram mixograph for determining and predicting functional properties of wheat flours. The Bakers Digest. April: 32-42.

Fustier, P., and Gelinas, P. 1998. Combining flour heating and chlorination to improve cake texture. Cereal Chemistry. 75(4): 568-570.

Gaines, C.S., and Donelson, J.R. 1982. Cake batter viscosity and expansion upon heating. Cereal Chemistry. 59(4): 237-240.

Gaines, C.S. 2000. Collaborative study of methods for solvent retention capacity profiles (AACC Method 56-11). Cereal Foods World. 45(7):303-306.

Gaou, I., Dubois, M., Pfohl-Leszkowicz, A., Coste, C., De Jouffrey, S., and Parent-Massin, D. 2005. Safety of Oxygreen®, an ozone treatment on wheat grains. Part 1. A four-week toxicity study in rats by dietary administration of treated wheat. Food Additives and Contaminants. 22(11):1113-1119.

Gouch, B.M., Whitehouse, M.E., and Greenwood, C.T. 1978. The role and function of chlorine in the preparation of high-ratio cake flour. CRC. Critical Reviews in Food Science and Nutrition. Sept: 91-113.

Graham, D.M. 1997. Use of ozone for food processing. Food Technology. 51(6):72-75.

Grosjean, E. and Grosjean, D. 1997. Gas phase reaction of alkenes with ozone: formation yields of primary carbonyls and biradicals. Environmental Science and Technology. 31(8):2421-2427.

Grosjean, D., Grosjean, E., and Williams II, E.L. 1994. Atmospheric chemistry of olefins: A product study of the ozone-alkene reaction with cyclohexane added to scavenge OH. Environmental Science and Technology. 28(1):186-196.

Gupta, R.B., Khan, K., and MacRitchie, F. 1993. Biochemical basis of flour properties in bread wheats. I. Effects of variation in the quality and size distribution of polymeric protein. Journal of Cereal Science. 18:23-41.

Han, Y., Floros, J.D., Linton, R.H., Nielsen, S.S., and Nelson, P.E. 2002. Response surface modeling for the inactivation of *Escherichia coli* O157:H7 on green peppers(*Capsicum annuum*) by ozone gas treatment. Journal of Food Science. 67(3):1188-1193.

Hoseney, R.C. 1994. Principles of Cereal Science and Technology. American Association of Cereal Chemists, Inc. St. Paul, Minnesota, USA

Hoseney, R.C. Atwell, W. A., and Linerback, D.R. 1977. Scanning electron microscopy of starch isolated from baked products. Cereal Foods World. 22:56.

Huang, G., Finn, J.W., and Varriano-Marston, E. 1982. Flour chlorination. II. Effect of water binding. Cereal Chemistry. 59(6):500-506.

Ibanoglu, S. 2002. Wheat washing with ozonated water: effects on selected flour properties. International Journal of Food Science and Technology. 37:579-584.

Ibanoglu, S. 2001. Influence of tempering with ozonated water on the selected properties of wheat flour. Journal of Food Engineering. 48:345-350.

Jacobsberg, F.R., and Daniels, N.W.R. 1974. Gelatinisation properties of high-ratio cake flours. Chemistry and Industry. 1007.

Johnson, A.C., Hoseney, R.C., and Varriano-Marston, E. 1979. Chlorine treatment of cake flours. I. Effect of lipids. Cereal Chemistry. 56(4):333-335.

Johnson, A.C., Hoseney, R.C., and Ghaisi, K. 1980. Chlorine treatment of cake flours. V. Oxidation of starch. Cereal Chemistry. 57(2):94-96.

Katai, A.A., and Schuerch, C. 1966. Mechanism of ozone attack on α-methyl glucoside and cellulosic materials. Journal of Polymer Science. 4(10): 2683-2703.

Kells, S., Mason, L.J., Maier, D.E., and Woloshuk, C.P. 2001. Efficacy and fumigation characteristics of ozone in stored maize. Journal of Stored Products Research. 37:371-382.

Khadre, M.A., Yousef, A.E., and Kim J.-G. 2001. Microbiological aspects of ozone applications in food: A review. Journal of Food Science. 66(9):1242-1252.

Kim, J.-G., Ahmed, E.Y., and Dave, S. 1999a. Application of ozone for enhancing the microbiological safety and quality of foods: A review. Journal of Food Protection. 62(9): 1071-1087.

Kim, J.-G. Yousef, A.E., and Chism, G.W. 1999b. Use of ozone to inactivate microorganisms on lettuce. Journal of Food Safety. 19:17-34.

Kim, J.-G, Yousef, A.E., and Khadre, M.A. 2003. Ozone and its current and future application in the food industry. Advances in Food and Nutrition Research. 45:167-218.

Kim, C.S., and Walker, C.E. 1992. Interaction between starches, sugars, and emulsifiers in high ratio cake model systems. Cereal Chemistry. 69(2):206-212.

Kissell, L.T., Donelson, J.R., and Clements, R.L. 1979. Functionality in white layer cake of lipids from untreated and chlorinated patent flours. I. Effects of free lipids. Cereal Chemistry. 56(1): 11-14.

Kroll, J.H., Clarke, J.S., Donahue, N.M., Anderson, J.G., and Demerjian, K.L. 2001a. Mechanism of HO_x formation in the gas-phase ozone-alkene reaction.1. Direct, pressure-dependent measurements of prompt OH yields. Journal of Physical Chemistry. 105:1554-1560.

Kroll, J.H., Sahay, S.R., Anderson, J.G., Demerjian, K.L., and Donahue, N.M. 2001b. Mechanism of HO_x formation in the gas-phase ozone-alkene reaction. 2. Prompt versus thermal dissociation of carbonyl oxides in form OH. Journal of Physical Chemistry. 105: 4446-4457.

Kulp, K. 1972. Some effects of chlorine treatment of soft wheat flour. The Bakers Digest. June: 26-32.

Kulp, K., and Tsen, C.C. 1972. Effect of chlorine on the starch component of soft wheat flour. Cereal Chemistry: 49: 194-200.

Langlias, B., Reckhow, D.A., and Brink, D.R. 1991. Ozone in Water Treatment Application and Engineering. Lewis Publishers, Inc. Michigan, USA.

Liew, C.L., and Prange, R.K. 1994. Effect of ozone and storage temperature on postharvest diseases and physiology of carrots (*Daucus carota* L.). Journal of the American Society for Horticultural Science. 119(3): 563-567.

MacRitchie, F., and Gras. P.W.1973. The role of flour lipids in baking. Cereal Chemistry. 50: 292-302.

MacRitchie, F. 2003. Fundamentals of dough formation. Cereal Foods World. 48(4):173-176.

Martinez, R.I., Herron, J.T., and Huie, R.E. 1981. The mechanism of ozone-alkene reactions in the gas phase. A mass Spectrometric study of the reactions of eight linear and branched-chain alkenes. Journal of the American Chemical Society. 103:3807-3820.

Mehlman, M.A., and Borek, C. 1987. Toxicity and biochemical mechanisms of ozone. Environmental Research. 42(1):36-53.

Mendez, F., Maier, D.E., Mason, L.J., and Woloshuk, C.P. 2003. Penetration of ozone into columns of stored grains and effects on chemical composition and processing performance. Journal of Stored Products Research. 39:33-44.

Morrison, W.R. 1988. Lipids in cereal starches: A review. Journal of Cereal Science. 8:1-15.

Mudd, J.B., Leavitt, R., Ongun, A., and McManus, T.T. 1969. Reaction of ozone with amino acids and proteins. Atmospheric Environment. 3:669-682.

Nakamura, M., and Kurata, T. 1997. Effect of L-ascorbic acid on the rheological properties of wheat flour-water dough. Cereal Chemistry. 74(5) 647-650.

Payne, J. 1995. Baking Science I Laboratory manual. Department of Grain Science and Industry, Kansas State University, Manhattan, Kansas, USA.

Pryor, W.A. Giamalva, D.H., and Church, D.F. 1984. Kinetics of ozonation. 2. Amino acids and model compounds in water and comparisons to rates in nonpolar solvents. Journal of the American Chemical Society. 106: 7094-7100.

Pyler, E.J. 1988. Baking Science and Technology. Sosland Publishing company., Kansas City, Missouri, USA.

Rakness, K., Gordon, G., Langlias, B., Masschelein, W., Matsumoto, N., Richard, Y., Robson, C.M., and Somiya, I. 1996. Guideline for measurement of ozone concentration in the process gas from an ozone generator. Ozone Science and Engineering. 18:209-229.

Rice, R.G., Farquhar, J.W., and Bollyky, L.J. 1982. Review of the applications of ozone for increasing storage times of perishable foods. Ozone Science and Engineering. 4:147-163.

Rice, R.G. 1997. Applications of ozone for industrial wastewater treatment- A review. Ozone Science and Engineering. 18: 477-515.

Rich, T. 1994. Match the ozone technology to your job. Some systems cost more, but produce more. Water Technology. 17:51-52.

Ruan, R., Lei, H., Chen, P., Deng, S., Lin, X., Li, Y., Wilcke, W., and Fulcher, G. 2004. Ozone-aided corn steeping process. Cereal Chemistry. 81(2):182-187.

Russo, J.V., and Doe, C.A.1970. Heat treatment of flour as an alternative to chlorination. Journal of Food Technology. 5:363-374.

Sayaslan, A., Chung, O.K., Seib, P.A., and Seitz, L.M. 2000. Volatile compounds in five starches. Cereal Chemistry. 77(2):248-253.

Schropp, P., Belitz, H., Seilmeier, W., and Wieser, H. 1995. Reoxidation of high molecular weight subunits of glutenins. Cereal Chemistry. 72(4): 406-410.

Seguchi, M., and Matsuki, J. 1977. Studies on pan-cake baking. I. Effect of chlorination of flour on pan-cake qualities. Cereal Chemistry. 54(2):287-299.

Seguchi, M. 1984. Oil binding capacity of prime starch from chlorinated wheat flour. Cereal Chemistry. 61(3): 241-244.

Seguchi, M. 1987. Effect of chlorination on the hydrophobicity of wheat starch. Cereal Chemistry. 64(4): 281-282.

Seguchi, M. 1990. Study of wheat starch granule surface proteins from chlorinated flours. Cereal Chemistry. 67(3):258-260.

Seitz, L.M. 1995. Volatile compounds in wheat cultivars from several locations in Kansas. In: Food Flavors:Generation, Analysis, and Process Influence. G. Charalambous (ed.). Elsevier Science B.V. Amsterdam.

Seitz, L.M., Ram, M.S., and Rengarajan, R. 1999. Volatiles obtained from whole and ground grain samples by supercritical carbon dioxide and direct helium purge methods: observations on 2,3-butanediols and halogenated anisoles. Journal of Agricultural and Food Chemistry. 47(3): 1051-1061.

Sinha, N.K., Yamamoto, H., and Ng, P.K.W. 1997. Effects of flour chlorination on soft wheat gliadins analyzed by reverse-phase high- performance liquid chromatography, differential scanning calorimetry and fluorescence spectroscopy. Food Chemistry. 59(3):387-393.

Sheldon, B.W. and Brown, A.L. 1986. Efficacy of ozone as a disinfectant for poultry carcasses and chill water. Journal of Food Science. 51(2):305-309.

Sollars, W.F. 1958. Cake and cookie flour fractions affected by chlorine bleaching. Cereal Chemistry. 35:100-110.

Spies, R.D., and Kirleis, A.W. 1978. Effect of free flour lipids on cake-baking potential. Cereal Chemistry. 55(5):699-704.

Spies, R.D, and Hoseney, R.C. 1982. Effect of sugars on starch gelatinization. Cereal Chemistry. 59(2): 128-131.

Staehelin, J. and Holgne, J. 1985. Decomposition of ozone in water in the presence of organic solutes acting as promoters and inhibitors of radical chain reactions. Environmental Science and Technology. 19:1206-1213.

Stauffer, C.E. 1990. Functional Additives for Bakery Foods. Van Nostrand Reinhold, New York, USA.

Thomasson, C.A., Miller, R.A., and Hoseney, R.C. 1995. Replacement of chorine treatment for cake flour. Cereal Chemistry. 72(6): 616-620.

Tsen, C.C., and Kulp, K. 1971. Effects of chlorine on flour proteins, dough properties, and cake quality. Cereal Chemistry. 48:247-254.

U.S. Environmental Protection Agency website. 2007. Available from: http://www. epa.gov/air/ozonepollution/ (Accessed November 15, 2007).

Varriano-Marston, E. 1985. Flour chlorination: New thoughts on an old topic. Cereal Foods World. 30(5):339-343.

Veraverbeke, W.S., Larroque, O.R., Bekes, F., and Delcour, J.A. 2000a. In vitro polymerization of wheat glutenin subunits with inorganic oxidizing agents. I. Comparison of single-step and stepwise oxidations of high molecular weight glutenin subunits. Cereal Chemistry: 77(5): 582-588.

Veraverbeke, W.S., Larroque, O.R., Bekes, F., and Delcour, J.A. 2000b. In vitro polymerization of wheat glutenin subunits with inorganic oxidizing agents. II. Stepwise oxidation of low molecular weight glutenin subunits and a mixture of high and low molecular weight glutenin subunits. Cereal Chemistry. 77(5):589-594.

Weak, E.D., Hoseney, R.C., Seib, P.A., and Biag, M. 1977. Mixograph studies. I. Effect of certain compounds on mixing properties. Cereal Chemistry. 54(4): 794-802.

Wei, C.-I., Cook, D.L., and Kirk, J.R. 1985. Use of chlorine compounds in the food industry. Food Technology. Jan: 107-115.

Whistler, R.L. Mittag, T.W., and Ingle, T.R.1966. Mechanism of starch depolymerization with chlorine. Cereal Chemistry. 43:362-371.

Williams, R.C., Sumner, S.S., and Golden, D.A. 2005. Inactivation of *Escherichia coli* O157:H7 and Salmonella in apple cider and orange juice treated with combinations of ozone, dimethyl dicarbonate, and hydrogen peroxide. Journal of Food Science. 70(4):197-201.

Xu, L. 1999. Use of ozone to improve the safety of fresh fruits and vegetables. Food Technology. 53(10):58-61.

Yang, P.P.W., and Chen, T.C. 1979. Stability of ozone and its germicidal properties on poultry meat microorganisms in liquid phase. Journal of Food Science. 44(2): 501-504.

Yvin, J.-C., Bailli, A. Joubert, J.-M, and Bertaud, O. 2001. Method and installation for making flour from ozone-treated grains. Australian Patent. Patent number 780378.

Zhao, J., and Cranston, P.M. 1995. Microbial decontamination of black pepper by ozone and the effect of the treatment on volatile oil constituents of the spice. Journal Science Food and Agriculture. 68:11-18.

Appendix A - Appendix for Part 1, 2, and 3

Table A.1 Volume of cakes made from flour treated with ozone gas for 10, 20, 30, 36, and 40 min.

Flours	Volume
10 min ozonated	113±1.00
20 min ozonated	114±4.24
30 min ozonated	118±2.12
36 min ozonated	121±4.25
40 min ozonated	113±2.12

Table A.2 Mixogram results of control (unchlorinated), chlorinated flour, flour treated with ozone gas for 10 min, and flour treated with ozone gas for 36 min.

Flours	Midline peak time (min)	Midline peak height	Width at peak
Control (Unchlorinated)	4.62±0.92	26.97±1.00	20.17±1.90
Chlorinated	4.90±0.34	25.70±0.65	20.40±0.90
10 min ozonated	5.47±0.62	26.07±2.60	21.27±2.75
36 min ozonated	8.51±0.97	26.06±2.60	21.16±2.75

Table A.3 Values of volume, firmness, and brightness of cakes made from all ozone treatments as a function of treatment time and temperature.

Treatments	Volume index	Firmness	Brightness
1	101.5±2.29	443.5±11.42	157.3±3.46
2	87.8±3.51	746.2±72.34	147.0±5.20
3	103.8±3.69	467.0±38.28	159.0±4.32
4	86.3±1.61	782.6±50.15	145.4±4.02
5	107.7±3.33	501.5±68.95	155.8±4.32
6	115.3±0.29	426.9±27.07	157.1±0.96
7	104.2±4.04	422.3±48.29	149.5±2.29
8	83.3±2.31	929.8±60.99	140.9±3.81
9	112.2±1.15	451.5±8.15	155.6±6.47
10	112.0±2.00	415.0±29.11	159.1±3.50
11	110.8±2.57	441.9±54.42	159.2±3.98
12	111.3±2.08	444.1±55.54	157.8±3.21
13	110.7±3.40	450.3±67.97	156.5±6.27

Table A.4 Mixographs of unchlorinated flour, chlorinated flour, flour treated with ozone for 5 min, flour treated with ozone for 10 min, and flour treated with ozone for 15 min.

Flours	Midline peak time (min)	Midline peak height	Width at peak
Control(Unchlorinated)	5.03	38.1	16.5
Chlorinated	7.14	36.0	15.2
5 min ozonated	6.85	36.2	18.5
10 min ozonated	8.00	41.0	32.3
15 min ozonated	9.10	45.4	42.1

Table A.5 Viscosity and specific gravity of cake batters made with unchlorinated flour and ozonated flours at different ozonation times.

Flours	Viscosity (cp)	Specific gravity
Control(Unchlorinated)	8.38±0.48	1.0414±0.008
5 min ozonated	10.05±0.58	1.0131±0.004
10 min ozonated	17.50±0.71	0.9390±0.004
15 min ozonated	20.00±1.41	0.8551±0.005
20 min ozonated	23.75±1.06	0.8449±0.003
25 min ozonated	25.50±0.71	0.8041±0.013