COMPARATIVE EFFECTS OF TWO OZONATION TREATMENTS ON WHEAT FLOUR TECHNOLOGICAL PROPERTIES

by

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Abstract

Ozone, a triatomic form of oxygen with a Generally Recognized As Safe (GRAS) status from the U. S. Food and Drug Administration, is a strong antimicrobial and sanitizing agent with numerous potential applications in the food industry. One of them is the improvement of wheat flour baking qualities, by replacement of the actual chlorination treatment.

Following recent developments realized by the company Goëmar (France) which invented and patented an ozone treatment device for wheat grain and a method for making flour from ozone-treated grains, this study aims to determine the effect of ozone treatment on wheat grain and on wheat flour, and to compare them. Three different ozone concentrations with different application times rendering three quantities of absorbed ozone have been investigated. Rheological, physicochemical and baking properties of soft wheat flours stemming from both treatments were evaluated and compared to untreated flour.

Results were overall significant and showed that the treatment of flour gives more marked results than the treatment on grain for retention capacity in sucrose and volume of cakes but decreases the α-amylase activity. On the other hand, action of ozone on grain augments the maximum viscosity of the flour. Bread volume was found to be increased by both treatments in similar proportions. The treatments were also analyzed in particular and showed specific characteristics. A single treatment has not been determined to enhance all characteristics of the flour. Hence, the modification of precise features of the flour has to be related to a specific treatment.

Table of Contents

List of Figures

List of Tables

Appendix B

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Bibliography

1. The Wheat, from kernel to flour

Wheat, member of the Gramineae family, is among the oldest and most extensively grown of all crops. Its world production as a cereal crop is ranked second behind corn. Different species of wheat exist but the most widely used are *Triticum aestivum* (hexaploid), also called common wheat or bread wheat, and *Triticum turgidum* subsp. *durum* (tetraploid), also called durum wheat or macaroni wheat (Orth and Shellenberger 1988).

1.1. Structure of the wheat grain (Hoseney 1994)

From a botanical point of view, the wheat grain is a single-seeded fruit called a caryopsis but it commonly goes by the denomination of kernel. It consists of a pericarp (or fruit coat), which surrounds the seed and adheres tightly to a seed coat. This seed is composed of an embryo or germ and an endosperm enclosed by a nucellar epidermis and a seed coat (Figure 1).

The caryopsis develops within modified leaves called glumes. They are readily removed during threshing and the grain is said to be naked since it has an uncovered caryopsis.

The color can vary from light buff or yellow to red-brown. It is due to the absence or presence of red pigmentation in the seed coat and is genetically controlled (Freed et al 1976). Wheat has then been consistently classified as red or white. Still, another variable affects the perception of grain color: the texture of the endosperm. It corresponds to air spaces in the endosperm at the many air-starch and air-protein interfaces. An absence of air results in a glassy appearance whereas a discontinuous matrix gives a chalky appearance (Evers and Bechtel 1988).

The wheat grain averages 8 mm in length and weighs about 35 mg. However, variations occur depending upon the cultivar and the location. Likewise, disparity happens in endosperm texture (or hardness), appearing to be related to binding forces in the endosperm. This point will be discussed later.

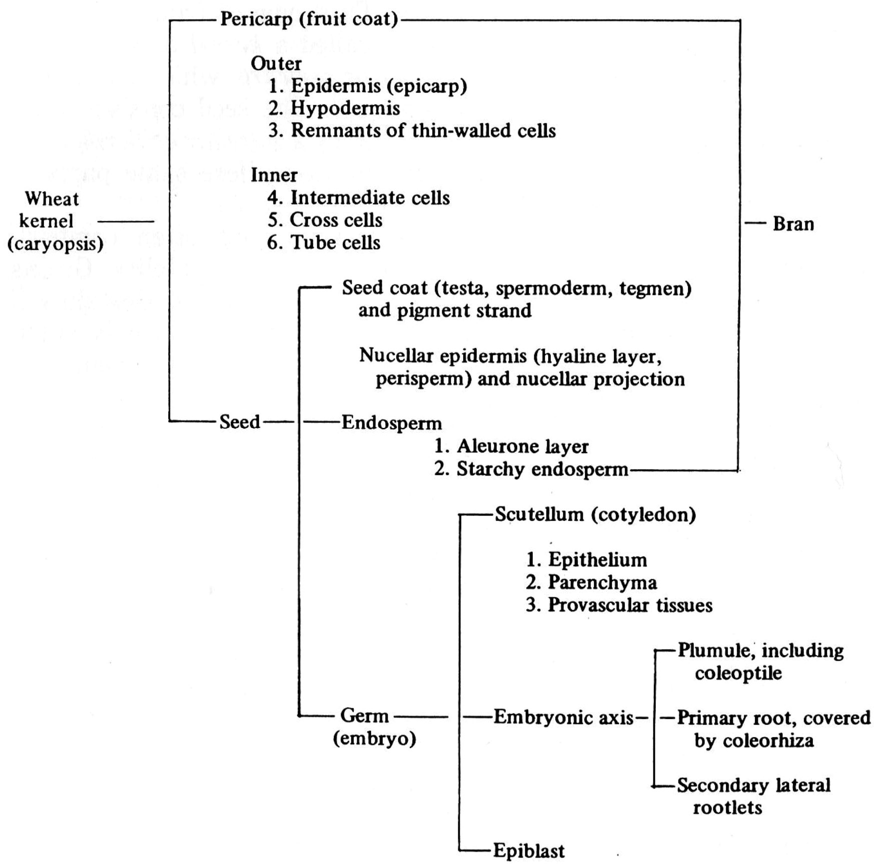

Figure 1: Parts of a wheat kernel (Hoseney 1994).

The grain itself has a more or less oval shape, is rounded on the dorsal side (the same side as the germ) and has a longitudinal crease on its ventral side (opposite the germ). It extends almost to the center of the grain and goes practically the entire length of the kernel. Even though it may be hidden by the flanks that touch each other, it remains a good place for microorganisms and dust to deposit. Both longitudinal and transverse sections are shown in Figure 2.

1.1.1. The pericarp

The pericarp is the first layer of the wheat grain. It is dead at harvest time and surrounds the entire seed. Most of the tissues are devoid of cytoplasm and have lignified walls. The outer pericarp is also called beeswing and its removal helps water move to the seed. The total pericarp represents about 5% of the kernel and consists of approximately 6% protein, 2% ash, 20% cellulose, and 0.5% fat, the remainder being nonstarch polysaccharides.

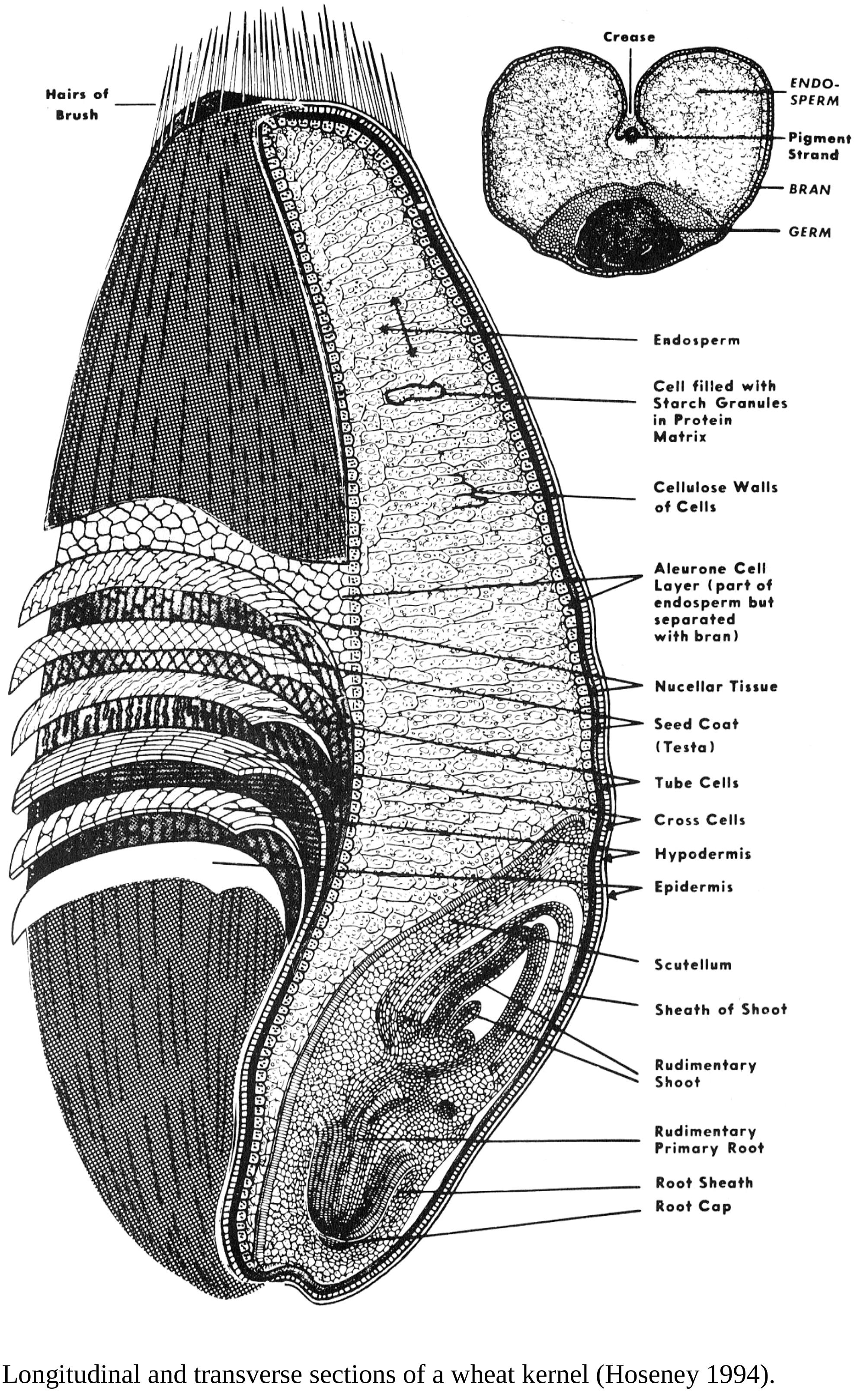

Figure 2: Longitudinal and transverse sections of a wheat kernel (Hoseney 1994).

1.1.2. The aleurone layer

The aleurone layer is the outermost layer of the endosperm tissue. It surrounds the grain over the starchy endosperm and the germ, and will be removed during milling, along with the nucellar epidermis, seed coat, and pericarp, being part of what millers call bran. Largely cellulosic in composition, the aleurone layer is relatively high in ash, protein, total phosphorus, fat and niacin. Moreover, the enzyme activity is high.

1.1.3. The germ

The germ lies on the lower dorsal side of the caryopsis. It comprises 2.5-3.5% of the kernel. Composed of the embryonic axis (rudimentary miniature living plant) and the scutellum (storage organ whose reserves are lipid droplets and protein bodies), the germ is relatively high in protein (25%), oil (16% of the embryonic axis and 32% of the scutellum are oil), and ash (5%).

1.1.4. The starchy endosperm

The cell walls of the starchy endosperm are made of pentosans, other hemicelluloses, and β-glucans but not cellulose. The thickness of these cell walls varies within the kernel; they are thicker near the aleurone layer.

The principal contents of endosperm cells, starch and protein, also vary with cell position. The peripheral cells have the lowest starch content and consequently have the highest protein content. Values as high as 54% protein have been found in subaleurone cells present in a flour of 12.5% protein (Kent 1966). These proteins in mature cells create a continuous matrix (the gluten) rather than a series of individual bodies. From there, the association of starch and protein develops the grain texture, which is affected by the degree of fenestration within the matrix, softer endosperm being characterized by interruptions with air spaces.

The denomination of soft and hard wheat has then been introduced. It is mainly referring to the point of fracture when the kernels are broken (MacRitchie 1980). In hard wheat kernels, the first point of fracture occurs at the cell wall rather than through the cell contents, and through some starch granules rather than at the starch-protein interface. On the contrary, in soft wheat, the fracture occurs primarily through the cell contents, and between the protein and starch

(Barlow et al 1973). Hardness was therefore related to adhesion between starch and protein matrix (Simmonds et al 1973). Greenwell and Schofield (1986) later found the presence of a M_r 15kD protein in markedly greater proportions in starch granules from soft wheat that may be responsible for preventing a closer association. This protein was first called friabilin (Morris et al 1992, Morrison et al 1992) and then puroindoline (Blochet, J.-E. et al 1993). A simple mutation in this protein causes the grain to be either soft or hard (Giroux and Morris 1997).

The starch granules present in the starchy endosperm are primarily either large, lenticular (lens-shaped) granules of up to 40 µm across the flattened side (type A) or small near-spherical granules averaging 2 to 8 µm in diameter (type B).

1.2. Production of flour

1.2.1. History

It is widely accepted that wheat has been a staple food for thousands of years, since people first began to settle in permanent communities. Wheat was a wild cereal but still a food grain that civilizations learned to select in order to produce superior plants with higher yield and better characteristics. The ultimate goal was the utilization for food and feed, a process still going on nowadays.

The whole grain itself in its integrity is not very desirable as food. For this reason, the idea of milling has been developed. Described as an ancient art, its objective is to make the cereals more palatable. It started with simple mortar and pestle or saddlestone, producing simple crushed grain, to be today modern electrically driven roller mills making the flour we know.

1.2.2. Milling of wheat (Bass 1988)

The milling is essentially a process of grinding and separating. Grinding is done on break rolls, sizing rolls, and reduction rolls. Separation is made using machines called sifters and purifiers.

The purpose of milling is to break open the grain, scrape off as much endosperm from the bran skin as possible and leave the germ (too high in oil that creates rancidity). Thus, after each grinding, the stock (or material going to the sieve) is sifted to remove the flour. The remainder can then be classified as: 1) pure, or relatively pure, endosperm; 2) composites of endosperm and bran varying in size, shape and proportion of the two; and 3) pure, or relatively pure, bran. This last part is definitely discarded whereas a judicious sequence of grinding, with corrugated and smooth rolls, sifting, and purification achieves an optimum separation of the endosperm and the bran (Figure 3). All the endosperm fractions and the flours produced along the milling will be reduced in size to pass through the very fine apertures of the sieves and be, by definition, flour. The bran, shorts (finer branny material) and germ form the by-products of the milling process and are known as millfeeds.

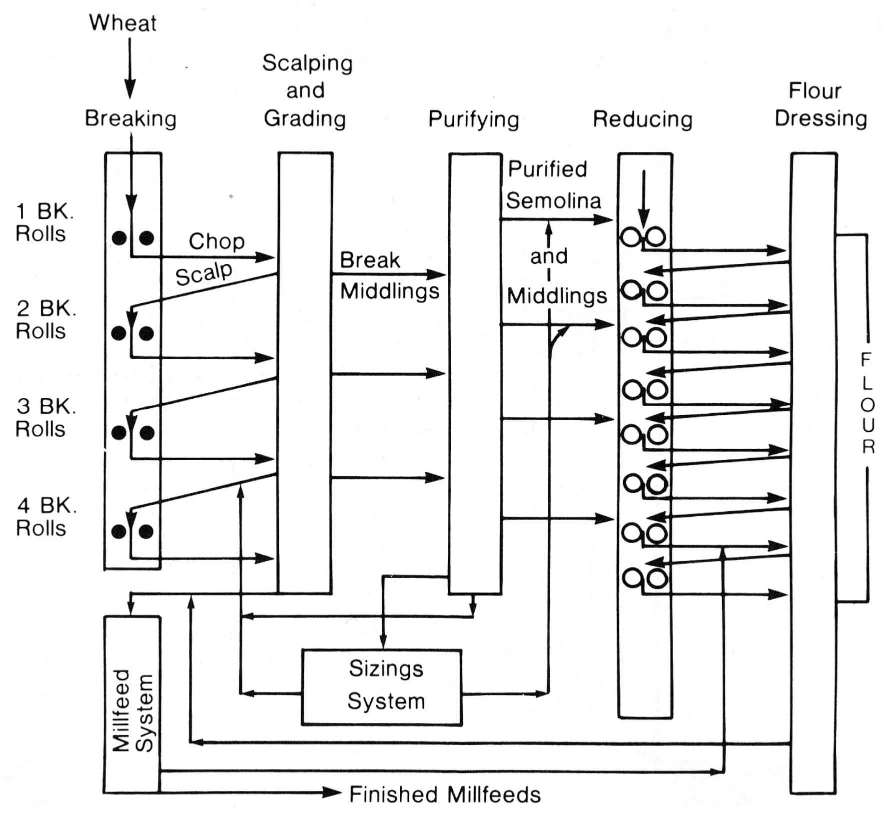

Figure 3: Schematic diagram of a simple mill flow.

1.2.3. Flour treatment

At the mill, flour may receive a number of treatments with a variety of additives to achieve any desired combination of the following objectives: 1) to bleach the flour, 2) to improve the bread-making quality of the flour, 3) to modify the gluten characteristics, 4) to supplement the natural amylase activity of the flour, or 5) to supplement the natural vitamin and mineral content of the flour. Since many chemicals exist and because they have multiple functions, only those specific to ensure a final flour with the desired functional properties are selected.

Bleaching agents are the primary chemicals added to the flour. Even though most of the pigment, giving freshly milled flour a creamy color, is bleached by natural oxidation, it requires a period of storage of three weeks. Being impractical for the millers, accelerated bleaching is then achieved by the addition of chemicals such as benzoyl peroxide ($(C_6H_5CO)_2O_2$). Chlorine gas (Cl_2) or chlorine dioxide (ClO_2) can also be used for the very white color it gives, but mostly on cake flour. Although safety evaluation has failed to detect any hazard associated with the consumption of products made from chlorinated flour, there remains concern about the introduction of organo-chlorine into food, and many countries, especially in the European Union (EU), do not permit its use (Greenwell and Brock 1996).

Flour improvers (also called maturing agents) are another type of chemical, used to improve the baking performance. The United States and Canada use potassium bromate ($KBrO_3$), azodicarbonamide ($(H_2NCON)_2$), acetone peroxide ($C_6H_{12}O_4$) and chlorine dioxide. In the EU, only ascorbic acid ($C_6H_8O_6$) is permitted.

Malted barley or malted wheat flour are added (2.5g/kg flour) to American wheat flours if they are low in amylase. Sufficient fermentable sugar is then generated for the conversion by yeast into carbon dioxide, improving loaf volume and reducing the harshness (rough texture) of the crumb. Vitamins (thiamin, riboflavin, niacin) and minerals (iron, calcium) supplementation also became popular, notably to replace the proportion lost during milling. In the EU, additives allowed for flour are protease, cystine and cysteine.

2. Chlorination, characteristics and effects

2.1. Introduction

From earliest recorded times, man has tried to secure a white flour, because it symbolized to him a pure and wholesome product. The desire of the consumer for an improved flour lead the millers to develop applications of bleaching agents to flour. The first bleaching agent used was nitrogen peroxide, introduced at the beginning of the 20^{th} century. Besides a slightly improved color, the flour was not modified as far as baking quality is concerned. In 1912, the chlorine treatment was introduced (Harrel 1952). This method has been found to improve color but first Montzheimer (1931) and then Smith (1932) reported that chlorine-treated flour gave a finer, more even texture to the crumb of cakes. Smith (1932) also noted an increase in volume and greater symmetry in treated cakes. Finnie et al (2006) found similar results for the quality of pancakes (Figure 4). Later, Bohn (1934) found that the use of chlorinated flour could prevent the decrease in cake volume occurring after removal from the oven. Chlorination allows traditional formulations, such as layer, genoese, yellow, madeira, and fruit cakes, to have greater proportions of sugar and liquor, as so-called “high-ratio cakes”.

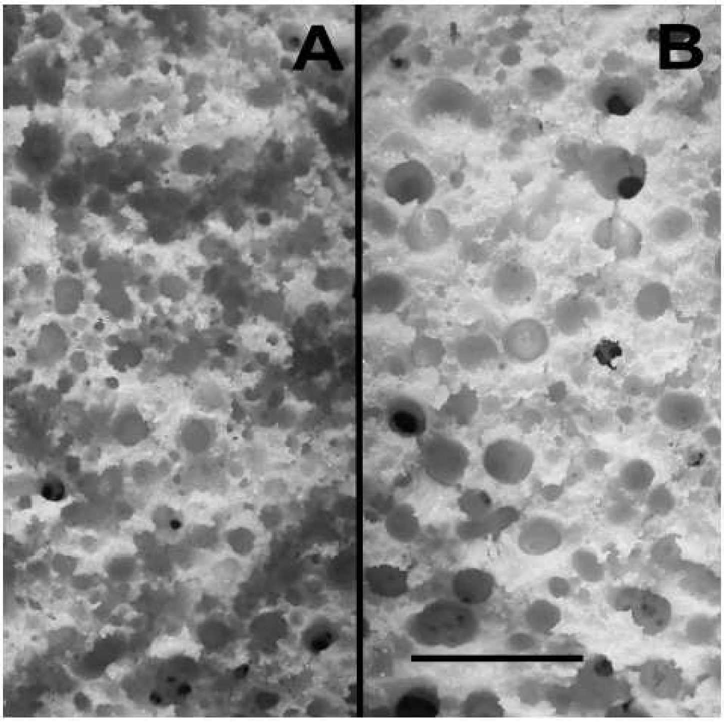

Figure 4: Photographs of pancake crumb structure and gas cell formation from (A) pancakes made from chlorinated flour and (B) pancakes made from untreated flour. The bar in the lower right corner represents one centimeter.

Nowadays, chlorine gas, whose role in effecting these important technological improvements is generally accepted, is widely used in the treatment of soft wheat flours and low-protein flours. The normal range of chlorination is 1,100-2,300 ppm (Hoseney et al 1988). The method consists in continuously injecting the gas into a stream of freshly milled flour. The production of hydrochloric acid (HCl) during the process induces a reduction in the pH of the flour, which is used as an analytical tool to monitor the extent of chlorination. The final pH found to be adequate is between 4.5 and 5.2 (Gough et al 1978). However, some specialty products may require slightly higher or lower levels of chlorination.

2.2. The modification induced by the chlorine

2.2.1. Association with the major components of the flour

Several workers have investigated the mode of action of chlorine in flour. Early, James and Huber (1927) assumed that gluten and starch were the main recipients. However, Hanson (1932) suggested the unsaturated fatty acids to react predominantly. In order to determine the distribution of chlorine in flour, Sollars (1961a) employed a fractionation technique in which the whole flour is chlorinated and then fractionated with subsequent analysis of the individual fractions. Using a water/acetic acid fractionation procedure, he found almost half of the chlorine (40%) present in the water-soluble protein fraction, suggesting that ionic chloride had been "washed out" of the insoluble fractions, and one third was present in the lipid fraction. Still, wet fractionation, known to transfer some free lipid to bound lipid (Olcott and Mecham 1947, Davies et al 1969), has been employed and may have induced the redistribution of the chlorine as well as changes in the flour fractions themselves. This approach is then subject to criticism. In an attempt to minimize the effects of sample preparation, Chamberlain (1962) air-classified chlorinated and unchlorinated flours into high-protein and low-protein fractions. He reported that one third of the chlorine was taken up by the lipids, one half by proteins and one seventh to one fifth by carbohydrates. Still using air-classification, Wilson et al (1964) found that the finer high-protein fractions would bind more chlorine (5 times in the experiment) than the coarse high-starch fractions. Results proved that the chlorine distribution was related to the size of the particles, chemical changes occurring being dependent upon their composition. As far as chemical modification is concerned, Ewart (1968) observed the transformation of cysteine and methionine into cysteic acid and methionine sulfoxide respectively when reacting with chlorine, and the destruction of tyrosine and histidine.

Consequently, it is clear that chlorine preferentially interacts with protein and lipid fractions. However, Lamb and Bode (1963) wet-fractionated flour, chlorinated the fractions and recombined them with unchlorinated fractions. Results from the cakes baked with recombined flours showed that chlorination of the starch was primarily responsible for the quality improvement. Likewise, Sollars (1958a) had indicated that both the starch and protein fractions were involved in the enhancement of the flour. Therefore, no correlation can be made between the quantitative distribution of the chlorine after reaction and the critical changes important to the cake baking.

2.2.2. Consequences on specific flour components

From the distribution of chlorine in chlorinated flour aforementioned, significant effects on the lipid fraction are expected. This idea is supported by the fact that the flour pigments (chiefly xanthophylls with small amounts of carotene), found in the lipid fraction, react with chlorine to form colorless addition compounds (Sollars 1961a, Sollars 1961b). The carotene content of the flour was found to fall rapidly upon chlorination until a dose of 2.0 oz/cwt, but no further decrease happened past this point (Tsen et al 1971).

Other research demonstrated substantial essential fatty acid destruction due to chlorination, at different levels of treatment (Coppock et al 1960, Daniels 1960, Daniels et al 1960). Further work using gas-liquid chromatography and infrared spectroscopy gave a more detailed analysis of the lipid products after chlorination (Daniels et al 1963). A reduction of the essential fatty acids by 60% has been determined as well as the creation of several new fatty acids, one of them being thought to be dichlorostearic acid (Table 1). It also appeared that chlorine preferred to react with monounsaturated oleic acid rather than with polyunsaturated linoleic and linolenic acids. The reaction of chlorine with flour lipids has then been shown to be very important. Changes in the lipid fraction could significantly modify the way starch and lipids interact (Gracza 1960, Youngquist et al 1969, Rees 1971, Seguchi 1984).

Chlorine treatment, g. per sack (280 lb.)	% Fatty acids as methyl esters				
	Palmitic	Oleic*	Linoleic	Linolenic	Undetected
None	18.9	12.5	64.4	4.3	Nil
50	19.2	12.8	57.6	4.8	5.6
150	22.5	10.0	41.4	3.2	22.9
250	21.3	4.5	25.3	0.9	48.0

*Including approximately 1% of stearic acid not separated on the chromatogram

Table 1: Effect of different levels of chlorine treatment on the fatty acids (Daniels et al 1963).

The reaction may occur with the individual amylose and amylopectin molecules, or the granule, as a structural unit, may be affected. Investigations have examined the action of excessively large doses of chlorine on semidry starch (Uchino and Whistler 1962, Ingle and Whistler 1964, Whistler et al 1966) and observed substantial oxidation of the glucose residues at C2 and C3, leading to depolymerization. Later, Johnson et al (1980) and Huang et al (1982a, 1982b) demonstrated that the oxidation damage at normal levels of chlorination was qualitatively similar. Starch, being the major fraction, remained the primary site affected by chlorine and its reaction resulted in an improvement in cake-baking quality (Sollars 1958a, Sollars 1958b, Lamb and Bode 1963, Sollars 1964, Sollars and Rubenthaler 1971, Johnson and Hoseney 1979). Frazier et al (1974) established the greater strength of the crumb from chlorinated flour, supporting the concept that the greater crumb strength of chlorinated flour produces better cakes. This point of view has then later been confirmed by Ngo et al (1985). However, chlorine does not appear to affect the crystallinity of the starch granule (Cauvain et al 1977, Huang et al 1982a) or the transition temperature and enthalpies of either flour or starch isolated from it by differential scanning calorimetry (DSC) (Jacobsberg and Daniels 1974, Allen et al 1982). Since amylose and amylopectin do not show any significant changes, speculations emerged that the main effect of chlorination involves the lipids or the protein-lipid complex on the starch granule. Therefore, Gough and Pybus (1971) proposed that the chlorination reaction disrupts the lipid-protein complex on the surface of the granule, allowing greater permeability by water. Varriano-Marston (1985) and Seguchi (1993) found evidence of changes on the surface of the starch granule suggesting that it should be rendered more hydrophobic after chlorination. Seguchi and Matsuki (1977) and Seguchi (1987) emphasized that starch from chlorinated flour appeared to be more hydrophobic than starch from untreated flour. Seguchi (1984) also found greater oil-binding capacity, supposing greater hydrophobicity. The increased hydration of the starch allows for even more total hydration (Kulp et al 1972), improved moisture retention during baking and a reduced tendency to collapse after baking.

Flour, chlorinated at the high levels necessary for cake making, is unfortunately unsuitable for use in bread since the treatment prevents the formation of an extensible gluten (James and Huber 1927, Harrel 1952). At the low pH attained on chlorination, the gluten is in a colloidal state which avoids dough formation (Alexander 1939) and the amount of water-extractable proteins increases with chlorine treatment (Kissel 1971) while the amount of proteins extractable in acetic acid decreases slightly with increasing levels of chlorination (Tsen et al 1971). Tsen also found evidence of chlorine reactions with tyrosine and sulphydryl groups. The other amino acids of the gluten primarily affected by chlorination are the methionine, the cysteine and the histidine (Ewart 1968). Such reactions are consistent with the increase in protein solubility observed with chlorination (Sollars 1958a, Kissel 1971). Only limited chemical evidence therefore exists about the nature of the chlorine-protein reactions occurring in flour, but the fact that some change does take place is demonstrated by the unsuitability of chlorinated cake flour for bread making purposes. This effect is presumably due to the loss of tertiary structure in the gluten, although chemical experiments alone are inadequate to determine what influence any such changes have upon cake quality (Gough et al 1978).

3. Ozonation, an alternative to chlorination

The world as we know it lives in a continuous evolution, scientifically, demographically, environmentally and many other ways. Such problems as the increasing population density throughout the world and the development of new microbiological strains (*Listeria, Escherichia coli*, and *Staphylococcus aureus*) have been emphasized for their involvement in human illnesses. Accumulation of chemicals in our environment have increased the international focus on the safe use of sanitizers, bleaching agents, pesticides, and other chemicals in industrial processing and other domains. The increasing need for more sanitizers to control infection and disease concurrent with the need to reduce the accumulation of chemical residues to maintain safe air, water and food supplies is paradoxical. Heavy metal salts, halogen compounds, reducing gases, oxidizers, and alcohols have been used as antimicrobial sanitizers in many specific applications.

3.1. Context

Chlorine in gaseous form and derivatives such as hypochlorite and chlorine dioxide are the most widely used sanitizing agents available for fresh produce, disinfection of food material, public water supplies and general sanitation. In the fresh fruit and vegetable industry, chlorine improves microbiological quality and controls pathogens. However, many research studies have indicated that it is limited in its ability to kill bacteria on fruit and vegetable surfaces (Rice et al

1982, Bott 1991, Graham 1997, Cena 1998). Environmental and health organizations have expressed concerns with traditional sanitizing agents with respect to the transformation of by-products, such as trihalomethanes (chemical compounds in which three of the four hydrogen atoms of methane are replaced by halogen atoms) and other chemical residues formed in the wastewater returned to the environment (Graham 1997, Cena 1998). Also, recognizing that food may be contaminated anywhere along the production chain, even on products thought to be pathogen-free, U. S. food processors have realized that some form of intervention to disinfect food, perhaps at several steps, is necessary (Majchrowicz 1999). But continued outbreaks of foodborne illnesses even after using conventional hot-water sprays, chlorine washes, and chemical treatments, have led to the examination of new alternative technologies to help assure the safety of their products. One of the approaches is to identify an alternative sanitizer to replace traditional sanitizing agents which can also be used to treat or recycle food processing wastewater. Research and commercial applications have indicated that ozone can replace chlorine with more benefits.

3.2. Presentation of the ozone

The familiar, fresh, clean smell in air following a thunderstorm characterizes ozone freshly generated in nature's environment. Ozone (O_3), or triatomic oxygen, is otherwise naturally produced by the action of UV irradiation on oxygen. It is a bluish gas at ambient pressures and temperatures that readily dissolves in water at acidic pH values (Gordon 1995), and decomposes, producing numerous free radical species, the most predominant being the hydroxyl radical (OH^-). Synthetically, ozone is a relatively unstable allotrope of oxygen that can be manufactured at low concentration (0.3 ppm) from oxygen in the air by radiation of 185-nm wavelength emitted by high transmission UV lamps (Ewell 1946) or by corona discharge generators, most widely used (Kim et al 1999a). The method consists in splitting the oxygen molecules, forming highly reactive free radicals that react with other oxygen molecules, forming ozone (Figure 5).

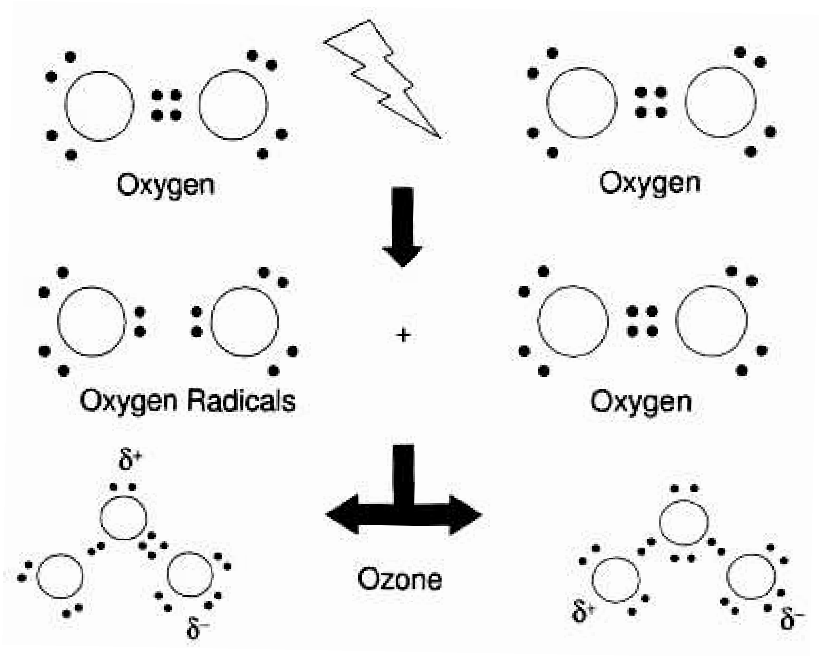

Figure 5: Formation of ozone molecules from oxygen radicals (Novak and Yuan 2007).

Historically, ozone was discovered by Schonbein in 1840, followed by a U.S. patent issued to Fewson in 1888 for an apparatus to produce ozone for deodorizing sewer gases (Graham 1997). In 1906, in Nice (France), the first commercial-scale disinfection of portable water with ozone was put into practice (Lebout 1959). From this time, ozonation has been adopted as standard practice for water treatment and disinfection by numerous cities in France, the Netherlands, Germany, Austria, Switzerland and many other countries. The United States would have to wait until 1940 to see the first potable water treatment plant to use ozone continuously, installed in Whiting, Indiana. Nowadays, more than 350 municipal water treatment plants are running in this country (Overbeck 2000). Most bottled water is treated with ozone as well, a practice stemming from a 1982 U. S. Food and Drug Administration (FDA) affirmation of ozone as Generally Recognized As Safe (GRAS) in this product (Majchrowicz 1998). As a gas, ozone is an alternative cleansing agent for water-sensitive products such as strawberries and raspberries, and was approved by the U.S. Department of Agriculture (USDA) for the storage of meat in 1957 (Majchrowicz 1999) and for the reconditioning of recycled poultry chilling water in 1997 (Güzel-Seydim et al 2004). The same year, an independent panel of experts sponsored by the Electric Power Research Institute (EPRI) decreed that ozone was a GRAS substance for use as a disinfectant and sanitizer for foods when used in accordance with good manufacturing practices (Graham 1997). Since the FDA did not object to the expert panel's findings, ozone has now been approved for use as a disinfectant or sanitizer in foods and food processing in the United States. An example is the approval for use as an antimicrobial agent in the treatment, storage and processing of meats and produce, issued by the FDA in 2001 (Novak and Yuan 2007).

3.3. Applications

3.3.1. Industrial wastewater treatment

Many industrial wastewaters contain impurities/contaminants which are amenable to oxidative destruction by ozone. Moreover, ozonation of some biorefractory organic materials can improve their biodegradability, thereby allowing an appropriate sequencing of ozone oxidation followed by an aerobic biological treatment step. Ozone is also coupled with ultraviolet radiation and/or hydrogen peroxide (advanced oxidation) for organic contaminants in groundwaters (hazardous wastes) or with activated carbon adsorption to remove colors and organics.

Ozone is the most powerful oxidizing agent available for the treatment of industrial wastewaters. It is introduced into water or wastewater as a gas to maximize the mass transfer of ozone from the gas phase to the aqueous phase. The chemical effects of ozone in water are a result of: 1) its direct reactions with dissolved compounds, 2) its decomposition into secondary oxidants, such as reactive free radicals (HO^-, HO_2^-), 3) the subsequent reactions of these secondary oxidants with solutes (Rice 1997). All of these reactions may occur simultaneously. In practice, however, one or the other reaction will predominate, depending on the reaction conditions and the chemical composition of the water or wastewater being treated.

3.3.2. Produce industry

Over the past several years, there has been increasing evidence that process water used by the food industry is not as free of pathogens as previously thought. Moreover, there is a certain level of pesticide and toxic compounds in the process water supply due to industrial activities. Normally, processing water is disinfected and sterilized with chlorine. However, chlorine cannot reduce the level of organic compounds and will produce chlorinated compounds. Ozone has then been proven to be an ideal replacement for chlorine for disinfection and sterilization of process water (Geering 1999, Rice 1999). Ozone can also destroy chlorine byproducts, pesticides, toxic organic compounds in the process water without any toxic residues, remove iron, manganese, sulfur, and control taste and color of fresh water. The practical applications of ozone to process water range from 0.5 to 5 ppm (depending on the water source), with less than 5 min contact time (Xu 1999).

The feasibility of using ozone in meat processing has been the focus of several studies. Kaess and Weidemann (1968) reported that the count of *Pseudomonas* spp. and *C. scottii* on contaminated beef decreased significantly at >2 µg/l gaseous ozone. The color of the muscle surface treated with < 0.6 µg/l ozone did not differ from that of the control. Ozone has also been tested in the process of tenderizing meats to control surface microflora. A simultaneous use of UV (0.2 $\mu W/cm^2$) and ozone (0.5 µg/l) produced a synergistic inhibitory effect against *Thamnidium* spp. and *Penicillium* spp (Kaess and Weidemann 1973). Spraying beef brisket fat with hydrogen peroxide (50 g/l) solution and ozonated water (5 g/l) was effective in reducing bacterial contamination, when compared to treatments with trisodium phosphate (120 g/l) and a commercial sanitizer (3 g/l) (Gorman et al 1995).

The utilization of ozone can also be useful for fruits and vegetables. One way is to wash with ozonated water to maintain or even improve the safety of those products. Two types of washing systems, spray or flume, can be used to reduce microbial counts on the surface of produce. Kim et al (1999b) used ozonated water to wash shredded lettuce and found a reduction of the microbial load by 1.5 to 1.9 logs in 5 min. Black peppercorns, contaminated with *Salmonella* spp., *S. aureus*, *B. cereus*, *Penicillium* spp., or *Aspergillus* spp., were immersed in water and sparged with gaseous ozone (6.7 mg/l) for 10 min at a flow rate of 6 l/min (Zhao and Cranston 1995). Ozone treatment decreased the microbial counts by 3 to 4 logs. Another way is to use gaseous ozone to prevent microbial activity on food surfaces and extend the shelf-life of products. It is mainly employed in cold storage to guard against mold and bacteria at a very low concentration, but also to destroy mold and bacteria present in the air and on the surface of produce, as well as to deodorize (Rice et al 1982). Many studies have been conducted on many different products. Ozone at 0.1 to 0.3 ppm in the atmosphere during blackberry storage suppressed fungal development for 12 days at 2°C and did not cause observable injury or defects

(Barth et al 1995). Grapes exposed for 20 min to ozone (8mg/l) had considerably reduced counts of bacteria, fungi and yeasts (Sarig et al 1996). Kuprianoff (1953) found that the shelf-life of apples could be increased by several weeks by applying 2-3 cm^3 of ozone per m^3 of air a few hours a day. However, ozone concentrations of 10 cm^3/m^3 resulted in apple damage.

Concerning the cereal grains, peas, beans and spices, *Bacillus* and *Microccus* are dominant bacterial genera that can be decreased by 1 to 3 logs by <50 mg/l ozone (Naito et al 1988). Naito et al (1987, 1988) studied the effects of ozone concentration (0.5 to 50 mg/l), exposure time (1 to 6 h), and temperature (5 to 50°C) on several cereal grains, cereal grain powders, peas, beans, and whole spices. They reported higher microbicidal activity for longer exposure time and lower temperature. A treatment of 0.5 to 50 ppm ozone for 6 h on wheat flour would inhibit microbial growth in namamen product and increase storage life two- to fivefold. Ibanoglu (2002) used ozonated water at a concentration of 1.5 mg/l to wash wheat grain during 30 min. The microbiological analysis showed that washing the kernels with ozonated water reduced the total and yeast/mould counts significantly (P=0.05) compared with washing with normal water. He suggested that ozonated water can be successfully used for wheat washing to reduce microbial populations. Besides the microbiological aspect, he indicated that washing flours from hard wheat samples with ozonated water did not significantly alter the chemical, physical or rheological properties and small but statistically significant differences were observed on extensograph values of flours milled from the soft wheat washed with ozonated water. Naito (1990) treated wheat flour (medium and soft flour) with an ozone-oxygen stream (0.05 to 50 ppm ozone) at a flow rate of 100 l/min at 10°C for 1 to 6 h. Physical dough testing properties showed 1) in a farinograph test, no significant change in the consistency of both flour doughs, 2) with an extensograph, an increase in the resistance to extension of both flours for the 0.5 to 50 ppm ozone treatment and a decrease in extensibility for the 0.05 to 50 ppm (soft flour) and the 5.0 to 50 ppm (medium flour) treatments. The intramolecular SH groups of wheat flour were decreased by about 30% by ozone treatment at 50 ppm for 1 hour, but intermolecular S–S bonds were increased by about 5% by the same treatment. Mendez et al (2003) realized a treatment with 50 ppm ozone penetrating into a column of stored grains for 30 days. He found no detrimental effect on popping volume of popcorn, fatty acid and amino acid composition of soybean, soft and hard red winter wheats and corn, milling characteristics of soft and hard red winter wheats and corn, bread-making characteristics of hard red winter wheat, and stickiness of rice. These data indicated that, if repeated ozone treatments are needed, such treatments should not decrease the quality of grain for end-users. (Dubois et al 2006) evaluated the effect of a new process called Oxygreen (described later) on vitamins, ferulic acid, phytates, proteins, carbohydrates and lipids. They used three treatments (5 g, 8 g, 12 g of ozone consumed per kg of grains) to compare with the control and concluded that there was no detectable substantial difference between ozone-treated grains and the untreated ones, although some quantitative differences can occur. The more detectable differences concern concentration of free sugars, and inhibition of some oxidative enzymes.

Nowadays, several patents utilizing ozone are currently available. Cantelli (1988) developed a method based on holding the produce in a sealed container while maintaining an electrical discharge that forms ozone and nitrogen oxides, at concentrations of ca. 0.05 ppm and 0.5 ppm, respectively. Karg (1990) obtained a patent for sterilization of heavily contaminated foods such as herbs, spices, fruits, and vegetables by ozone treatment. His process comprises an initial conditioning phase, treatment of gas mixture containing ozone, and elimination of residual ozone. Mitsuda et al (1991) patented a method to sterilize foods such as fish, fruits, vegetables, and beef, in a processing room, packing receptacles, or a refrigerator using a gas mixture that includes O_3, CO_2, and/or N_2. Hurst (1993) developed a method for sanitizing food products by immersion of the product in a bath supplied with a continuous stream of ozone-containing bubbles. Rosenthal (1995) obtained a patent for sanitizing fruits with an apparatus consisting of UV, infrared radiation, and ozone water. Yvin et al (2001) created a method for making flour with high food safety level from ozone-treated grains, ozone being produced from a carrier gas in an amount ranging between 0.5 and 20 grams of O3 per kilogram of grain. This equipment is part of the Oxygreen® process.

3.4. The example of the Oxygreen® process

The Oxygreen® process has been developed by Goëmar Laboratories, in France. It enables the treatment of a batch of grain in five different ways, in one single operation: eliminate microorganisms, control mycotoxins, destroy pesticides (without producing metabolites) and eliminate insects during storage. It may be used in flour production for human consumption (baking, industry, standard flours and technological flours) and animal consumption.

The flour decontaminated by the Oxygreen® process provides a high level of safety in food. It also makes it possible to produce technological flour without having to add synthetic products.

The Oxygreen® process works naturally in the transformation of grain to flour, without changing the intrinsic concept. Grain treatment is carried out at the same output as that of a standard mill. A minimum of two reactors alternately treat the grain (Figure 6). One reactor is filled and emptied alternately while the treatment phase takes place in the other (batch system). The ozone production necessary to the operation is made continuously in situ at . It is obtained by passing a current of air and oxygen in variable proportions between two electrodes put under different high alternative potential. The ozone is released into the reactors under light pressure by a perfectly adapted apparatus. The ozone and its vector gas go through in an ascendant flow to the grain, the grain then follows a descendant trajectory. This double transfer permanently assures the renewal of the reaction interface between the ozone and the grain to be treated. The Oxygreen® process ensures a perfectly homogeneous treatment. This way, every single grain is treated.

3.5. Limitations of ozone

An often-cited disadvantage of using ozone as a disinfectant is that, unlike chlorine, it is extremely unstable (Gordon 1995, Graham 1997, Rice 1997, Novak and Yuan 2007). It is difficult to predict how ozone reacts in the presence of organic matter. It can oxidize or ionize the compounds or spontaneously decompose to oxygen and free radicals.

Surface oxidation of food may result from excessive use of ozone (Rice et al 1982). The authors stressed that ozone is not universally beneficial and, in some cases, may promote oxidative spoilage. Fournaud and Lauret (1972) detected discoloration and undesirable odors in ozone-treated meat. Ozone also changed the surface color of some fruits and vegetables such as peaches (Badiani et al 1996) and carrots (Liew and Prange 1994). Ozone had a negative effect on the sensory quality of other commodities such as grains (Naito et al 1988) and milk powder (Ipsen 1989) due to lipid oxidation. However, other studies reported that ozone treatment improved the sensory quality in beef and eggs (Dondo et al 1992, Bailey et al 1996). Therefore, alterations in the sensory attributes depend on the chemical composition of food, ozone dose and treatment condition.

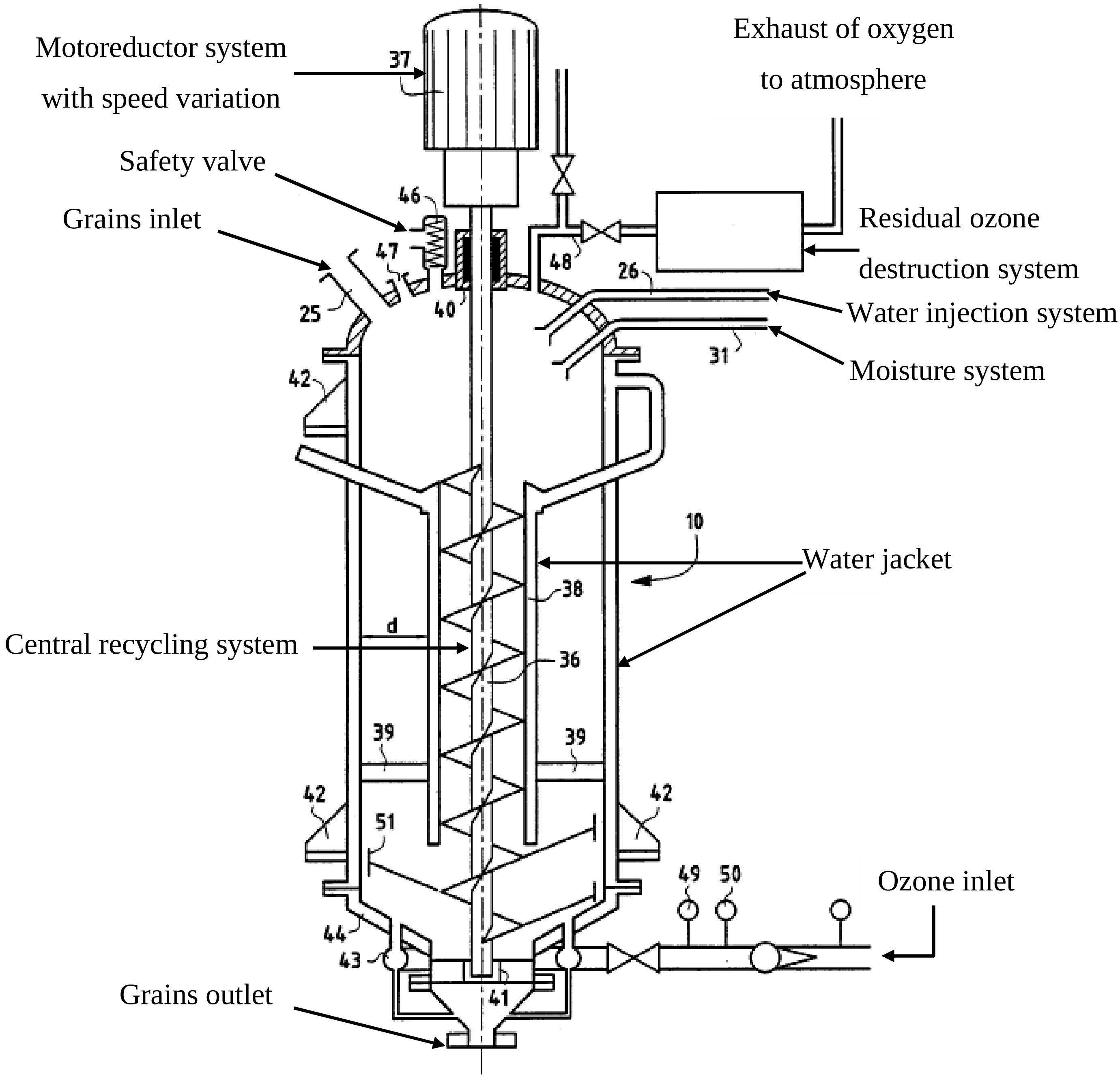

Figure 6: The Oxygreen reactor (Yvin et al 2001).

3.6. Conclusion

There is great potential for using the reactive, antimicrobial properties of a natural environmental compound such as ozone when synthesized in a controlled system for food-based applications. Studies are there to indicate that ozone can be used as a safe and effective antimicrobial agent in many food applications. When compared with chlorine and other disinfectants, lower concentrations of ozone and shorter contact times are sufficient in controlling or reducing microbial population. Ozone does not produce significant toxic residues in the environment after treatment. Ultimately system design and monitoring will enable this technology to succeed for future applications whether based on water purification recycling, air quality improvement, product extended storage and/or equipment surface sanitations.

Objectives

The utilization of ozone for wheat in order to improve the technological properties of the flour is a process that needs consideration. Few studies have been reported and more information is required to determine the exact mechanism involved and the repercussions on the flour. Until now, the few researches made have been realized on soft and hard wheat flours, and compared with chlorinated flour.

As seen previously, ozone is used in its gas phase. The application on wheat can therefore be on either the kernel itself or on the flour.

The objectives of this study are then to determine: 1) the effects of the ozone when applied on the wheat kernel, 2) the effects of the ozone when applied on the wheat flour, and 3) to compare the two ozonation treatments: ozone applied on the wheat kernel or on the wheat flour.

Materials and Methods

1. Wheat samples

For this experiment, soft white wheat samples given by the Agro-Physiology Laboratory (Toulouse, France) have been used.

For one part of the experiment, nine samples were constituted with 500 g of grain each. The grains had been humidified 48 hours before treatment in order to have a kernel humidity of 12 to 17%. Ten minutes before ozonation, an additional water quantity of 3% has been mixed with the sample to increase the absorption of ozone by the kernel.

For the other part, a 4 kg fraction free of treatment was kept aside. After milling, one tenth of the flour was kept as a control whereas the remainder was divided in nine equal portions and ozonated.

2. Ozone treatment

Ozonation of the wheat grain has been realized by the Agro-Physiology Laboratory. The conditions in the reactor were a debit of 0.4 m^3 of ozone per hour, a pressure of 500 mbars and a humidity of 3%. Temperature was the one of the room.

Three different ozone concentrations were used (110, 95 and 80 g of ozone per m^3) with different exposure time in order to obtain three different quantities of ozone (5, 10 and 20 g of ozone per kg of grain) for each concentration. Nine ozonated samples were then produced besides the non-ozonated grain.

The ozonation of the wheat flour stemming from the untreated grain has been realized with an ozone test setup (Figure 1) developed by O_3Co. (Aberdeen, ID, United States), requested by Dr. Bhadriraju from the Grain Science and Industry Department of Kansas State University (Manhattan, KS, United States). The ozone analyzer is a model IN2000 (In USA, Inc., Norwood, MA, United States).

The ozonation has been made with the same conditions as the ozonation of the grain. The concentration was measured after the ozone generator using the ozone analyzer.

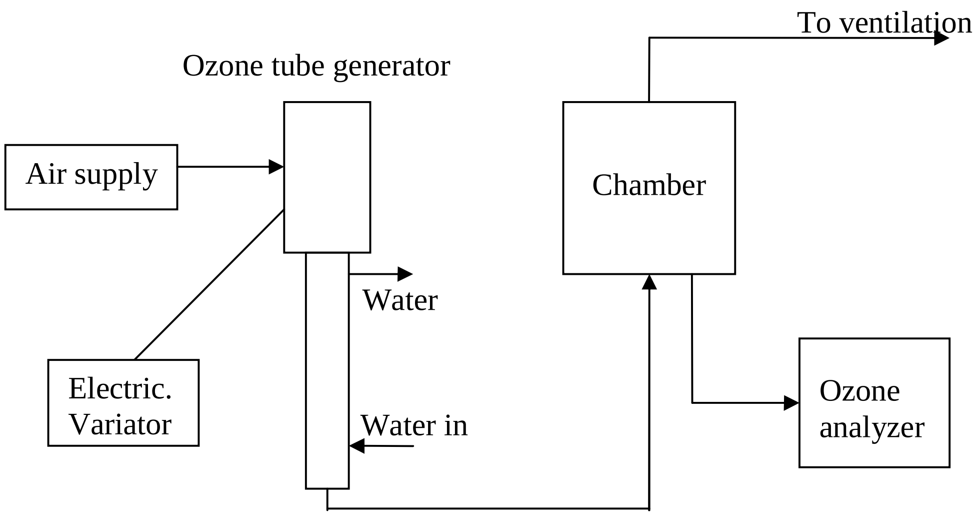

Figure 1: The ozone test setup

3. Laboratory milling

The wheat samples were milled in a Bühler Experiment Mill (Bühler Inc., Minneapolis, MN, USA) to short straight grade flour with an average extraction of 70%. All flours were stored in air-tight plastic bags at room temperature.

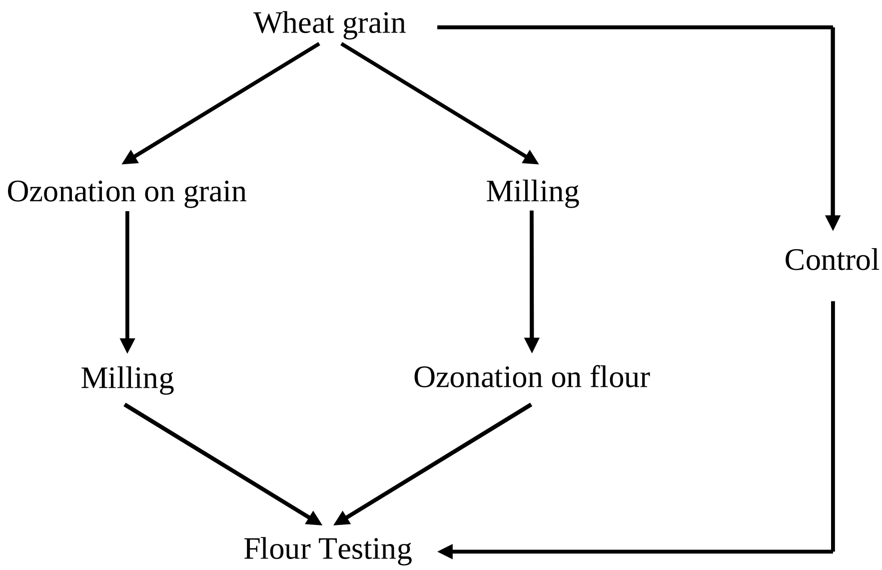

Figure 2: Diagram of the experiment

4. Analysis

4.1. Water activity

The water activity was measured with a water activity meter CX-1 updated CX-2 from Decagon Devices, Inc. (Pullman, WA, USA). Temperature and water activity values were given by the display of the instrument.

4.2. Physicochemical tests

4.2.1. Solvent retention capacity

The solvent retention capacity was determined according to AACC method 56-11 (AACC 2000). The following four solvents were used: deionized water from a Barnstead deionizer (model D8971, Barnstead International, Dubuque, IA, USA); sucrose (Fisher Scientific, Fair Lawn, NJ, USA), 50% (w/w) solution; sodium carbonate (Fisher Scientific), 5% (w/w) solution; lactic acid (MCB Manufacturing Chemists, Inc., Cincinnati, OH, USA), 5% (w/w) solution.

Due to the small quantity of flour, the test was run with 2.0 ml microfuge tubes and 0.2 g of sample. A volume of 1.0 ml of appropriate solvent was added to each tube containing flour.

4.2.2. Determination of Falling Number

The falling number values were reported according to AACC method 56-81B (AACC 2000) using a type 1800 falling number apparatus (Perten Inst., Huddinge, Sweden). No calculations were required since the instrument has a digital display.

4.3. Starch

Based on the AACC method 76-21 (AACC 2000) using a Brabender Micro Visco-Amylo-Graph® (Brabender OHG, Duisburg, Germany), the maximum viscosity, breakdown and setback were evaluated. The quantity of flour used in this case was 10 g (14% moisture basis) with 71.4 ml of distilled water (14% moisture basis). The test profile followed corresponds to the standard 1 profile of AACC method 76-21. The total length of the test is 13 minutes.

4.4. Baking quality

4.4.1. Cake test

The cake test is based on AACC method 10-90 (AACC 2000). The difference is on the quantity of ingredients used. The original formulation requires 200 g (14% moisture basis) of flour whereas only 100 g have been used due to the small quantity of flour available. Nevertheless, the proportions were kept (Table 1).

Ingredients	Amount (g)	Bakers %
Flour (14% moisture basis)	100	100
Sugar	130	130
Non-fat dry milk	12	12
Dried eggs	18	18
Salt	3	3
Baking Powder	6	6
Cake Shortening	50	50
Water	135	135

Table 1: Cake formula for the cake test, based on AACC method 10-90.

The batter was mixed with a Hobart mixer model N-50 (The Hobart Mfg. Co., Troy, OH, USA). All dry ingredients were sifted and mixed in first speed with 80% of the water for 30 seconds. Batter was scraped down and mixed a second time in second speed for 4 minutes. 10% of water was then added to the batter and mixed for 30 seconds in first speed. Batter was scraped down and mixed in second speed for 2 minutes. The 10% of water remaining was added to the batter and mixed in first speed for 30 seconds. Batter was again scraped down and mixed in second speed for 2 minutes. The mixed batter was finally placed into a 6 inch pan. Each cake represented 200 g of batter. Volume index, contour index and symmetry were calculated using a template (Figure 3) 2 hours after the cake was taken out of the oven. Calculations were as follow:

Volume index = B + C + D   Contour index = 2C – B – D   Symmetry = | B – D |

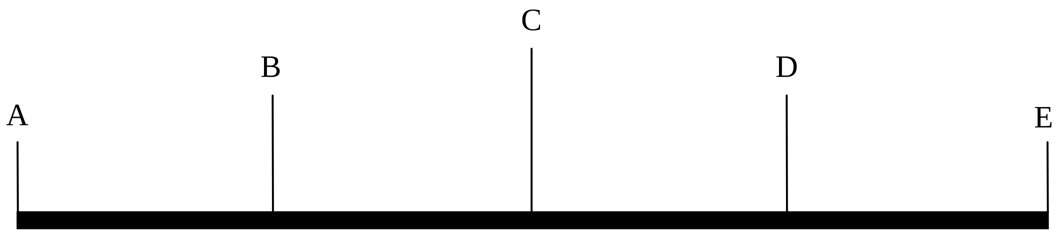

Figure 3: Template used to measure the cakes

4.4.2. Bread test

The formula for the bake does not include any maturing agents, dough improvers or additive such as malt, ascorbic acid, potassium bromate, soy flour, non-fat dry milk and whey solids (Table 2) like other methods propose.

Ingredients	Amount (g)	Bakers %
Flour (14% moisture basis)	35.0	100.0
Water	Variable (optimum)	Variable (optimum)
Yeast	0.95	2.7
Sucrose	2.10	6.0
Salt	0.52	1.5
Bread Shortening	1.05	3.1

Table 2: Bread formula for the bread test

The dough was mixed with a 35 g mixograph® (National Mfg. Co., Lincoln, NE, USA) to optimum dough development, according to AACC method 54-40A (AACC 2000). The fermentation process is more a short time fermentation procedure. The dough was sheeted at 5/16 in, molded and transferred to greased pan. After 20 minutes placed in a proofing cabinet (86°F and 85% humidity), the dough was re-sheeted, re-molded and transferred to a pan. A second proofing time of 40 minutes was done before transferring to the oven. Breads were baked at 200°C for 20 min. Volume measurement was made by rapeseed displacement after breads had cooled down during 2 hours.

5. Colorimetry

Color of flours was measured using a Minolta CR-210 colorimeter (Konica Minolta, Osaka, Japan) and refers to the L*a*b color space (also referred to as the CIELAB space) defined by the CIE (Commission Internationale de l'Eclairage) in 1976 (Oliver et al 1992). The instrument was calibrated against a standard white tile (No: 17033201, L=97.83, a=–0.41 and b=1.90), where L indicates the lightness, –a to +a indicates green to red and –b to +b indicates blue to yellow.

6. Statistical analysis

The results were analyzed by pair using Tukey's grouping after an analysis of variances with the SAS program.

Chapter 1 - Effect of ozone treatment on wheat grain

Ozonation of wheat grain is a quick and easy process that could be realized during the storage. The ozone is in direct contact with the grain and modifies immediately the properties of the wheat. However, the ozone has to go through the pericarp and the seed coat to reach the endosperm, which represents the greater proportion of the short grade flour. It is therefore expected that not all of the ozone will penetrate the endosperm and the flour that follows will have specific characteristics.

1. Results from the analytical tests

1.1. The effect of the ozonation on the water activity

The results from the water activity test show that ozonation of the grain has a significant effect on the flour at both concentration and quantity levels (Table 1).

Source	d.f.	Aw
Concentration	2	628.20***
Quantity	2	57.85***

*, ** and ***, significant at P<0.05, P<0.01 and P<0.001, respectively.
F-values were derived from Type III Sums of Squares.

Table 1: F-values from analysis of variance of the water activity for soft white wheat flour from ozonated grain using different concentrations and quantities of ozone.

These results can be related to the fact that water has been added to the grain before the ozonation. This excess amount of water has not been removed during the milling process and is found to increase the water activity (Table 2 and 3), and to differ from the control, which has not received any additional water. More detailed results are displayed in Appendix A, Table I.

Ozone Concentration	Control	80 g/m^3	95 g/m^3	110 g/m^3
Aw	0.462 ± 0.004^a	0.598 ± 0.003^b	0.562 ± 0.029^c	0.535 ± 0.024^d

Means with the same letter are not significantly different (P=0.05).

Results are average of duplicate measurements ± standard deviation.

Table 2: Water activity results of soft white wheat flour from ozonated grain, at different average concentrations of ozone.

Ozone Quantity	Control	5 g/kg	10 g/kg	20 g/kg
Aw	0.462 ± 0.004^a	0.571 ± 0.022^b	0.554 ± 0.037^c	0.570 ± 0.042^b

Means with the same letter are not significantly different (P=0.05).

Results are average of duplicate measurements ± standard deviation.

Table 3: Water activity results of soft white wheat flour from ozonated grain, for different average quantities of ozone.

It appears that the lower concentration induces a higher augmentation in water activity whereas the higher concentration induces a lower augmentation. Also, a quantity of 10 g of ozone per kg of grain creates a higher water activity compared to the two other quantities.

1.2. Influence on physicochemical tests

1.2.1. The solvent retention capacity

The ozonation process has been demonstrated to have a significant effect on the sodium carbonate, lactic acid and deionized water retention capacities (Table 4). Only the retention in sucrose is not significantly driven by either the concentration or the quantity.

Source	d.f.	Sucrose Retention	Sodium Carbonate Retention	Lactic Acid Retention	Deionized Water Retention
Concentration	2	1.48^{ns}	12.48***	2.12^{ns}	1.20^{ns}
Quantity	2	1.70^{ns}	9.64***	12.97***	7.98**

*, ** and ***, significant at P<0.05, P<0.01 and P<0.001, respectively.

F-values were derived from Type III Sums of Squares.

Table 4: F-values from analysis of variance of the solvent retention capacity test for soft white wheat flour from ozonated grain using different concentrations and quantities of ozone.

However, it is interesting to see that the concentration of 80 g/m^3 impacts on almost all the solvent retention values (Table 5). Both the sucrose and the sodium carbonate retention capacities are reduced whereas the retention in lactic acid is increased. Only the deionized water retention is not modified. On the other hand, the concentration of 95 g/m^3 does not change any retention capacity except the one of lactic acid that is increased. The retention of this last solvent is easily altered by the ozonation since all the concentrations are significantly different from the control. All three concentrations augment the retention capacity of the lactic acid whereas none of them modifies the retention by deionized water.

Ozone Concentration	Control	80 g/m^3	95 g/m^3	110 g/m^3
Sucrose (%)	112.0 ± 2.6^{a}	105.5 ± 4.8^{b}	108.0 ± 3.5^{a}	106.8 ± 3.7^{a}
Sodium Carbonate (%)	109.9 ± 2.4^{a}	101.1 ± 3.1^{b}	$106.5 \pm 4.0^{a,c}$	$103.7 \pm 3.4^{b,c}$
Lactic Acid (%)	123.1 ± 3.5^{a}	129.1 ± 3.6^{b}	130.1 ± 2.9^{b}	131.0 ± 4.6^{b}
Deionized Water (%)	81.8 ± 1.9^{a}	82.3 ± 3.1^{a}	83.6 ± 1.5^{a}	83.4 ± 3.9^{a}

Means with the same letter within a row are not significantly different (P=0.05).

Results are average of quadruplicate measurements ± standard deviation.

Table 5: Solvent retention capacity results of soft white wheat flour from ozonated grain, at different average concentrations of ozone.

Ozone Quantity	Control	5 g/kg	10 g/kg	20 g/kg
Sucrose	112.0 ± 2.6^{a}	106.2 ± 3.2^{b}	$108.2 \pm 4.8^{a,b}$	105.8 ± 3.6^{b}
Sodium Carbonate	109.9 ± 2.4^{a}	101.2 ± 2.9^{b}	$105.9 \pm 3.6^{a,c}$	104.2 ± 4.4^{c}
Lactic Acid	123.1 ± 3.5^{a}	130.5 ± 3.2^{b}	132.3 ± 3.2^{b}	127.4 ± 3.4^{c}
Deionized Water	81.8 ± 1.9^{a}	82.0 ± 2.4^{a}	85.2 ± 1.8^{b}	82.1 ± 3.4^{a}

Means with the same letter within a row are not significantly different (P=0.05).

Results are average of quadruplicate measurements ± standard deviation.

Table 6: Solvent retention capacity results of soft white wheat flour from ozonated grain, for different average quantities of ozone.

Table 6 shows that the quantities of 5 g/kg and 20 g/kg significantly modify the retention in sucrose, sodium carbonate and lactic acid. In the two first cases, they decrease the capacity whereas for the lactic acid, they increase it. Both quantities have no effect on the deionized water retention. Only the quantity of 10 g/kg has. It increases it as well as the one of lactic acid.

Once again, the lactic acid retention capacity is increased by all three quantities. More detailed results are shown in Appendix A, Table II.

1.2.2. Falling Number

Table 7 clearly shows that neither the concentration nor the quantity of ozone has a significant effect on the Falling Number.

Source	d.f.	Falling Number
Concentration	2	1.34[ns]
Quantity	2	0.29[ns]

*, ** and ***, significant at P<0.05, P<0.01 and P<0.001, respectively.
F-values were derived from Type III Sums of Squares.

Table 7: F-values from analysis of variance of the Falling Number determination for soft white wheat flour from ozonated grain using different concentrations and quantities of ozone.

Likewise, Tables 8 and 9 show similar results. It appears that this type of treatment is totally ineffective in modifying the falling number. More detailed results are shown in Appendix A, Table III.

Ozone Concentration	Control	80 g/m^3	95 g/m^3	110 g/m^3
Falling Number (s)	404.0 ± 8.5[a]	385.3 ± 15.2[a]	374.3 ± 23.2[a]	372.2 ± 17.7[a]

Means with the same letter are not significantly different (P=0.05).
Results are average of duplicate measurements ± standard deviation.

Table 8: Falling Number results of soft white wheat flour from ozonated grain, at different average concentrations of ozone.

Ozone Quantity	Control	5 g/kg	10 g/kg	20 g/kg
Falling Number (s)	404.0 ± 8.5a	380.8 ± 14.0a	374.3 ± 24.2a	376.7 ± 19.9a

Means with the same letter are not significantly different (P=0.05).

Results are average of duplicate measurements ± standard deviation.

Table 9: Falling Number results of soft white wheat flour from ozonated grain, for different average quantities of ozone.

1.3. The change in viscosity

The ozonation on grain has significant effects on the maximum viscosity and the setback (Table 10), measured by the micro visco-amylo-graph®. However, the first samples that have been run encountered problems in the cooling phase. Therefore, the setback, occurring during the cooling phase, is different from what it should be and gives questionable results. On the other hand, the beginning of the gelatinization and the breakdown are not significantly modified.

Source	d.f.	Beginning of Gelatinization	Maximum Viscosity	Breakdown	Setback
Concentration	2	0.33^{ns}	15.84***	2.13^{ns}	14.69***
Quantity	2	1.40^{ns}	4.62*	1.93^{ns}	11.69***

*, ** and ***, significant at P<0.05, P<0.01 and P<0.001, respectively.

F-values were derived from Type III Sums of Squares.

Table 10: F-values from analysis of variance of micro visco-amylo-graph® parameter for soft white wheat flour from ozonated grain using different concentrations and quantities of ozone.

As said above, only the maximum viscosity and the setback are significantly different from the control (Table 11 and 12). All three concentrations go the same way: the viscosity is increased and the setback is reduced. The concentration of 80 g/m^3 is the one to have the greatest difference compared to the control. For the quantities, this tendency is also found. In this case, it is the 5 g/kg that has the greatest difference with the control, followed by the 10 g/kg and the 20 g/kg. More detailed results are shown in Appendix A, Table IV.

Ozone Concentration	Control	80 g/m^3	95 g/m^3	110 g/m^3
Begin Gelatinization (BU)	35.0 ± 7.2^a	29.9 ± 7.7^a	28.3 ± 3.7^a	30.2 ± 5.9^a
Maximum Viscosity (BU)	816.3 ± 3.8^a	873.7 ± 11.3^b	846.0 ± 9.4^c	860.7 ± 22.4^b
Breakdown (BU)	270.0 ± 3.0^a	284.1 ± 10.3^a	278.8 ± 14.5^a	270.0 ± 23.5^a
Setback (BU)	235.3 ± 3.1^a	133.4 ± 9.3^b	138.0 ± 19.2^b	176.3 ± 59.3^c

Means with the same letter within a row are not significantly different (P=0.05).

Results are average of triplicate measurements ± standard deviation.

Table 11: Micro visco-amylo-graph® parameter results of soft white wheat flour from ozonated grain, at different average concentrations of ozone.

Ozone Quantity	Control	5 g/kg	10 g/kg	20 g/kg
Begin Gelatinization (BU)	35.0 ± 7.2[a]	31.0 ± 6.2[a]	27.1 ± 4.8[a]	30.3 ± 6.3[a]
Maximum Viscosity (BU)	816.3 ± 3.8[a]	868.6 ± 18.3[b]	857.4 ± 18.3[b,c]	854.3 ± 18.9[c]
Breakdown (BU)	270.0 ± 3.0[a]	283.3 ± 15.0[a]	279.4 ± 22.1[a]	270.1 ± 12.8[a]
Setback (BU)	235.3 ± 3.1[a]	125.0 ± 27.8[b]	161.8 ± 47.1[c]	161.0 ± 35.1[c]

Means with the same letter within a row are not significantly different (P=0.05).

Results are average of triplicate measurements ± standard deviation.

Table 12: Micro visco-amylo-graph® parameter results of soft white wheat flour from ozonated grain, for different average quantities of ozone.

1.4. Effects on baking quality

1.4.1. The cake test

Table 13 shows that the concentration of ozone has very significant consequences on the volume and symmetry of the cake. The quantity has a significant effect only on the symmetry.

Source	d.f.	Cake Volume	Cake Contour	Cake Symmetry
Concentration	2	10.99**	0.16[ns]	24.29***
Quantity	2	1.98[ns]	0.76[ns]	6.41*

*, ** and ***, significant at P<0.05, P<0.01 and P<0.001, respectively.

F-values were derived from Type III Sums of Squares.

Table 13: F-values from analysis of variance of cake test for soft white wheat flour from ozonated grain using different concentrations and quantities of ozone.

The only concentrations that modify the properties of the flour are 80 g/m^3 and 95 g/m^3. They have a detrimental effect on the volume of the cake (Table 14). The same trend is found with all three quantities (Table 15). Like the two lowest concentrations, none of them significantly modifies the symmetry of the cake. Overall, this treatment is not to be used for cake purposes. More detailed results are shown in Appendix A, Table V.

Ozone Concentration	Control	80 g/m^3	95 g/m^3	110 g/m^3
Volume Index	96.0 ± 1.4^a	69.3 ± 1.0^b	72.0 ± 4.1^b	85.7 ± 11.8^a
Contour Index	9.0 ± 11.3^a	8.7 ± 3.4^a	5.5 ± 3.8^a	10.8 ± 2.0^a
Symmetry	3.0 ± 0.0^{a,b}	1.0 ± 0.0^a	2.5 ± 1.0^a	5.2 ± 3.3^b

Means with the same letter within a row are not significantly different (P=0.05).

Results are average of duplicate measurements ± standard deviation.

Table 14: Cake test results of soft white wheat flour from ozonated flour, at different average concentrations of ozone.

Ozone Quantity	Control	5 g/kg	10 g/kg	20 g/kg
Volume Index	96.0 ± 1.4^a	72.2 ± 5.3^b	75.8 ± 10.2^b	79.0 ± 13.5^b
Contour Index	9.0 ± 11.3^a	6.8 ± 3.9^a	8.7 ± 3.8^a	9.5 ± 3.7^a
Symmetry	3.0 ± 0.0^{a,b}	1.8 ± 1.0^a	4.0 ± 3.5^b	2.8 ± 2.5^{a,b}

Means with the same letter within a row are not significantly different (P=0.05).

Results are average of duplicate measurements ± standard deviation.

Table 15: Cake test results of soft white wheat flour from ozonated flour, for different average quantities of ozone.

1.4.2. The bread test

If the treatment has some very significant effect, the bread volume is one of them (Table 16). Both the concentration and quantity are sources of modification.

Source	d.f.	Bread Volume
Concentration	2	12.31***
Quantity	2	9.67**

*, ** and ***, significant at P<0.05, P<0.01 and P<0.001, respectively.

F-values were derived from Type III Sums of Squares.

Table 16: F-values from analysis of variance of bread test for soft white wheat flour from ozonated grain using different concentrations and quantities of ozone.

To prove the action of this treatment, Table 17 and 18 show that the volumes are greater than the control. The concentration of 80 g/ m^3 is significantly different from the control and the one of 95 g/m3 is not significantly different from either the control or the lowest concentration. The same is observed for the quantity of 5 g/kg and 10 g/kg. Therefore, the lowest concentration and quantity have the best results. More detailed results are shown in Appendix A, Table VI.

Ozone Concentration	Control	80 g/m^3	95 g/m^3	110 g/m^3
Bread Volume (cm^3)	134.3 ± 4.0^{a,c}	148.3 ± 4.0^{b}	143.9 ± 8.5^{a,b}	134.8 ± 9.8^{c}

Means with the same letter are not significantly different (P=0.05).
Results are average of triplicate measurements ± standard deviation.

Table 17: Bread test results of soft white wheat flour from ozonated grain, at different average concentrations of ozone.

Ozone Quantity	Control	5 g/kg	10 g/kg	20 g/kg
Bread Volume (cm^3)	134.3 ± 4.0^{a}	148.9 ± 3.6^{b}	142.3 ± 9.5^{a,b}	138.5 ± 10.0^{a}

Means with the same letter are not significantly different (P=0.05).
Results are average of triplicate measurements ± standard deviation.

Table 18: Bread test results of soft white wheat flour from ozonated grain, for different average quantities of ozone.

2. Colorimetry

The direct ozonation on the flour did not change in any way the color of the flour (Appendix A, Table VII).

3. Conclusion

This type of treatment has clearly modified the properties of the flour. Besides the increase in water activity due to the preparation of the samples, it has been seen to decrease the retention in sodium carbonate and increase the one in lactic acid, which means that this flour would increase the spread of cookies and ameliorate long fermentation products such as sour dough, respectively. Such a modified flour would also create bigger bread volumes but would be detrimental for use in cakes (reduced volume and bad symmetry). In regard to this, the ozonation of grain confers to the flour a better viscosity that can be related to a reduction of the α-amylase activity. Therefore, the ozonation of grain would be useful for breads and sour dough, as well as in cookie formulas to develop the spread of cookies.

Chapter 2 - Effect of ozone treatment on wheat flour

In regard to ozonation of the grain, ozonation of the flour seems to be better since its action is directly on the material. However, the process is not as easy as the first one and requires a specific infrastructure to treat. But the main interest is on the characteristics that the treatment can have on the flour.

1. Results from the analytical tests

1.1. The water activity

Totally opposite to the treatment of the grain, this treatment does not influence the water activity (Table 1). Nevertheless, since no water has been added before the treatment, contrary to the first treatment, this result may be normal. More detailed results are shown in Appendix B, Table I.

Source	d.f.	Aw
Concentration	2	1.11^{ns}
Quantity	2	0.94^{ns}

*, ** and ***, significant at P<0.05, P<0.01 and P<0.001, respectively.
F-values were derived from Type III Sums of Squares.

Table 1: F-values from analysis of variance of the water activity for ozonated soft white wheat flour using different concentrations and quantities of ozone.

1.2. Effects on physicochemical tests

1.2.1. Solvent Retention Capacity

The solvent retention capacity test is one of the tests that is really significant. The capacity to retain sucrose and lactic acid are very significantly related to the concentration and quantity applied to the flour during this treatment (Table 2). It also appears that the deionized water retention capacity is significantly dependent on the quantity.

Source	d.f.	Sucrose Retention	Sodium Carbonate Retention	Lactic Acid Retention	Deionized Water Retention
Concentration	2	8.19**	1.51^{ns}	4.58*	2.58^{ns}
Quantity	2	7.12**	2.31^{ns}	105.38***	4.71*

*, ** and ***, significant at P<0.05, P<0.01 and P<0.001, respectively.

F-values were derived from Type III Sums of Squares.

Table 2: F-values from analysis of variance of the solvent retention capacity for ozonated soft white wheat flour using different concentrations and quantities of ozone.

Table 3 confirms the relationship. All three concentrations are significantly different from the control for the sucrose and the lactic acid. The retention capacity is clearly increased. Also, the deionized water retention capacity is significantly different from the control.

Ozone Concentration	Control	80 g/m^3	95 g/m^3	110 g/m^3
Sucrose (%)	112.0 ± 2.6^a	125.3 ± 5.3^b	122.5 ± 6.1^{b,c}	120.5 ± 2.5^c
Sodium Carbonate (%)	109.9 ± 2.4^a	98.8 ± 11.4^b	102.5 ± 3.9^a	103.1 ± 6.1^a
Lactic Acid (%)	123.1 ± 3.5^a	125.7 ± 1.1^b	126.8 ± 6.0^b	127.0 ± 6.3^b
Deionized Water (%)	81.8 ± 1.9^a	90.3 ± 3.8^b	86.8 ± 6.2^{a,b}	88.7 ± 5.8^b

Means with the same letter within a row are not significantly different (P=0.05).

Results are average of quadruplet measurements ± standard deviation.

Table 3: Solvent retention capacity results of ozonated soft white wheat flour, at different average concentrations of ozone.

The results in Table 4 are less notable. All quantities increase the sucrose retention whereas only the 5 g/kg quantity increases the lactic acid retention capacity. On the other hand, both 5 g/kg and 20 g/kg increase the retention in deionized water compared to the control. The quantity of 10 g/kg increases only the sodium carbonate retention capacity. More detailed results are shown in Appendix B, Table II.

Ozone Quantity	Control	5 g/kg	10 g/kg	20 g/kg
Sucrose (%)	112.0 ± 2.6^a	124.0 ± 5.0^b	120.1 ± 4.6^c	124.0 ± 5.0^b
Sodium Carbonate (%)	109.9 ± 2.4^a	105.0 ± 3.8^a	99.1 ± 1.8^b	100.8 ± 12.2^a
Lactic Acid (%)	123.1 ± 3.5^a	132.2 ± 4.8^b	124.3 ± 2.4^a	123.5 ± 1.4^a
Deionized Water (%)	81.8 ± 1.9^a	89.5 ± 6.6^b	85.8 ± 5.4^a	90.7 ± 2.7^b

Means with the same letter within a row are not significantly different (P=0.05).

Results are average of quadruplet measurements ± standard deviation.

Table 4: Solvent retention capacity results of ozonated soft white wheat flour, for different average quantities of ozone.

1.2.2. The Falling Number

Whereas the action of ozone on grain has no effect on the Falling Number, the ozonation of flour has a slightly significant effect between the quantity and the Falling Number (Table 5).

Source	d.f.	Falling Number
Concentration	2	2.33^{ns}
Quantity	2	5.78*

*, ** and ***, significant at P<0.05, P<0.01 and P<0.001, respectively.

F-values were derived from Type III Sums of Squares.

Table 5: F-values from analysis of variance for the Falling Number determination for ozonated soft white wheat flour using different concentrations and quantities of ozone.

As shown in Table 6, all concentrations are significantly different from the control. The increase in falling number follows the increase in concentration.

Ozone Concentration	Control	80 g/m^3	95 g/m^3	110 g/m^3
Falling Number (s)	404.0 ± 8.5^a	487.2 ± 54.4^b	499.5 ± 20.0^b	517.3 ± 31.5^b

Means with the same letter are not significantly different (P=0.05).

Results are average of duplicate measurements ± standard deviation.

Table 6: Falling Number results of ozonated soft white wheat flour, at different average concentrations of ozone.

The falling number increases also with the quantity applied (Table 7). Like the concentration, as the quantity increases, the Falling Number increases. The treatment can then be related to a decrease in the amylase activity. More detailed results are shown in Appendix B, Table III.

Ozone Quantity	Control	5 g/kg	10 g/kg	20 g/kg
Falling Number (s)	404.0 ± 8.5^{a}	473.7 ± 37.1^{b}	515.8 ± 29.6^{b}	514.3 ± 35.7^{b}

Means with the same letter are not significantly different (P=0.05).

Results are average of duplicate measurements ± standard deviation.

Table 7: Falling Number results of ozonated soft white wheat flour, for different average quantities of ozone.

1.3. The ineffective action on viscosity

Unlike the treatment on grain, the treatment of flour does not have any significant effect on any of the parameters of the micro visco-amylo-graph® (Table 8).

Source	d.f.	Beginning of Gelatinization	Maximum Viscosity	Breakdown	Setback
Concentration	2	0.27^{ns}	0.82^{ns}	3.35^{ns}	1.24^{ns}
Quantity	2	1.30^{ns}	0.61^{ns}	0.65^{ns}	0.27^{ns}

*, ** and ***, significant at P<0.05, P<0.01 and P<0.001, respectively.

F-values were derived from Type III Sums of Squares.

Table 8: F-values from analysis of variance of the micro visco-amylo-graph® parameter for ozonated soft white wheat flour using different concentrations and quantities of ozone.

However, Tables 9 and 10 show that the maximum viscosity and the breakdown are modified by the three concentrations and the three quantities. Contrary to the treatment on grain, both parameters are decreased by the action of ozone on flour, meaning an increasing α-amylase activity. More detailed results are shown in Appendix B, Table IV.

Ozone Concentration	Control	80 g/m^3	95 g/m^3	110 g/m^3
Begin Gelatinization (BU)	35.0 ± 7.2^a	33.7 ± 5.0^a	35.7 ± 4.4^a	34.4 ± 8.8^a
Maximum Viscosity (BU)	816.3 ± 3.8^a	791.7 ± 5.4^b	793.2 ± 4.8^b	789.4 ± 8.9^b
Breakdown (BU)	270.0 ± 3.0^a	232.9 ± 8.8^b	228.9 ± 9.4^b	239.3 ± 12.8^b
Setback (BU)	235.3 ± 3.1^a	221.6 ± 5.3^a	224.9 ± 5.0^a	232.2 ± 22.7^a

Means with the same letter within a row are not significantly different (P=0.05).

Results are average of triplicate measurements ± standard deviation.

Table 9: Micro visco-amylo-graph® parameter results of ozonated soft white wheat flour, at different average concentrations of ozone.

Ozone Quantity	Control	5 g/kg	10 g/kg	20 g/kg
Begin Gelatinization (BU)	35.0 ± 7.2^a	34.7 ± 6.9^a	32.3 ± 4.9^a	36.8 ± 6.5^a
Maximum Viscosity (BU)	816.3 ± 3.8^a	792.3 ± 6.5^b	789.6 ± 8.1^b	792.4 ± 5.2^b
Breakdown (BU)	270.0 ± 3.0^a	233.2 ± 9.7^b	231.7 ± 9.6^b	236.2 ± 13.9^b
Setback (BU)	235.3 ± 3.1^a	229.0 ± 13.5^a	225.7 ± 13.7^a	224.0 ± 15.9^a

Means with the same letter within a row are not significantly different (P=0.05).

Results are average of triplicate measurements ± standard deviation.

Table 10: Micro visco-amylo-graph® parameter results of ozonated soft white wheat flour, for different average quantities of ozone.

1.4. Effects on baking quality

1.4.1. The cake test

As for the action of ozone on grain, the concentration and quantity of ozone applied to flour are significantly related to the change in cake volume but not to the cake symmetry (Table 11). Meanwhile, only the quantity modifies the cake contour.

Source	d.f.	Cake Volume	Cake Contour	Cake Symmetry
Concentration	2	6.53*	0.64^{ns}	0.64^{ns}
Quantity	2	7.61**	4.45*	0.93^{ns}

*, ** and ***, significant at P<0.05, P<0.01 and P<0.001, respectively.

F-values were derived from Type III Sums of Squares.

Table 11: F-values from analysis of variance of the cake test for ozonated soft white wheat flour using different concentrations and quantities of ozone.

Table 12 and 13 show that all concentrations and quantities have greater volumes, but due to a large standard deviation they are not significantly different from the control. Similar conclusions can be given to the contour index and the symmetry. More detailed results are shown in Appendix B, Table V.

Ozone Concentration	Control	80 g/m^3	95 g/m^3	110 g/m^3
Volume Index	$96.0 \pm 1.4^{a,b}$	$110.2 \pm 14.4^{a,b}$	96.3 ± 14.0^{a}	111.2 ± 19.5^{b}
Contour Index	9.0 ± 11.3^{a}	2.8 ± 9.8^{a}	4.7 ± 3.9^{a}	7.3 ± 10.8^{a}
Symmetry	3.0 ± 0.0^{a}	5.2 ± 2.5^{a}	4.3 ± 3.3^{a}	3.3 ± 2.3^{a}

Means with the same letter within a row are not significantly different (P=0.05).

Results are average of duplicate measurements ± standard deviation.

Table 12: Cake test results of ozonated soft white wheat flour, at different average concentrations of ozone.

Ozone Quantity	Control	5 g/kg	10 g/kg	20 g/kg
Volume Index	$96.0 \pm 1.4^{a,b}$	96.5 ± 6.0^{a}	$106.8 \pm 8.4^{a,b}$	114.3 ± 25.5^{b}
Contour Index	9.0 ± 11.3^{a}	3.0 ± 4.9^{a}	0.2 ± 5.3^{a}	11.7 ± 10.1^{a}
Symmetry	3.0 ± 0.0^{a}	5.0 ± 2.1^{a}	4.8 ± 3.3^{a}	3.0 ± 2.4^{a}

Means with the same letter within a row are not significantly different (P=0.05).

Results are average of duplicate measurements ± standard deviation.

Table 13: Cake test results of ozonated soft white wheat flour, for different average quantities of ozone.

1.4.2. The bread test

As well as the treatment on grain, the concentration and quantity of the treatment on flour are significantly related to the bread volume (Table 14).

Source	d.f.	Bread Volume
Concentration	2	7.32**
Quantity	2	6.04**

*, ** and ***, significant at P<0.05, P<0.01 and P<0.001, respectively.
F-values were derived from Type III Sums of Squares.

Table 14: F-values from analysis of variance of the bread test for ozonated soft white wheat flour using different concentrations and quantities of ozone.

The action is confirmed by Tables 15 and 16. All treatments of different concentrations and quantities show greater volumes than the control. Once again, the concentration of 80 g/m^3 and the quantity of 5 g/kg have the best results whereas the greater concentration and quantity have lower differences with the control. More detailed results are shown in Appendix B, Table VI.

Ozone Concentration	Control	80 g/m^3	95 g/m^3	110 g/m^3
Bread Volume (cm^3)	134.3 ± 4.0^{a}	147.8 ± 3.6^{b}	146.2 ± 3.0^{b,c}	141.0 ± 7.2^{a,c}

Means with the same letter are not significantly different (P=0.05).
Results are average of triplicate measurements ± standard deviation.

Table 15: Bread test results of ozonated soft white wheat flour, at different average concentrations of ozone.

Ozone Quantity	Control	5 g/kg	10 g/kg	20 g/kg
Bread Volume (cm^3)	134.3 ± 4.0^{a}	147.8 ± 2.5^{b}	146.4 ± 5.5^{b,c}	141.4 ± 6.0^{a,c}

Means with the same letter are not significantly different (P=0.05).
Results are average of triplicate measurements ± standard deviation.

Table 16: Bread test results of ozonated soft white wheat flour, for different average quantities of ozone.

2. Colorimetry

The direct ozonation on the flour did not change in any way the color of the flour (Appendix B, Table VII).

3. Conclusion

This type of treatment has also clearly demonstrated its possibilities to modify the properties of the flour. Its main characteristics are to increase the volumes of breads and cakes. Besides that, the increase in sucrose and lactic acid retention capacity shows that the direct ozonation ofo flour is suitable for flour destined for high sugar level products such as sweet breads and long fermentation products such as sour dough, respectively.

Chapter 3 - Comparison of the treatments on wheat grain and flour

The ozonation of wheat is a growing subject of research. Many experiments need to be done in order to establish the real effects of this treatment. In this part, two treatments are compared to determine the specificities of each of them.

1. Comparison from the analytical tests

1.1. The water activity

We have seen that the treatment on wheat grain requires an addition of water to increase the introduction of ozone into the grain. This results in an increase of the water activity of the flour. It may or may not be a problem, depending on the conditions of storage and maintenance, but the effect still remains. A similar experiment should be run without addition of water to see if any difference occurs compared to the one used in this experiment.

On the other hand, the treatment on flour did not increase the water activity. Of course, no water has been added before the treatment. It is obvious that the addition of water, like in the previous treatment, is more difficult.

In any case, it could be concluded that none of the treatments would actually modify the water activity of the final flour.

1.2. The physicochemical tests

1.2.1. The Solvent Retention Capacity

This test has shown very significant results for both treatments. They proved that they increase the retention in sucrose and lactic acid. But when compared, it appears that they do not have significant differences except in the retention of sucrose (Table 1 and 2). The treatment on flour is significantly greater than the treatment on grain.

Ozone Concentration	80 g/m^3		95 g/m^3		110 g/m^3	
Treatment	Grain	Flour	Grain	Flour	Grain	Flour
Sucrose (%)	105.5 ± 4.8^a	125.3 ± 5.3^b	108.0 ± 3.5^a	122.5 ± 6.1^b	106.8 ± 3.7^a	120.5 ± 2.5^b
Sodium Carbonate (%)	101.1 ± 3.1^a	98.8 ± 11.4^a	106.5 ± 4.0^a	102.5 ± 3.9^a	103.7 ± 3.4^a	103.1 ± 6.1^a
Lactic Acid (%)	129.1 ± 3.6^a	125.7 ± 1.1^a	130.1 ± 2.9^a	126.8 ± 6.0^a	131.0 ± 4.6^a	127.0 ± 6.3^a
Deionized Water (%)	82.3 ± 3.1^a	90.3 ± 3.8^b	83.6 ± 1.5^a	86.8 ± 6.2^a	83.4 ± 3.9^a	88.7 ± 5.8^a

Means with the same letter within a row of a specific concentration column are not significantly different (P=0.05).

Results are average of quadruplicate measurements ± standard deviation.

Table 1: Solvent retention capacity results of soft white wheat flour from ozonated grain and ozonated flour, as a function of average concentrations of ozone.

Ozone Quantity	5 g/kg		10 g/kg		20 g/kg	
Treatment	Grain	Flour	Grain	Flour	Grain	Flour
Sucrose (%)	106.2 ± 3.2^a	124.0 ± 5.0^b	108.2 ± 4.8^a	120.1 ± 4.6^b	105.8 ± 3.6^a	124.0 ± 5.0^b
Sodium Carbonate (%)	101.2 ± 2.9^a	105.0 ± 3.8^a	105.9 ± 3.6^a	99.1 ± 1.8^b	104.2 ± 4.4^a	100.8 ± 12.2^a
Lactic Acid (%)	130.5 ± 3.2^a	132.2 ± 4.8^a	132.3 ± 3.2^a	124.3 ± 2.4^b	127.4 ± 3.4^a	123.5 ± 1.4^a
Deionized Water (%)	82.0 ± 2.4^a	89.5 ± 6.6^a	85.2 ± 1.8^a	85.8 ± 5.4^a	82.1 ± 3.4^a	90.7 ± 2.7^b

Means with the same letter within a row of a specific quantity column are not significantly different (P=0.05).

Results are average of quadruplicate measurements ± standard deviation.

Table 2: Solvent retention capacity of soft white wheat flour from flour from ozonated grain and ozonated flour, as a function of average quantities of ozone.

1.2.2. The Falling Number

This test shows the real differences that can occur between two treatments. From any point of view, concentration (Table 3) or quantity (Table 4), the treatment of flour demonstrates significantly that it gives better results than the treatment on grain.

The action of ozone directly on the flour seems to greatly modify the physicochemical properties of the flour for higher falling numbers.

Ozone Concentration	80 g/m³		95 g/m³		110 g/m³	
Treatment	Grain	Flour	Grain	Flour	Grain	Flour
Falling Number (s)	385.3 ± 15.2[a]	487.2 ± 54.4[b]	374.3 ± 23.2[a]	499.5 ± 20.0[b]	372.2 ± 17.7[a]	517.3 ± 31.5[b]

Means with the same letter within a row of a specific concentration column are not significantly different (P=0.05).
Results are average of duplicate measurements ± standard deviation.

Table 3: Falling Number determination results of soft white wheat flour from ozonated grain and ozonated flour, as a function of average concentrations of ozone.

Ozone Quantity	5 g/kg		10 g/kg		20 g/kg	
Treatment	Grain	Flour	Grain	Flour	Grain	Flour
Falling Number (s)	380.8 ± 14.0[a]	473.7 ± 37.1[b]	374.3 ± 24.2[a]	515.8 ± 29.6[b]	376.7 ± 19.9[a]	514.3 ± 35.7[b]

Means with the same letter within a row of a specific quantity column are not significantly different (P=0.05).
Results are average of duplicate measurements ± standard deviation.

Table 4: Falling Number determination results of soft white wheat flour from ozonated grain and ozonated flour, as a function of average quantities of ozone.

1.3. The modification of starch

The viscosity parameters are another example of the differences present between the two treatments. It comes from the results that the treatment on grain gives significantly greater maximum viscosity and breakdown values than the treatment on flour, both depending on the concentration (Table 5) and on the quantity (Table 6). On the other hand, the beginning of gelatinization stays unchanged, no matter which concentration or quantity of ozone is used.

The quantity of ozone appears to be important for the setback values (Table 6). Even though the variation in concentration does not give significant differences between the two treatments, the variation in quantity does. The treatment on flour has greater setback values than the treatment on grain. However, as stated earlier (Chapter 1, 1.3), problems occurred during the cooling phase of some samples for the treatment on grain. The instrument did not cool down as much as supposed, resulting in setback values higher than expected. Therefore, the significant difference between the two treatments for the setback values is questionable. It would be more probable to have no significant differences.

Ozone Concentration	80 g/m^3		95 g/m^3		110 g/m^3	
Treatment	Grain	Flour	Grain	Flour	Grain	Flour
Begin Gelatinization (BU)	29.9 ± 7.7^a	33.7 ± 5.0^a	28.3 ± 3.7^a	35.7 ± 4.4^a	30.2 ± 5.9^a	34.4 ± 8.8^a
Maximum Viscosity (BU)	873.7 ± 11.3^a	791.7 ± 5.4^b	846.0 ± 9.4^a	793.2 ± 4.8^b	860.7 ± 22.4^a	789.4 ± 8.9^b
Breakdown (BU)	284.1 ± 10.3^a	232.9 ± 8.8^b	278.8 ± 14.5^a	228.9 ± 9.4^b	270.0 ± 23.5^a	239.3 ± 12.8^a
Setback (BU)	133.4 ± 9.3^a	221.6 ± 5.3^a	138.0 ± 19.2^a	224.9 ± 5.0^a	176.3 ± 59.3^a	232.2 ± 22.7^a

Means with the same letter within a row of a specific concentration column are not significantly different (P=0.05).

Results are average of triplicate measurements ± standard deviation.

Table 5: Micro visco-amylo-graph® parameter results of soft white wheat flour from ozonated grain and ozonated flour, as a function of average concentrations of ozone.

Ozone Quantity	5 g/kg		10 g /kg		20 g/kg	
Treatment	Grain	Flour	Grain	Flour	Grain	Flour
Begin Gelatinization (BU)	31.0 ± 6.2^a	34.7 ± 6.9^a	27.1 ± 4.8^a	32.3 ± 4.9^a	30.3 ± 6.3^a	36.8 ± 6.5^a
Maximum Viscosity (BU)	868.6 ± 18.3^a	792.3 ± 6.5^b	857.4 ± 18.3^a	789.6 ± 8.1^b	854.3 ± 18.9^a	792.4 ± 5.2^b
Breakdown (BU)	283.3 ± 15.0^a	233.2 ± 9.7^b	279.4 ± 22.1^a	231.7 ± 9.6^b	270.1 ± 12.8^a	236.2 ± 13.9^b
Setback (BU)	125.0 ± 27.8^a	229.0 ± 13.5^b	161.8 ± 47.1^a	225.7 ± 13.7^b	161.0 ± 35.1^a	224.0 ± 15.9^b

Means with the same letter within a row of a specific quantity column are not significantly different (P=0.05).

Results are average of triplicate measurements ± standard deviation.

Table 6: Micro visco-amylo-graph® parameter results of soft white wheat flour from ozonated grain and ozonated flour, as a function of average quantities of ozone.

1.4. The baking quality tests

1.4.1. The cake test

Once again, significant differences appear between the two treatments (Tables 7 and 8). The action of the ozone directly on the flour modifies it so that the volume index of the cakes is significantly greater than when grain is treated with ozone, with every concentration and quantity. For the other parameters, differences are not significant.

Ozone Concentration	80 g/m³		95 g/m³		110 g/m³	
Treatment	Grain	Flour	Grain	Flour	Grain	Flour
Volume Index	69.3 ± 1.0[a]	110.2 ± 14.4[b]	72.0 ± 4.1[a]	96.3 ± 14.0[b]	85.7 ± 11.8[a]	111.2 ± 19.5[b]
Contour Index	8.7 ± 3.4[a]	2.8 ± 9.8[a]	5.5 ± 3.8[a]	4.7 ± 3.9[a]	10.8 ± 2.0[a]	7.3 ± 10.8[a]
Symmetry	1.0 ± 0.0[a]	5.2 ± 2.5[b]	2.5 ± 1.0[a]	4.3 ± 3.3[a]	5.2 ± 3.3[a]	3.3 ± 2.3[a]

Means with the same letter within a row of a specific concentration column are not significantly different (P=0.05).

Results are average of duplicate measurements ± standard deviation.

Table 7: Cake test results of soft white wheat flour from ozonated grain and ozonated flour, as a function of average concentrations of ozone.

Ozone Quantity	5 g/kg		10 g/kg		20 g/kg	
Treatment	Grain	Flour	Grain	Flour	Grain	Flour
Volume Index	72.2 ± 5.3[a]	96.5 ± 6.0[b]	75.8 ± 10.2[a]	106.8 ± 8.4[b]	79.0 ± 13.5[a]	114.3 ± 25.5[a]
Contour Index	6.8 ± 3.9[a]	3.0 ± 4.9[a]	8.7 ± 3.8[a]	0.2 ± 5.3[a]	9.5 ± 3.7[a]	11.7 ± 10.1[a]
Symmetry	1.8 ± 1.0[a]	5.0 ± 2.1[b]	4.0 ± 3.5[a]	4.8 ± 3.3[a]	2.8 ± 2.5[a]	3.0 ± 2.4[a]

Means with the same letter within a row of a specific quantity column are not significantly different (P=0.05).

Results are average of duplicate measurements ± standard deviation.

Table 8: Cake test results of soft white wheat flour from ozonated grain and ozonated flour, as a function of average quantities of ozone.

1.4.2. The bread test

In contrast to the cake test, the bread test does not show any significant difference between the treatments, neither by concentration (Table 9) nor quantity (Table 10). Both treatments seem to modify similarly the flour.

Ozone Concentration	80 g/m³		95 g/m³		110 g/m³	
Treatment	Grain	Flour	Grain	Flour	Grain	Flour
Bread Volume (cm³)	148.3 ± 4.0[a]	147.8 ± 3.6[a]	143.9 ± 8.5[a]	146.2 ± 3.0[a]	134.8 ± 9.8[a]	141.0 ± 7.2[a]

Means with the same letter within a row of a specific concentration column are not significantly different (P=0.05).

Results are average of triplicate measurements ± standard deviation.

Table 9: Bread test results of soft white wheat flour from ozonated grain and ozonated flour, as a function of average concentrations of ozone.

Ozone Quantity	5 g/kg		10 g/kg		20 g/kg	
Treatment	Grain	Flour	Grain	Flour	Grain	Flour
Bread Volume (cm^3)	148.9 ± 3.6[a]	147.8 ± 2.5[a]	142.3 ± 9.5[a]	146.4 ± 5.5[a]	138.5 ± 10.0[a]	141.4 ± 6.0[a]

Means with the same letter within a row of a specific quantity column are not significantly different (P=0.05).
Results are average of triplicate measurements ± standard deviation.

Table 10: Bread test results of soft white wheat flour from ozonated grain and ozonated flour, as a function of average quantities of ozone.

2. Colorimetry

As previously stated in Chapters 1 and 2, no difference in colorimetry has been determined neither compared to the control nor between the two treatments. The action of ozone seems to not deteriorate the color of the flour, in any way. Hence, the ozone is definitely not a bleaching agent.

3. Conclusion

The results given by the various analytical tests show clearly that each treatment has its own characteristics. They modify specific properties of the flour. The action of the ozone on the grain will specifically modify the viscosity of the flour and its breakdown whereas the other treatment will not. But, the action of ozone directly on the flour will give greater results on the retention of sucrose, the activity of α-amylase (falling number) and cake volume.

One important point to retain is the fact that when significant differences occur, they occur for both the concentration and the quantity average. No analytical test leads to a difference in only one of the two variables. Would it mean that the treatments are significant or not, no matter the variables? Such a conclusion cannot be made since differences have been shown in Chapters 1 and 2.

Finally, beside the disparity of the treatments, we need to look simultaneously for the effect within a treatment and between the treatments.

Conclusion

Since recently, the ozone is viewed as a new compound that could be used to treat the flour. Especially in countries were the chlorination is forbidden, the ozone treatment could be of a big interest if it was showing significant and reliable modification of the flour.

Lately, such treatment has been developed by the Goëmar laboratory and is now industrialized. But many questions remain and need answers, especially on the actual action of the ozone, its modification and the variation in the results that can be realized. This last point is what this research has been looking for.

In a first time, it has been put in light that the ozonation on the grain confers greater sodium carbonate retention capacity (useful for long fermentation products such as sour dough), lactic acid retention capacity (allowing the cookie to spread), increases the maximum viscosity and the volume of the bread, but gives detrimental results on the volume and symmetry of the cakes.

In a second time, the ozonation directly on flour has proven that it grants the flour with greater sucrose retention capacity (important for the use in high ratio sugar formulas), lactic acid retention capacity and increases the bread and cake volumes, but the α-amylase activity is shown to decrease.

In a third time, the comparison of the two treatments demonstrates that the treatment on flour has overall a superior effect than the treatment on grain, except for the maximum viscosity determined by visco-amylo-graph®. The principal points of advantage concern the greater retention capacity in sucrose, the decrease in α-amylase activity and the larger volume of the cakes. On the other hand, both treatments are similar for the retention capacity in sodium carbonate, lactic acid and deionized water and bread volume.

To conclude, the treatment on flour seems to overcome the treatment on grain. However, the effects of each treatment should stay explicit and related to each test. A flour with a specific treatment is intended for a specific use. Treatments have to be chosen conscientiously.

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Appendix A - Detailed results from Chapter 1

This appendix contains all the detailed results that have not been shown in Chapter 1.

Table I: Water activity results from soft white wheat flour from ozonated grain.

Results are average of duplicate measurements ± standard deviation.

Ozone Concentration	80 g/m^3			95 g/m^3			110 g/m^3		
Ozone Quantity	5 g/kg	10 g/kg	20 g/kg	5 g/kg	10 g/kg	20 g/kg	5 g/kg	10 g/kg	20 g/kg
Aw	0.597 ± 0.001^a	0.601 ± 0.004^a	0.597 ± 0.001^a	0.550 ± 0.003^a	0.537 ± 0.001^b	0.599 ± 0.002^c	0.565 ± 0.005^a	0.524 ± 0.005^b	0.516 ± 0.001^b

Ozone Quantity	5 g/kg			10 g/kg			20 g/kg		
Ozone Concentration	80 g/m^3	95 g/m^3	110 g/m^3	80 g/m^3	95 g/m^3	110 g/m^3	80 g/m^3	95 g/m^3	110 g/m^3
Aw	0.597 ± 0.001^a	0.550 ± 0.003^b	0.565 ± 0.005^c	0.601 ± 0.004^a	0.537 ± 0.001^b	0.524 ± 0.005^b	0.597 ± 0.001^a	0.599 ± 0.002^a	0.516 ± 0.001^b

Means with the same letter within a row of a specific concentration or quantity column are not significantly different (P=0.05).

Table II: Solvent Retention Capacity results of soft white wheat flour from ozonated grain.

Results are average of quadruplicate measurements ± standard deviation.

Ozone Concentration	80 g/m^3			95 g/m^3			110 g/m^3		
Ozone Quantity	5 g/kg	10 g/kg	20 g/kg	5 g/kg	10 g/kg	20 g/kg	5 g/kg	10 g/kg	20 g/kg
Sucrose (%)	106.0 ± 4.7[a]	105.3 ± 6.3[a]	105.2 ± 4.4[a]	106.6 ± 3.0[a]	109.4 ± 3.9[a]	107.8 ± 3.9[a]	106.0 ± 3.9[a,b]	110.0 ± 2.8[a]	104.3 ± 1.5[b]
Sodium Carbonate (%)	99.5 ± 0.8[a]	103.0 ± 4.0[a]	100.8 ± 2.9[a]	102.9 ± 3.5[a]	108.1 ± 2.8[a]	108.4 ± 3.4[a]	101.3 ± 3.0[a]	106.6 ± 2.1[b]	103.4 ± 3.3[a,b]
Lactic Acid (%)	129.6 ± 3.0[a]	132.4 ± 1.8[a]	125.2 ± 1.0[b]	128.1 ± 2.0[a]	130.9 ± 4.0[a]	131.3 ± 1.2[a]	133.7 ± 1.5[a]	133.6 ± 3.2[a]	125.8 ± 3.1[b]
Deionized Water (%)	80.3 ± 1.1[a]	86.0 ± 1.5[b]	80.8 ± 2.1[a]	84.2 ± 1.5[a]	84.1 ± 1.6[a]	82.6 ± 1.1[a]	81.7 ± 2.7[a]	85.6 ± 2.1[a]	83.0 ± 5.7[a]

Ozone Quantity	5 g/kg			10 g/kg			20 g/kg		
Ozone Concentration	80 g/m^3	95 g/m^3	110 g/m^3	80 g/m^3	95 g/m^3	110 g/m^3	80 g/m^3	95 g/m^3	110 g/m^3
Sucrose (%)	106.0 ± 4.7[a]	106.6 ± 3.0[a]	106.0 ± 3.9[a]	105.3 ± 6.3[a]	109.4 ± 3.9[a]	110.0 ± 2.8[a]	105.2 ± 4.4[a]	107.8 ± 3.9[a]	104.3 ± 1.5[a]
Sodium Carbonate (%)	99.5 ± 0.8[a]	102.9 ± 3.5[a]	101.3 ± 3.0[a]	103.0 ± 4.0[a]	108.1 ± 2.8[a]	106.6 ± 2.1[a]	100.8 ± 2.9[a]	108.4 ± 3.4[b]	103.4 ± 3.3[a,b]
Lactic Acid (%)	129.6 ± 3.0[a]	128.1 ± 2.0[a]	133.7 ± 1.5[b]	132.4 ± 1.8[a]	130.9 ± 4.0[a]	133.6 ± 3.2[a]	125.2 ± 1.0[a]	131.3 ± 1.2[b]	125.8 ± 3.1[b]
Deionized Water (%)	80.3 ± 1.1[a]	84.2 ± 1.5[b]	81.7 ± 2.7[a,b]	86.0 ± 1.5[a]	84.1 ± 1.6[a]	85.6 ± 2.1[a]	80.8 ± 2.1[a]	82.6 ± 1.1[a]	83.0 ± 5.7[a]

Means with the same letter within a row of a specific concentration or quantity column are not significantly different (P=0.05).

Table III: Falling Number results of soft white wheat flour from ozonated grain.

Results are average of duplicate measurements ± standard deviation.

Ozone Concentration	80 g/m^3			95 g/m^3			110 g/m^3		
Ozone Quantity	5 g/kg	10 g/kg	20 g/kg	5 g/kg	10 g/kg	20 g/kg	5 g/kg	10 g/kg	20 g/kg
Falling Number (s)	389.5 ± 21.9[a]	388.0 ± 21.2[a]	378.5 ± 9.2[a]	377.5 ± 13.4[a]	349.5 ± 9.2[a]	396.0 ± 15.6[a]	375.5 ± 9.2[a]	385.5 ± 23.3[a]	355.5 ± 0.7[a]

Ozone Quantity	5 g/kg			10 g/kg			20 g/kg		
Ozone Concentration	80 g/m^3	95 g/m^3	110 g/m^3	80 g/m^3	95 g/m^3	110 g/m^3	80 g/m^3	95 g/m^3	110 g/m^3
Falling Number (s)	389.5 ± 21.9[a]	377.5 ± 13.4[a]	375.5 ± 9.2[a]	388.0 ± 21.2[a]	349.5 ± 9.2[a]	385.5 ± 23.3[a]	378.5 ± 9.2[a]	396.0 ± 15.6[a]	355.5 ± 0.7[a]

Means with the same letter within a row of a specific concentration or quantity column are not significantly different (P=0.05).

Table IV: Micro Visco-Amylo-Graph® results of soft white wheat flour from ozonated grain.

Results are average of triplicate measurements ± standard deviation.

Ozone Concentration	80 g/m³			95 g/m³			110 g/m³		
Ozone Quantity	5 g/kg	10 g/kg	20 g/kg	5 g/kg	10 g/kg	20 g/kg	5 g/kg	10 g/kg	20 g/kg
Begin Gelatinization (BU)	37.7 ± 5.1[a]	24.7 ± 0.6[a]	27.3 ± 8.4[a]	27.0 ± 4.4[a]	29.7 ± 4.6[a]	28.3 ± 3.2[a]	28.3 ± 2.5[a]	27.0 ± 7.2[a]	35.3 ± 4.5[a]
Maximum Viscosity (BU)	868.0 ± 7.0[a]	879.7 ± 5.9[a]	873.3 ± 18.0[a]	850.0 ± 8.7[a]	846.7 ± 13.2[a]	841.3 ± 6.8[a]	887.7 ± 12.3[a]	846.0 ± 4.0[b]	848.3 ± 14.2[b]
Breakdown (BU)	284.0 ± 8.2[a]	290.7 ± 13.1[a]	277.7 ± 7.6[a]	276.0 ± 7.8[a]	289.7 ± 17.7[a]	270.7 ± 13.3[a]	290.0 ± 25.1[a]	258.0 ± 20.7[a]	262.0 ± 15.5[a]
Setback (BU)	136.7 ± 9.0[a]	126.3 ± 7.1[a]	137.3 ± 10.0[a]	135.7 ± 22.4[a]	139.0 ± 28.0[a]	139.3 ± 13.3[a]	102.7 ± 37.2[a]	220.0 ± 16.7[b]	206.3 ± 6.1[b]

Ozone Quantity	5 g/kg			10 g/kg			20 g/kg		
Ozone Concentration	80 g/m³	95 g/m³	110 g/m³	80 g/m³	95 g/m³	110 g/m³	80 g/m³	95 g/m³	110 g/m³
Begin Gelatinization (BU)	37.7 ± 5.1[a]	27.0 ± 4.4[b]	28.3 ± 2.5[a,b]	24.7 ± 0.6[a]	29.7 ± 4.6[a]	27.0 ± 7.2[a]	27.3 ± 8.4[a]	28.3 ± 3.2[a]	35.3 ± 4.5[a]
Maximum Viscosity (BU)	868.0 ± 7.0[a,b]	850.0 ± 8.7[a]	887.7 ± 12.3[b]	879.7 ± 5.9[a]	846.7 ± 13.2[b]	846.0 ± 4.0[b]	873.3 ± 18.0[a]	841.3 ± 6.8[a]	848.3 ± 14.2[a]
Breakdown (BU)	284.0 ± 8.2[a]	276.0 ± 7.8[a]	290.0 ± 25.1[a]	290.7 ± 13.1[a]	289.7 ± 17.7[a]	258.0 ± 20.7[a]	277.7 ± 7.6[a]	270.7 ± 13.3[a]	262.0 ± 15.5[a]
Setback (BU)	136.7 ± 9.0[a]	135.7 ± 22.4[a]	102.7 ± 37.2[a]	126.3 ± 7.1[a]	139.0 ± 28.0[a]	220.0 ± 16.7[b]	137.3 ± 10.0[a]	139.3 ± 13.3[a]	206.3 ± 6.1[b]

Means with the same letter within a row of a specific concentration or quantity column are not significantly different (P=0.05).

Table V: Cake test results of soft white wheat flour from ozonated grain.

Results are average of duplicate measurements ± standard deviation.

Ozone Concentration	80 g/m^3			95 g/m^3			110 g/m^3		
Ozone Quantity	5 g/kg	10 g/kg	20 g/kg	5 g/kg	10 g/kg	20 g/kg	5 g/kg	10 g/kg	20 g/kg
Volume Index	68.5 ± 0.7^a	70.5 ± 0.7^a	69.0 ± 0.0^a	74.0 ± 7.1^a	70.0 ± 4.2^a	72.0 ± 0.0^a	74.0 ± 7.1^a	87.0 ± 11.3^a	96.0 ± 5.7^a
Contour Index	5.0 ± 2.8^a	12.0 ± 1.4^a	9.0 ± 0.0^a	4.0 ± 1.4^a	5.0 ± 4.2^a	7.5 ± 6.4^a	11.5 ± 0.7^a	9.0 ± 1.4^a	12.0 ± 2.8^a
Symmetry	1.0 ± 0.0^a	1.0 ± 0.0^a	1.0 ± 0.0^a	3.0 ± 0.0^a	3.0 ± 1.4^a	1.5 ± 0.7^a	1.5 ± 0.7^a	8.0 ± 2.8^a	6.0 ± 0.0^a

Ozone Quantity	5 g/kg			10 g/kg			20 g/kg		
Ozone Concentration	80 g/m^3	95 g/m^3	110 g/m^3	80 g/m^3	95 g/m^3	110 g/m^3	80 g/m^3	95 g/m^3	110 g/m^3
Volume Index	68.5 ± 0.5^a	74.0 ± 1.7^a	74.0 ± 7.1^a	70.5 ± 0.7^a	70.0 ± 4.2^a	87.0 ± 11.3^a	69.0 ± 0.0^a	72.0 ± 0.0^a	96.0 ± 5.7^b
Contour Index	5.0 ± 2.8^a	4.0 ± 1.4^a	11.5 ± 0.7^a	12.0 ± 1.4^a	5.0 ± 4.2^a	9.0 ± 1.4^a	9.0 ± 0.0^a	7.5 ± 6.4^a	12.0 ± 2.8^b
Symmetry	1.0 ± 0.0^a	3.0 ± 0.0^b	$1.5 \pm 0.7^{a,b}$	1.0 ± 0.0^a	3.0 ± 1.4^a	8.0 ± 2.8^a	1.0 ± 0.0^a	1.5 ± 0.7^a	6.0 ± 0.0^b

Means with the same letter within a row of a specific concentration or quantity column are not significantly different (P=0.05).

Table VI: Bread test results of soft white wheat flour from ozonated grain.

Results are average of triplicate measurements ± standard deviation.

Ozone Concentration	80 g/m³			95 g/m³			110 g/m³		
Ozone Quantity	5 g/kg	10 g/kg	20 g/kg	5 g/kg	10 g/kg	20 g/kg	5 g/kg	10 g/kg	20 g/kg
Bread Volume (cm^3)	148.3 ± 4.0[a]	151.3 ± 3.2[a]	145.3 ± 3.2[a]	151.3 ± 2.5[a]	139.5 ± 0.7[a]	139.3 ± 10.6[a]	146 ± 2.8[a]	131.5 ± 4.9[a]	127.0 ± 7.1[a]

Ozone Quantity	5 g/kg			10 g/kg			20 g/kg		
Ozone Concentration	80 g/m³	95 g/m³	110 g/m³	80 g/m³	95 g/m³	110 g/m³	80 g/m³	95 g/m³	110 g/m³
Bread Volume (cm^3)	148.3 ± 4.0[a]	151.3 ± 2.5[a]	146 ± 2.8[a]	151.3 ± 3.2[a]	139.5 ± 0.7[b]	131.5 ± 4.9[b]	145.3 ± 3.2[a]	139.3 ± 10.6[a]	127.0 ± 7.1[a]

Means with the same letter within a row of a specific concentration or quantity column are not significantly different (P=0.05).

Table VII: Colorimetric results of soft white wheat flour from ozonated grain.

Ozone Concentration	Control	80 g/m^3			95 g/m^3			110 g/m^3		
Ozone Quantity		5 g/kg	10 g/kg	20 g/kg	5 g/kg	10 g/kg	20 g/kg	5 g/kg	10 g/kg	20 g/kg
L	96.33	96.19	96.89	96.95	96.56	96.05	96.44	95.95	95.28	96.67
a*	-0.97	-0.79	-1.06	-1.04	-1.08	-1.07	-0.76	-1.13	-0.97	-0.87
b*	7.60	8.13	7.61	7.54	7.64	7.63	7.56	7.03	7.73	7.53

Appendix B - Detailed results from Chapter 2

This appendix contains all the detailed results that have not been shown in Chapter 2.

Table I: Water activity results of soft white wheat flour being ozonated.

Results are average of duplicate measurements ± standard deviation.

Ozone Concentration	80 g/m^3			95 g/m^3			110 g/m^3		
Ozone Quantity	5 g/kg	10 g/kg	20 g/kg	5 g/kg	10 g/kg	20 g/kg	5 g/kg	10 g/kg	20 g/kg
Aw	0.439 ± 0.018^a	0.452 ± 0.001^a	0.433 ± 0.012^a	0.456 ± 0.003^a	0.426 ± 0.030^a	0.428 ± 0.018^a	0.438 ± 0.014^a	0.461 ± 0.001^a	0.447 ± 0.006^a

Ozone Quantity	5 g/kg			10 g/kg			20 g/kg		
Ozone Concentration	80 g/m^3	95 g/m^3	110 g/m^3	80 g/m^3	95 g/m^3	110 g/m^3	80 g/m^3	95 g/m^3	110 g/m^3
Aw	0.439 ± 0.018^a	0.456 ± 0.003^a	0.438 ± 0.014^a	0.452 ± 0.001^a	0.426 ± 0.030^a	0.461 ± 0.001^a	0.433 ± 0.012^a	0.428 ± 0.018^a	0.447 ± 0.006^a

Means with the same letter within a row of a specific concentration or quantity column are not significantly different (P=0.05).

Table II: Solvent Retention Capacity results of soft white wheat flour being ozonated.

Results are average of quadruplicate measurements ± standard deviation.

Ozone Concentration	80 g/m^3			95 g/m^3			110 g/m^3		
Ozone Quantity	5 g/kg	10 g/kg	20 g/kg	5 g/kg	10 g/kg	20 g/kg	5 g/kg	10 g/kg	20 g/kg
Sucrose	121.4 ± 3.3^a	125.0 ± 5.2^a	129.5 ± 4.5^a	129.8 ± 2.6^a	116.1 ± 0.6^b	121.6 ± 2.0^c	120.7 ± 2.1^a	119.3 ± 1.0^a	121.4 ± 3.5^a
Sodium Carbonate	100.3 ± 1.3^a	101.4 ± 0.6^a	94.8 ± 21.0^a	106.7 ± 1.3^a	98.7 ± 1.1^b	102.2 ± 3.4^b	108.2 ± 1.1^a	97.6 ± 0.6^b	104.5 ± 7.4^{a,b}
Lactic Acid	125.8 ± 0.7^a	126.3 ± 1.6^a	124.8 ± 0.5^a	134.7 ± 0.7^a	121.8 ± 1.2^b	123.9 ± 1.1^c	136.2 ± 0.8^a	124.7 ± 2.0^b	122.0 ± 0.5^c
Deionized Water	86.2 ± 1.5^a	92.8 ± 3.9^b	91.9 ± 1.5^b	88.8 ± 10.9^a	84.3 ± 1.2^a	87.5 ± 1.9^a	93.4 ± 0.8^a	81.4 ± 1.4^b	92.2 ± 1.8^a

Ozone Quantity	5 g/kg			10 g/kg			20 g/kg		
Ozone Concentration	80 g/m^3	95 g/m^3	110 g/m^3	80 g/m^3	95 g/m^3	110 g/m^3	80 g/m^3	95 g/m^3	110 g/m^3
Sucrose	121.4 ± 3.3^a	129.8 ± 2.6^b	120.7 ± 2.1^a	125.0 ± 5.2^a	116.1 ± 0.6^b	119.3 ± 1.0^b	129.5 ± 4.5^a	121.6 ± 2.0^b	121.4 ± 3.5^b
Sodium Carbonate	100.3 ± 1.3^a	106.7 ± 1.3^b	108.2 ± 1.1^b	101.4 ± 0.6^a	98.7 ± 1.1^b	97.6 ± 0.6^b	94.8 ± 21.0^a	102.2 ± 3.4^a	104.5 ± 7.4^a
Lactic Acid	125.8 ± 0.7^a	134.7 ± 0.7^b	136.2 ± 0.8^b	126.3 ± 1.6^a	121.8 ± 1.2^b	124.7 ± 2.0^{a,b}	124.8 ± 0.5^a	123.9 ± 1.1^a	122.0 ± 0.5^b
Deionized Water	86.2 ± 1.5^a	88.8 ± 10.9^a	93.4 ± 0.8^a	92.8 ± 3.9^a	84.3 ± 1.2^b	81.4 ± 1.4^b	91.9 ± 1.5^a	87.5 ± 1.9^b	92.2 ± 1.8^a

Means with the same letter within a row of a specific concentration or quantity column are not significantly different (P=0.05).

Table III: Falling Number results of soft white wheat flour being ozonated.

Results are average of duplicate measurements ± standard deviation.

Ozone Concentration	80 g/m^3			95 g/m^3			110 g/m^3		
Ozone Quantity	5 g/kg	10 g/kg	20 g/kg	5 g/kg	10 g/kg	20 g/kg	5 g/kg	10 g/kg	20 g/kg
Falling Number (s)	431.0 ± 14.1[a]	544.5 ± 12.0[b]	486.0 ± 39.6[a,b]	485.5 ± 3.5[a]	500.5 ± 6.4[a]	512.0 ± 35.4[a]	504.5 ± 29.0[a]	502.5 ± 41.7[a]	545.0 ± 8.5[a]

Ozone Quantity	5 g/kg			10 g/kg			20 g/kg		
Ozone Concentration	80 g/m^3	95 g/m^3	110 g/m^3	80 g/m^3	95 g/m^3	110 g/m^3	80 g/m^3	95 g/m^3	110 g/m^3
Falling Number (s)	431.0 ± 14.1[a]	485.5 ± 3.5[a]	504.5 ± 29.0[a]	544.5 ± 12.0[a]	500.5 ± 6.4[a]	502.5 ± 41.7[a]	486.0 ± 39.6[a]	512.0 ± 35.4[a]	545.0 ± 8.5[a]

Means with the same letter within a row of a specific concentration or quantity column are not significantly different (P=0.05).

Table IV: Micro Visco-Amylo-Graph® results of soft white wheat flour being ozonated.

Results are average of triplicate measurements ± standard deviation.

Ozone Concentration	80 g/m³			95 g/m³			110 g/m³		
Ozone Quantity	5 g/kg	10 g/kg	20 g/kg	5 g/kg	10 g/kg	20 g/kg	5 g/kg	10 g/kg	20 g/kg
Begin Gelatinization	36.7 ± 6.7[a]	33.0 ± 1.0[a]	31.3 ± 5.7[a]	38.0 ± 1.7[a]	32.7 ± 4.6[a]	36.3 ± 5.5[a]	29.3 ± 8.7[a]	31.3 ± 8.4[a]	42.7 ± 3.2[a]
Maximum Viscosity	789.7 ± 7.1[a]	793.3 ± 5.5[a]	792.0 ± 5.2[a]	794.0 ± 1.0[a]	793.7 ± 5.7[a]	792.0 ± 7.5[a]	793.3 ± 10.0[a]	781.7 ± 7.6[a]	793.3 ± 4.9[a]
Breakdown	235.0 ± 12.3[a]	235.3 ± 7.1[a]	228.3 ± 7.6[a]	236.0 ± 2.0[a]	221.0 ± 6.2[a]	229.7 ± 11.9[a]	228.7 ± 13.3[a]	238.7 ± 3.8[a]	250.7 ± 10.0[a]
Setback	222.7 ± 4.0[a]	220.0 ± 6.9[a]	222.0 ± 6.6[a]	229.0 ± 3.0[a]	229.0 ± 3.0[a]	223.7 ± 6.5[a]	235.3 ± 24.2[a]	235.0 ± 22.3[a]	226.3 ± 30.1[a]

Ozone Quantity	5 g/kg			10 g/kg			20 g/kg		
Ozone Concentration	80 g/m³	95 g/m³	110 g/m³	80 g/m³	95 g/m³	110 g/m³	80 g/m³	95 g/m³	110 g/m³
Begin Gelatinization	36.7 ± 6.7[a]	38.0 ± 1.7[a]	29.3 ± 8.7[a]	33.0 ± 1.0[a]	32.7 ± 4.6[a]	31.3 ± 8.4[a]	31.3 ± 5.7[a]	36.3 ± 5.5[a]	42.7 ± 3.2[a]
Maximum Viscosity	789.7 ± 7.1[a]	794.0 ± 1.0[a]	793.3 ± 10.0[a]	793.3 ± 5.5[a]	793.7 ± 5.7[a]	781.7 ± 7.6[a]	792.0 ± 5.2[a]	792.0 ± 7.5[a]	793.3 ± 4.9[a]
Breakdown	235.0 ± 12.3[a]	236.0 ± 2.0[a]	228.7 ± 13.3[a]	235.3 ± 7.1[a,b]	221.0 ± 6.2[a]	238.7 ± 3.8[b]	228.3 ± 7.6[a]	229.7 ± 11.9[a]	250.7 ± 10.0[a]
Setback	222.7 ± 4.0[a]	229.0 ± 3.0[a]	235.3 ± 24.2[a]	220.0 ± 6.9[a]	229.0 ± 3.0[a]	235.0 ± 22.3[a]	222.0 ± 6.6[a]	223.7 ± 6.5[a]	226.3 ± 30.1[a]

Means with the same letter within a row of a specific concentration or quantity column are not significantly different (P=0.05).

Table V: Cake test results of soft white wheat flour being ozonated.

Results are average of duplicate measurements ± standard deviation.

Ozone Concentration	80 g/m^3			95 g/m^3			110 g/m^3		
Ozone Quantity	5 g/kg	10 g/kg	20 g/kg	5 g/kg	10 g/kg	20 g/kg	5 g/kg	10 g/kg	20 g/kg
Volume Index	101.0 ± 7.1[a]	103.5 ± 7.8[a]	126.0 ± 12.7[a]	94.5 ± 7.8[a]	111.5 ± 6.4[a]	83.0 ± 7.1[a]	94.0 ± 2.8[a]	105.5 ± 13.4[a,b]	134.0 ± 4.2[b]
Contour Index	2.5 ± 3.5[a]	-4.5 ± 4.9[a]	10.5 ± 14.8[a]	7.5 ± 0.7[a]	2.5 ± 6.4[a]	4.0 ± 2.8[a]	-1.0 ± 5.7[a]	2.5 ± 3.5[a]	20.5 ± 2.1[b]
Symmetry	4.5 ± 0.7[a]	5.5 ± 4.9[a]	5.5 ± 2.1[a]	5.5 ± 3.5[a]	5.5 ± 4.9[a]	2.0 ± 0.0[a]	5.0 ± 2.8[a]	3.5 ± 0.7[a]	1.5 ± 2.1[a]

Ozone Quantity	5 g/kg			10 g/kg			20 g/kg		
Ozone Concentration	80 g/m^3	95 g/m^3	110 g/m^3	80 g/m^3	95 g/m^3	110 g/m^3	80 g/m^3	95 g/m^3	110 g/m^3
Volume Index	101.0 ± 7.1[a]	94.5 ± 7.8[a]	94.0 ± 2.8[a]	103.5 ± 7.8[a]	111.5 ± 6.4[a]	105.5 ± 13.4[a]	126.0 ± 12.7[a]	83.0 ± 7.1[b]	134.0 ± 4.2[a]
Contour Index	2.5 ± 3.5[a]	7.5 ± 0.7[a]	-1.0 ± 5.7[a]	-4.5 ± 4.9[a]	2.5 ± 6.4[a]	2.5 ± 3.5[a]	10.5 ± 14.8[a]	4.0 ± 2.8[a]	20.5 ± 2.1[a]
Symmetry	4.5 ± 0.7[a]	5.5 ± 3.5[a]	5.0 ± 2.8[a]	5.5 ± 4.9[a]	5.5 ± 4.9[a]	3.5 ± 0.7[a]	5.5 ± 2.1[a]	2.0 ± 0.0[a]	1.5 ± 2.1[a]

Means with the same letter within a row of a specific concentration or quantity column are not significantly different (P=0.05).

Table VI: Bread test results of soft white wheat flour being ozonated.

Results are average of triplicate measurements ± standard deviation.

Ozone Concentration	80 g/m^3			95 g/m^3			110 g/m^3		
Ozone Quantity	5 g/kg	10 g/kg	20 g/kg	5 g/kg	10 g/kg	20 g/kg	5 g/kg	10 g/kg	20 g/kg
Bread Volume (cm^3)	149.0 ± 2.6[a]	150.7 ± 1.5[a]	143.7 ± 1.5[b]	147.3 ± 3.5[a]	147.3 ± 0.6[a]	144.0 ± 3.6[a]	147.0 ± 1.7[a]	138.5 ± 4.9[a]	136.7 ± 8.7[a]

Ozone Quantity	5 g/kg			10 g/kg			20 g/kg		
Ozone Concentration	80 g/m^3	95 g/m^3	110 g/m^3	80 g/m^3	95 g/m^3	110 g/m^3	80 g/m^3	95 g/m^3	110 g/m^3
Bread Volume (cm^3)	149.0 ± 2.6[a]	147.3 ± 3.5[a]	147.0 ± 1.7[a]	150.7 ± 1.5[a]	147.3 ± 0.6[a]	138.5 ± 4.9[b]	143.7 ± 1.5[a]	144.0 ± 3.6[a]	136.7 ± 8.7[a]

Means with the same letter within a row of a specific concentration or quantity column are not significantly different (P=0.05).

Table VII: Colorimetric results of soft white wheat flour being ozonated.

Ozone Concentration	Control	80 g/m^3			95 g/m^3			110 g/m^3		
Ozone Quantity		5 g/kg	10 g/kg	20 g/kg	5 g/kg	10 g/kg	20 g/kg	5 g/kg	10 g/kg	20 g/kg
L	96.33	94.68	94.51	94.76	94.59	94.33	94.59	95.02	94.63	94.40
a*	-0.97	-0.93	-0.91	-0.87	-0.88	-0.93	-0.86	-0.88	-0.89	-0.94
b*	7.60	8.55	8.26	8.50	8.53	8.23	8.34	8.53	8.42	8.40