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Ozone degradation of off-flavors in catfish

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OZONE DEGRADATION OF OFF-FLAVORS IN CATFISH

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ABSTRACT

In the United States aquaculture is a billion dollar industry. Aquaculture is essentially the production of aquatic organisms under controlled conditions. Although conditions are controlled to a certain degree, absolute control is not possible. This reason alone is a huge problem that can cost the industry millions of dollars a year. In catfish, geosmin and 2-methylisoborneol are two compounds are primarily responsible for imparting a musty/earthy off-flavor. Off- flavors are secondary metabolites of some blue-green algae and actinomycete bacteria. When present in fish tissue they create an undesirable taste.

Different approaches have been used in order to alleviate the problem of catfish off-flavor, however as of yet no permanent solution has been found. Ozone, a very powerful oxidizer, is currently being used in a wide range of industries from wastewater to food. Previous research has shown that ozone is effective in eliminating off-flavors in water, while current research is exploring how it can further be applied.

The purpose of this research is to determine the effects of ozone on off-flavors in catfish. Twenty grams was cut from fresh catfish fillets and spiked with 0 or 10 ppb of the off-flavor 2-Methylisoborneol. After being stored at 4ºC for at least 12 hours the catfish was exposed to oxygen or ozone treatment for 0, 30 or 60 minutes. The concentrations were determined with SPME GC-MS analysis. Quality tests such as moisture, fat and color were also determined. The study indicates that oxygen and ozone treatments did differ, indicating that ozone treatment was successful in reducing catfish off-flavor. Moisture was unaffected, however color was significantly changed, while it was unclear if ozone was the cause of changes in fat.

INTRODUCTION

Aquaculture is the rearing of aquatic organisms under controlled conditions. One-third of the world's fishery products are produced via aquaculture (Schrader and Rimando 2003). In the United States aquaculture is the fastest growing agriculture, with the production of channel catfish making up 46% (Tucker et al 2004). Although catfish production is a very lucrative industry, there is a challenge to produce and maintain good quality fish. The threat due to the ongoing problem of catfish off-flavor is a problem for all catfish farmers. Off-flavor causes inconsistent quality in catfish.

Off-flavor is a problem worldwide; however it's most expensive in the US due to the weather and climate. Alabama, Arkansas, Louisiana, and Mississippi are currently the four main catfish producing states. The weather is hot & humid, causing the causative agents of the off-flavors to flourish. Off-flavors are compounds that, when present in fish tissue, create an undesirable taste. Although there are many types of off-flavors, the Geosmin and 2-Methylisoborneol are the most common in channel catfish. Geosmin and MIB are described to be earthy and musty. Catfish can acquire off-flavor when the odorous molecules are absorbed through the gills & into the blood where they are transferred into the flesh and deposited. When catfish are deemed to be off-flavor they cannot be sold because they are unacceptable to the consumer, which can delay harvesting resulting in a loss millions of dollars per year.

Ozone is what protects us from harmful UV rays but it is also a powerful oxidizer that is currently being used in a variety of industries, including protecting our food and water from unsafe pathogens. Ozone is a three atomed molecule that can be made naturally or artificially. When in contact with any biological material it will oxidize it by a "direct kill attack".

Researchers at Louisiana State University Department of Food Science along with the cooperation of researchers from the United States Department of Agriculture have conducted research to determine whether the off-flavor 2-Methylisoborneol could be degraded by the use of ozone. Samples were analyzed both quantitatively and qualitatively.

LITERATURE REVIEW

Catfish

Catfish come in a variety of size, shapes and colors. Infact there are over 1,250 species of catfish known to man, with less than 50 species situated in North America; however only eight species are common (Lee 1991 & Forrester 1999). Those include channel catfish, blue catfish, white catfish, flathead catfish, speckled bullhead catfish, brown bullhead catfish, black bullhead catfish, and yellow bullhead catfish (Lee 1991 & Forrester 1999). Although common in freshwater, catfish can be found in both saltwater and freshwater. Catfish can be classified in several different ways which include habitat, spawning season, and scientific classification (Lee 1991).

The origin of the name "catfish" can be attributed to the fact they have two traits common to the housecat. First, when out of water they make purring noises and second, they possess barbells on their head which are said to resemble whiskers. The most frequently encountered catfish is the bullhead, which include: speckled bullhead (*Ictalurus nebulosus marmoratus*), brown bullhead (*Ictalurus nebulosus*), black bullhead (*Ictalurus melas*), and the yellow bullhead (*Ictalurus natalis*). They are extremely resilient and due to the presence of an air bladder that acts like a lung and allows them to breathe air directly they can survive in waters that others could not (Limburg 1980). Surprisingly, these types of catfish are hardly ever raised commercially as they are slow growers, very vulnerable to disease, have a poor food conversion ratio and are very unappealing to consumers (Limburg 1980).

It is the channel catfish (*Ictalurus punctatus*) that is the leading cultured catfish in the United States (Forrester 1999). They favor clear, flowing water but are adaptable to ponds. They are described as being handsome, slender, light blue fish that withstand crowding well

(Limburg 1980). They have deeply forked tails, rounded anal fins and spots on their body (Lee 1991). Channel catfish are often confused with blue catfish (Lee 1991).

Blue catfish (*Ictalurus furcatus*) have the capability of being trained to feed at the surface of the water. However they grow slow, don't grow uniformly and don't convert feed to flesh very well. Flathead catfish (*Pylodictis olivaris*) are too aggressive and cannibalistic, while white catfish (*Ictalurus catus*) are slow growers and their big heads cut dress out weight (Limburg 1980). Although the white catfish can survive overcrowding, high temperatures and turbid water better than Ictalurus punctatus, channel catfish are the predominant species used in catfish farming. Channel catfish are useful to cultivate due to the fact that they are a healthy source of protein, have exceptional flavor, efficiently convert feed to flesh, and well suited for intensive culture and catfish ponds can be established on land that would be inadequate for other crops (Lee 1991). Catfish farming is more than capturing fish; it involves managing the environment of the catfish from the egg to adult. In order to "farm" catfish, one must be knowledgeable in how catfish live, reproduce, and grow (Lee 1991).

Aquaculture

Aquaculture is derived from the Latin words "aqua" which means water and "culture" which means "to till", "to cultivate" or "to grow" (Lee 1991). Aquaculture is essentially the production of aquatic organisms under controlled conditions. Although conditions are controlled to a certain degree, absolute control is not possible in these environments. This reason alone is a huge problem that can cost the industry millions of dollars a year in loss (Tucker 2000). Aquaculture production requires the environment to be stable. This stability has been attributed to the phytoplankton and bacterial communities that live within these ponds (Conte et. al 1996b; Perschbacher 1995). Phytoplanktons are single-celled plants that produce oxygen as a byproduct

of photosynthesis (Lutz et. al. 1992). In a commercial catfish production pond the phytoplankton provide not only oxygen but also food. They also help remove waste by digesting ammonia as a nitrogen source for growth reducing the buildup of unionized ammonia that is toxic to fish (Forrester 1999). However just as phytoplanktons help the pond ecosystem, they can also be a factor in destroying it (Conte et. al. 1996b).

Algal Blooms

Algal blooms (dense aggregations) are made up by actinomycetes, green algae, and blue-green algae and can form near the surface of the water restricting light from penetrating it (Johnsen & Dionigi 1994). Although there are no environmental factors that control the growth of blue-green algae blooms, light plays an important role (Millie et. al. 1990). Phytoplankton can reduce underwater light intensity that allows low light tolerant algae (example: blue-green algae) to flourish (Forrester 1999). Absence of light can also prevent nuisance plants from growing but it can limit photosynthesis, pond maintenance, and harvesting of fish (Johnsen & Dionigi 1994; Lutz et. al. 1992). However, killing these algal blooms will not improve the situation; it only makes the problem worse. When photosynthetic algae die & decompose the cells rupture, releasing odorous metabolites into the water resulting in a loss of oxygen and ultimately the suffocation of fish (Arganosa & Flick, Jr. 1992). Due to high fish densities, these ponds receive large nutrient inputs, which include fish excretions, sediment mineralization/resuspension and excess fish feed (Zimba et. al. 2001). For example, it is common practice in an intensive catfish production system to stock a pond at 20,000 to 30, 000 fish per hectare, which is approximately 1.6 to 2.5 fish per meter cubed (Masser 1995). Unfortunately because these pond systems are “static” systems (no water goes in or out) and high amounts of nutrients are added daily, algal blooms & bacteria are encouraged to grow (Conte et.

al.1996; Lutz et. al 1992). Researchers suggest that many of these organisms release odorous metabolites into the water that produce off-flavor in water and fish.

Off-Flavor and Off-Flavor Production

Off-flavors are compounds that, when present in fish tissue, create undesirable taste (Conte et. al. 1996c). Although there are many off-flavors known to man, in catfish, geosmin and 2-Methylisoborneol (MIB) are two compounds that are primarily responsible for imparting a musty/earthy off-flavor (Korth et. al.1991; Arganosa & Flick, Jr. 1992). Geosmin & MIB are secondary metabolites of some species of blue-green algae and actinomycete bacteria; and are among the most potent olfactory stimuli known to man (Korth et. al. 1991; Arganosa & Flick, Jr. 1992). Geosmin is an off-flavor causative compound of not only fish, but also beets and beans (Dupuy et. al; Hensarling & Waage 1990). Geosmin is said to have an earthy or pond-bottom taste, while MIB produces a musty flavor (Conte et. al. 1996a; Schlenk 1994).

Uptake of Off-Flavors

Channel catfish acquire off-flavor when the odorous molecules are absorbed through the gills & into the blood where they are transferred into the flesh and deposited. In catfish, the diffusion of the off-flavors between water & blood are increased due to the off-flavor structure and function (Tucker 2000). These off-flavor molecules are fat-soluble and likely to deposit themselves under the skin (Van der Ploeg et. al. 2001). A study done in 1992 suggests that the fat content of channel catfish could affect the uptake & depuration of odorous compounds, mainly MIB. Johnsen & Lloyd (1992) used several fish of different fat content and held them in water containing MIB. The fish that had greater than 2.5% muscle fat were considered to be fat, and the fish that had 2% or lower muscle fat were considered to be lean. Within a 24 hour period the fatter fish took in 3 times more MIB than the leaner fish, and when purged it took $1/6^{th}$ the

time for the leaner fish to purge the MIB. This study concluded that attempting to rear leaner fish would be more profitable because they would have lower levels of off-flavor compounds and have an increased rate of purging (Johnsen & Lloyd 1992).

Gills are not the only routes of entry for odorous compounds; they can also be absorbed via the alimentary canal while feeding. Off-flavors are also absorbed on or through the skin, but are usually removed from skin mucosa during the processing of the fish (Persson 1984). The rate that the off-flavor is absorbed at depends on the water temperature, length of exposure, and concentration of compounds in the water. For example Johnsen (1989) used temperature-controlled experimental tanks to demonstrate the effect of temperature on catfish. When catfish are exposed to 1 ppb of geosmin at 20ºC, the geosmin off-flavor was rapidly taken up (Arganosa & Flick, Jr. 1992). Another study done at Mississippi State University revealed uptake of the off-flavor MIB was increased in warmer temperatures, and also that off-flavors were developed within hours when exposed to high concentrations of MIB (van der Ploeg et. al 2001). The rate of uptake can also be affected by the species of fish and physiological state (Persson 1984).

Effects of Off-Flavors

Although MIB & geosmin are not mutagenic or cytotoxic to consumers, they still have a detrimental effect financially (Tucker 2000; Dionigi et. al 1998). It is estimated that off-flavor can cost the catfish industry an extra $0.10 to $0.26 per kilogram due to the increased production time, feed & possible diseases (van der Ploeg et. al 1994; Lorio et. al 1992). In Finland, for example, there was an occurrence of a musty off-flavor in fish from the Oulu Sea. This episode not only costs the industry a financial loss, but also a temporary loss of jobs for hundreds of fisherman (Persson 1980). On August 15, 1977 in Cedar Lake, Manitoba, a consumer reported a moldy/earthy off-flavor in a commercial walleye. After careful inspection of other shipments of

fish from Cedar Lake, it was discovered that they were all afflicted by the same geosmin & MIB off-flavor chemicals. In this instance the commercial fishing season was closed just ten days after the complaint was received (Yurkowski & Tabachek 1980).

Detection of Off-flavors

The use of instrumentation to detect flavor thresholds can often be expensive & complicated (Bett et. al. 1997). The methods associated with quantitative chemical analysis are very time-consuming & tedious in order to extract off-flavors from seafood. These methods cannot be used as routine quality control because they aren't sensitive enough to detect off-flavors at low levels. In these cases where there are low levels or even when the causative compounds are unknown it is best to use sensory evaluation (Bett et. al. 1997). Sensory evaluation is a method in which trained analyzers are used as "instruments" in order to test fish flavor quality. This "taste-testing" method is widely used because low levels of odorous compounds can be detected, discriminated and flavor intensity identified (van der Ploeg 1992).

Sensory Evaluation

Sensory evaluation is defined as a scientific discipline used to evoke, measure, analyze and interpret reactions to those characteristics of foods and materials as they are perceived by the senses of sight, smell, taste, touch and hearing (IFT 1975). Consumers most often complain about the taste of fish when the cause of the problem is really its odor. There are only four basic taste sensations, which are sweet, salty, bitter & sour. Anything other than these four sensations that are taken into the mouth is actually detected by the olfactory system (Young et. al 1996).

Just as mechanical instruments have limitations, so do humans. Controlled environments are conducive to the accuracy of these evaluations of off-flavors, but there is always a possibility for human error (Bett and Diognigi, 1997). For example, repeating a test of the same sample

may be necessary if the off-flavor is very strong, due to olfactory receptors that can quickly fatigue (van der Ploeg et. al. 1992).

Infact in 2004 a study was published suggesting that dogs may provide "practical early detection" of off-flavor problems that can occur within catfish ponds. Since early detection means that producers mat be able to take corrective action before off-flavors accumulate in the fish, Shelby et. al trained six dogs to detect MIB and GSM in water prepared in the lab. The three dogs that reacted better to the training were given additional guidance in order to identify real catfish ponds that were in the early stages of becoming off-flavor. The responses for identifying on-flavor water samples were correct 96% of the time and the off-flavor water samples varied from 30-95% (Shelby et. al. 2004).

Mechanical Instrumentation

When looking at methods of analysis to detect the presence of off-flavor compounds it is important that they have a good sample turnover rate and have accurate & reproducible results (Watson et. al 2000). For example in 1986, Dupuy et. al wanted a simple, quick, yet effective test that would detect geosmin, because when present in water it would be taken up by the fish and render them unmarketable. This method required fish to be steamed in order to break up the tissue, the residual oil phase was then centrifuged and injected into the GC in order to detect geosmin (Dupuy et. al 1986). As time progressed so did technology and in 1998 Palmentier et. al discussed a method known as Isotope Dilution High-Resolution Mass Spectrometry. This method enabled large amounts of samples to be handled at one time and still have a reasonable turnaround time. Lloyd et. al 1998 used a variation of SPME (Solid Phase Micro-Extraction) methods in order to analyze geosmin & MIB in water samples. They concluded that the SPME method was most efficient when coupled with gas chromatography, because it was less

expensive, portable, and a faster more reliable method of analyses of off-flavor compounds in water (Lloyd et. al 1998). Another type of SPME method was studied by Watson et. al in 2000. Headspace SPME coupled with GC/MS provided "excellent quantification" of geosmin & MIB in water at levels lower than human odor threshold concentrations (OTC).

Off-Flavor Management/Control Measures

Due to the year-round production and harvesting of catfish some type of off-flavor management plan should be in effect to ensure schedules are maintained (Johnsen & Dionigi 1994). One possible way to control the production of MIB & geosmin is to decrease stocking densities. By doing this the amount of nutrients that are added into the pond are minimized and therefore the growth of blue-green algae & actinomycetes is discouraged (Conte et. al. 1996). Readying catfish to be harvested between July and August is also a manner in which off-flavor can be prevented, given that blue-green algae is normally present in ponds from late spring to early fall (van der Ploeg & Tucker 1994). Due to the seasonal appearance of geosmin & MIB it is best to harvest fish before the warm temperatures start to encourage their growth (Dupuy et. al 1986; Arganosa & Flick, Jr. 1992). Dividing large ponds into smaller ones can also be useful.

Elimination of Off-Flavors

Although off-flavor is easy to acquire (sometimes within a matter of minutes), once these chemicals are present it may take several days to bring fish back "on-flavor" (Heikes 1993). A common practice is to purge odorous chemicals out of fish. However as stated previously this method can be very costly & time-consuming. Purging is achieved by holding fish in a smaller pond and continuously flushing them with well water (free of off-flavor chemicals) until off-flavor is gone. MIB can usually be purged within 3-5 days. Geosmin is harder to purge and can take up to 3-4 weeks to be flushed. During this process little to no food is added, consequently

fish often lose 1-5% of their body weight. This is why off-flavor compounds should be identified before choosing this as a recovery method (van der Ploeg et. al. 2001).

Forrester et al. (2002) attempted to establish whether geosmin and MIB found in catfish fillets post-harvest could be degraded chemically or masked with treatment of an antioxidant, citric acid. The use of vacuum tumbling allowed for the citric acid solution to be massaged into the catfish tissue rapidly without deterioration in color or formation of emulsions. The results of this study showed that by using a 2% acid treatment coupled with vacuum tumbling; the catfish fillets had an increase in moisture, lightness, and protein concentration and a decrease in fat (Forrester et al. 2002). Another study has suggested that the best conditions for depuration of fish are those in which the fish are lean & the water temperatures are warm. In those conditions the musty flavor of MIB can be purged within sixty hours (Tucker 2000). Not many studies have been able to accurately estimate rates of purging in fish; however studies have shown that the rate of purging is affected by water quality, holding conditions, water temperatures, concentrations of odorous compounds in fish, and fat content (van der Ploeg 1992). A study done by the USDA's Agricultural Research Service, Southern Regional Research Center suggested that MIB and geosmin could be purged within 2-3 days provided they are contained within water free of off-flavor metabolites (Heikes 1993).

In order to prevent the harvesting of catfish with off-flavor certain quality control methods are put into place (van der Ploeg 1992). Various methods, such as sensory evaluation (which was previously discussed), have been discovered in order to detect levels of geosmin & MIB preharvest. Quality control practices may vary within the industry, however it is routine to collect samples frequently from ponds before harvesting them (Dionigi et. al. 1998). Normally 2-3 fish from each pond are taken to a processing facility for preharvest flavor testing (Dionigi

et. al. 1998; van der Ploeg 1992). If fish are deemed to be unacceptable they are not harvested (Johnsen et. al. 1996).

Ozone as an Oxidizing Agent

The Encarta World English Dictionary describes ozone as being "a form of oxygen gas with three oxygen atoms in its molecule; formed by electrical discharge in oxygen, acting as a strong oxidizing agent, and used in water purification" (Encarta 1999). Ozone (O_3) under normal atmospheric conditions is a colorless gas that can be detected by humans at levels of 0.01ppm to 0.02 ppm (Graham et. al 1997; Singh & Wheaton 1999). Ozone is said to be one of the most powerful oxidizing agents known to man (Roy-Arcand & Archibald 1996). Ozone has a chlorine-like scent at low concentrations and an unpleasantly pungent odor at high concentrations (Graham et. al 1997; Singh & Wheaton 1999).

History of Ozone

The earliest history of ozone is dated back to 1840 when a German scientist, C.F. Schonbein, first produced and identified it (Graham et. al 1997). In 1888, a U.S. patent was issued for an apparatus that could produce O_3 to deodorize sewer gases (Graham 1997). Ozone has proven to be very soluble at low temperatures and is best used in an aqueous solution to disinfect polluted water (Graham et. al 1997). In 1891, the use of ozone as a bactericidal agent was proven and two years later the Dutch began to use ozone commercially to disinfect water (Graham et. al 1997). Discoveries like this eventually led to the development of the 1st full-scale ozone-generating water treatment plant in Germany in 1902 (Graham 1997). Although ozone was widely being used as a standard practice across Europe in the early 1900's, it wasn't until 1940 that the 1st potable water treatment plant to continuously use ozone was constructed in the United States (Graham 1997; Kaminski & Prendiville 1996). In 1980, there were reportedly 5

plants that used ozone as treatment for potable water, and by 1987 over 200 plants in the US were using ozone (Kaminski & Prendiville 1996; Graham 1997).

Generating Ozone

Ozone occurs naturally in nature at the earth's upper atmosphere and when lightning strikes or by UV rays from the sun (Marriott 1999). However these two naturally occurring phenomenon's can be mimicked in order to produce ozone. Ozone is usually generated in two ways, corona discharge or ultraviolet. Ultraviolet (UV) ozone generation is done by passing an oxygen containing gas through a source of ultraviolet radiation. Air is passed over an ultraviolet lamp, which emits 185nm, thereby splitting the oxygen (O2) molecules. The resulting oxygen atoms (O1) attach to other oxygen (O2) molecules in order to stabilize and accordingly form ozone (O3). The resulting gas can then be injected into water in order to inactivate contaminants (to be discussed later). The UV method is similar to how UV rays from the sun produce ozone. Passing dried oxygen containing gas through a high-energy electric field carries out Corona Discharge. This causes the oxygen molecules (O2) to split into oxygen atoms (O1). The oxygen atoms want to be more stable and therefore attach to other oxygen molecules in order to form ozone. Due to the fact that electrical energy is being put into the system/generator, a method to remove the heat is required. A cooling method must also be used. This method is used mostly to produce large amounts of ozone (Kim et al. 1999). The corona discharge method is similar to how lightning produces ozone.

Another method of ozone production is electrolysis. Lynntech, Inc. has developed a newer method of producing ozone (College Station, TX), in which the oxygen atoms in water provide the source of oxygen in order to form ozone. Only a power supply and water are needed to achieve this. This electrochemical process splits water into hydrogen and oxygen atoms by

electrolysis (Kim et. al 1999). The hydrogen molecules are separated from the gas and water mixture. The oxygen atoms (O1) will combine to form ozone (O3) and diatomic oxygen (O2). This method of producing ozone is said to supply concentrations of three to four times that of corona discharge (Kim et. al 1999). The purer the water used, the longer the life of the ozone apparatus.

How Ozone Works

Now that we know how to make ozone we will discuss the manner in which it works to destroy microorganisms. Generally the sterilization effect of ozone is achieved by a "direct kill attack" and oxidation of the biological material. When a chemical compound in an aqueous environment is exposed to ozone, it is either directly attacked by ozone and/or by free radicals from ozone (Kim et. al 1999).

It is believed that the bacterial cell surface is the object of ozone (Kim et. al 1999). Ozone will approach the bacteria in a solution and bind with the organic compounds in the cell wall of the bacteria. The free radicals of ozone breakdown the double bonds in the cell wall and destroys the cell permeability of the structure resulting in break down and eventual lysis of the cell (Kim et. al 1999). Ozone will continuously oxidize the bacteria and any released organics. The effectiveness of ozone will depend on a variety of factors including, but not limited to, pH, temperature, humidity, amount of organic matter present, and additives.

Applications of Ozone

General Uses in Various Industries

The use of ozone in the U.S. food industry was approved in 1997 due to an endeavor led by Dr. Dee Graham. His efforts resulted in the Generally Recognized as Safe (GRAS) affirmation of the use of ozone in food processing in the United States (Graham et. al 1997). The

USDA did not object the GRAS decision to use ozone as a disinfectant or sanitizer in food processing. On June 26, 2001 the United States Food and Drug Administration formally approved the use of ozone as an "antimicrobial agent for the treatment, storage and processing of foods in gas and aqueous phases" (Sopher et. al 2002). On December 21, 2001 the use of ozone was approved for direct contact with meat and poultry by the United States Department of Agriculture's Food Safety and Inspection Service. It can be used for raw and fresh cooked products up until they are to be packaged (Sopher et. al 2002). Due to these developments there has been ongoing research in the application of ozone to meet safety and quality requirements in the following fields: aquaculture, beef processing, poultry processing and wastewater treatment to name a few.

Specific Uses in the Food Industry

Wastewater Treatment

Water quality is a very important issue nationwide. Therefore the search continues for the most effective treatment to disinfect water (Kaminski & Prendiville 1996). The most commonly used disinfectant of public water supplies and general sanitation in the U.S. has been chlorine (Graham 1997). Although chlorine is a very good disinfectant, the oxidizing capabilities of ozone make it a more powerful oxidizer that reacts 3000 times faster than chlorine and ozone is one and half times more reactive than chlorine (Chang & Sheldon 1988; Lazar 1998). Chlorination also produces chlorine clouds and harmful hydrocarbon byproducts (THMs) during treatment. Ozone on the other hand produces no toxic clouds, no residual ozone odor or taste, and no hydrocarbon byproducts (Graham et. al 1997). The two largest ozonation facilities in the United States are housed in Dallas, TX and Los Angeles, CA (Kaminski & Prendiville 1996).

Although the application of ozone in wastewater treatment facilities is expensive it has been ideal because it not only kills microorganisms, but it also helps improve color, odor and taste (Singh & Wheaton 1999; Graham et. al 1997). Although most wastewater has offensive odors there are certain treatment methods that can actually add to the odorous compounds (Hwang et. al 1994). In 1993 in Milwaukee, over 400,000 people were afflicted after drinking water contaminated with *Cryptosporidium*. *Cryptosporidium* is a microorganism that causes gastrointestinal illness in humans and can often mean death for those already afflicted with immune system deficiencies. In order to assure that this did not happen again, the city introduced an agenda that would upgrade the waterworks facilities and provide safer water in the future. While the city was in compliance with all state and federal regulations before, during and after this emergency the root of this problem was never determined. Milwaukee's intent was simple, they wanted to kill the *Cryptosporidium* in the raw water to prevent future contamination, control the odorous compounds in the water, reduce the levels of byproducts that may result due to treatment, and protect public health all while staying within the guidelines of federal and city regulations. Considering the fact that ozone is the only disinfectant that can kill *Cryptosporidium*, as well as the fact that the pre-ozonation would require little to no disruption, they decided to use ozonation. The results were positive and Milwaukee was one of the first facilities that had been specifically designed to inactivate *Cryptosporidium.* (Kaminski & Prendiville 1996). In 1990, Glaze et. al reported on a study that carefully assessed nine oxidizing agents in removing six tastes & odor compounds that were present in Colorado River water. One of the discoveries made was that, while other traditional oxidants could not get rid of geosmin & MIB in the water, ozone could. Ozone was able to oxidize the two compounds without the addition of any other oxidant (Glaze et. al 1990).

Aquaculture

In the United States aquaculture is a billion dollar industry (Tucker 2000). This is why it should be no surprise that the seafood processors are investigating new ways to rid water and fish surfaces of microorganisms and extend shelf life. When fish are harvested/captured the opportunity presents itself to maintain quality by detouring the growth of microbes that cause reduction of shelf life (daSilva et. al 1998). In 1982, it was reported that Pacific salmon packed in ozonated ice were preserved for 6 days and in 1984, shrimp that were chilled with ozonated ice were extended 1-2 days (daSilva et. al 1998). An ice making company in Seattle was featured in a January 1998 article of Fish Farming International; they suggested that using ozonated water to make ice would produce a "bactericidal ice". When used to chill fish this ice could prolong shelf life up to a week, depending on the fish (FFI 1998). The extended shelf life was apparently due to the "bactericidal ice" reducing the number of microbes present (daSilva et. al 1998). This has led some commercial fishing vessels to install ozone-generating equipment on board (daSilva et. al 1998).

A recirculating aquacultural system is an alternative method to rear fish commercially in which the culture water is continuously treated and recycled within the system (Singh & Wheaton 1999). The advantages of recirculating aquacultural systems over "traditional" aquaculture are many. The first is of is water conservation, due to the continuing recycling of the water. Also fish can be grown year round with better environmental controls that result in a consistency of final product (Singh & Wheaton 1999). The fact that recirculating aquaculture still hasn't reached its growth potential means that no SOPs (Standard Operating Procedures) are in place, although most have requirements for waste removal. Singh & Wheaton (1999) go on in this article to discuss the use of ozone application and how it can be used as a water treatment

process in recirculating aquaculture due to its ability to dissolve organic and inorganic waste, amplify nitrification on the biofilter, control suspended solids and disinfect. The only problems that may arise are due to the ozone toxicity to the fish. However since there are no specifications for the application of ozone it is believed that more research should be done in this area of aquaculture in order to predict optimum conditions in which ozonation should be carried out (Singh & Wheaton 1999).

Another application of ozonation in aquaculture has been to reduce color in fish. Ozone has previously been used in the food industry to remove color, taste, odor, iron, and manganese from water (Kim et. al 1997; Park et. al 1999). A study aimed at improving the color of horse mackerel mince was undertaken in 1997, with the expectation that the use of the "ozone flotation washing" method would improve the color of the mince with a shorter washing period (Chen et. al 1997; Kim et. al 1999). The idea was that ozone would damage the porphin of myoglobin or hemoglobin in order to be successful in the discoloration of the fish. Although several methods were used, the use of ozonated water in washing helped reduce the wash time required (Chen et. al 1997). Another study that was done in 1998 compared the effects of alkaline washing versus ozonation on color & texture of mackerel surimi gels (Jiang et. al 1998). In this experiment Jiang et. al (1998) concluded that the dark color of mackerel mince could be enhanced by 30 minutes of ozonation at a pH of 3.0.

Beef Processing

The government has a "zero tolerance" policy for contamination of beef (Reagan et. al 1996). In order to ensure that all beef products are safe and wholesome the USDA has set forth regulations that require physical contamination (such as fecal matter and other materials) to be trimmed off all beef carcasses before being washed and chilled (Reagan et. al 1996). This

trimming process is done in an attempt to remove physical and microbiological contaminants from the tissue. Although the effectiveness of this process had not yet been studied, in 1996 the trimming method was one of the beef industries most common practices.

In 1996, a study was undertaken in six beef carcass conversion plants, operated by four different companies that spanned across five states. In these studies the beef carcasses were purposely contaminated with fecal matter in an attempt to evaluate the best decontamination method to be used in a commercial setting (Reagan et. al 1996). No scientific in-plant studies had ever compared the washing/trimming method to the use of ozonation. Reagan et. al (1996) reported that the industries current practice of trimming and washing the carcasses continually showed a decrease in bacterial populations however the use of the ozone and hydrogen peroxide treatments had minimal effects on the reduction of bacteria on the beef carcasses. Another study done by Fournaud and Lauret (1972) concluded that the use of gaseous ozone treatment (100 ppm) for 30 minutes did not significantly decrease the amounts of bacteria on surface of the beef. They believed that ozone was an unacceptable treatment because odor & discoloration developed (Kim et. al 1999).

Poultry Processing

It is estimated that in a year a poultry processing plant uses between 25–46 billion gallons of water. The need to decrease the amount of water usage as well as decrease the amount of bacteria in poultry processing, has led to the use of ozone (Lazar 1998; Sheldon & Brown 1986). The need for a method to disinfect and treat water for poultry chilling was brought about due to the Federal Poultry Products Inspection Act that allowed for the recycling of chilling water (Sheldon & Brown 1986; Chang & Sheldon 1988). In 1986, Sheldon & Brown began a study in which they proved ozone to be effective as a disinfectant of chiller water (suited for recycling),

and a major factor in reducing the number of pathogenic and spoilage microorganisms on poultry carcasses processed in ozonated chiller water while consistently meeting USDA regulations (Sheldon & Brown 1986; Chang & Sheldon 1988). In 1988, Chang & Sheldon investigated the reconditioning of overflow prechiller, neck chiller, and final carcass rinse waters from a boiler-processing plant by using wastewater treatments in combination with ozone. They concluded that the highest quality of water was produced when a combination of screening, DE filtration and ozonation were used. Afterwards they were able to replace every 1-gallon of fresh water with 1.1 gallons of reconditioned water (Chang & Sheldon 1988).

Recycling chiller water can definitely have a positive economic impact on the poultry processing industry by reducing the amount of water used, however this is not the only process in which ozone proves to be profitable. Poultry processors in Florida designed an apparatus that utilizes ozonation to process and reduce the amount of bacteria on their chicken breasts by 90%. They pass the chicken breasts under UV light in order to kill the surface bacteria (Lazar 1998).

Possible Downfalls of the Application of Ozone

Although the application of ozone can be a positive method of disinfection, it can also be detrimental if used incorrectly (Garratt 1997). Ozone is a very lethal weapon that is known to have unfriendly effects on crops, human health, and various other materials (Garratt 1997; Jenkin & Hayman 1999). When using ozone it is very important to remember the following:
In general ozone is an irritant to eyes and mucous membranes as all oxidizers are (Graham et. al 1997). Ozone is non-selective in its actions and attacks anything it comes in contact with (Gooch 1998). This reason is why any materials that are involved in the ozonation process must be able to withstand ozone (Singh & Wheaton). If generators are incorrectly installed dangerous levels of ozone can be leaked into the atmosphere, which are toxic to humans (Gooch 1998;

Singh & Wheaton 1999). In aquaculture the application of ozone can kill fish by damaging gills, killing beneficial bacteria and/or destroy feeding structures of the fish (Garrett 1997). In beef processing high exposure to ozone can lead to shrinkage weight loss and possible darkening of meat (Graham et. al 1997). In poultry processing ozonation can possibly cause skin to become discolored, develop darkening, rancidity or even off-flavors (Graham et. al 1997).

Evaluation of Quality

Evaluating quality of fish is very important for catfish producers and seafood processors. The quality of the product as it leaves the producer significantly influences the quality of the product that is presented to the consumer. Quality can't be improved at the processing or retail level; it has to be assured by the producers. For processors it is very important so that they will not purchase poor quality fish. Also as the government it is their duty to uphold and protect the public health of consumers by ensuring that safe products reach the market.

Nutritional Qualities of Catfish

While trying to rid catfish of off-flavors to increase quality you do not want to decrease the quality of the nutrients. Fish, in general, are primarily known for being good sources of omega-3 fatty acids, which are linked to the reduction in the risk of cancer and heart disease. They are also a low fat, low calorie food that provides generous amounts of complete proteins, and an array of vitamins and minerals (Piggott and Tucker 1990).

MATERIALS AND METHODS

Preparation of Samples

All fresh catfish samples used were obtained locally from Tony's Seafood, located at 5215 Plank Road in Baton Rouge, Louisiana. Live catfish were chosen based on length then gutted, skinned and filleted, leaving the fillet attached to the tailbone. Leaving the fish attached to the tailbone allowed us to keep track of which fillets came from the same fish. Catfish were then brought to a lab in the LSU Food Science Department where treated. The catfish fillets were cut off of the tailbone using a sharp knife. Because each catfish has two fillets, one fillet was used as a control to assure that the fish did not have any off-flavor to begin with and the other was treated (as specified later). Each control fillet was cut to obtain 20g ± 0.05g pieces. Each treated fillet was cut into a 60g piece and then into three 20g ± 0.05g pieces (these pieces are used for spiked control, spiked oxygen control and spiked ozone treated). The fish were cut in a rectangular shape towards the bottom center of the fish to ensure the sample contained the fatty tissue of the fish where off-flavors are almost likely to deposit themselves. The spiked and/or unspiked, untreated control was then ready to be analyzed for quality or extracted for off-flavor analyses and the remaining samples were now ready to be treated (Figure 1).

Treatment of Samples

To determine percent recovery of the MIB analysis, one control used was fish spiked with off-flavors only. Twenty grams of a catfish fillet sample was placed into a large plastic weigh boat and spiked in several spots with a syringe (Supelco, Bellefonte, PA) just underneath the skin with 10 ppb of 2-methylisoborneol (Supelco, Bellefonte, PA). Afterwards the weigh boat was placed into a plastic Ziploc bag (SC Johnson, Racine, WI) labeled with a black permanent marker and stored for at least 12 hours at 4ºC before analysis.

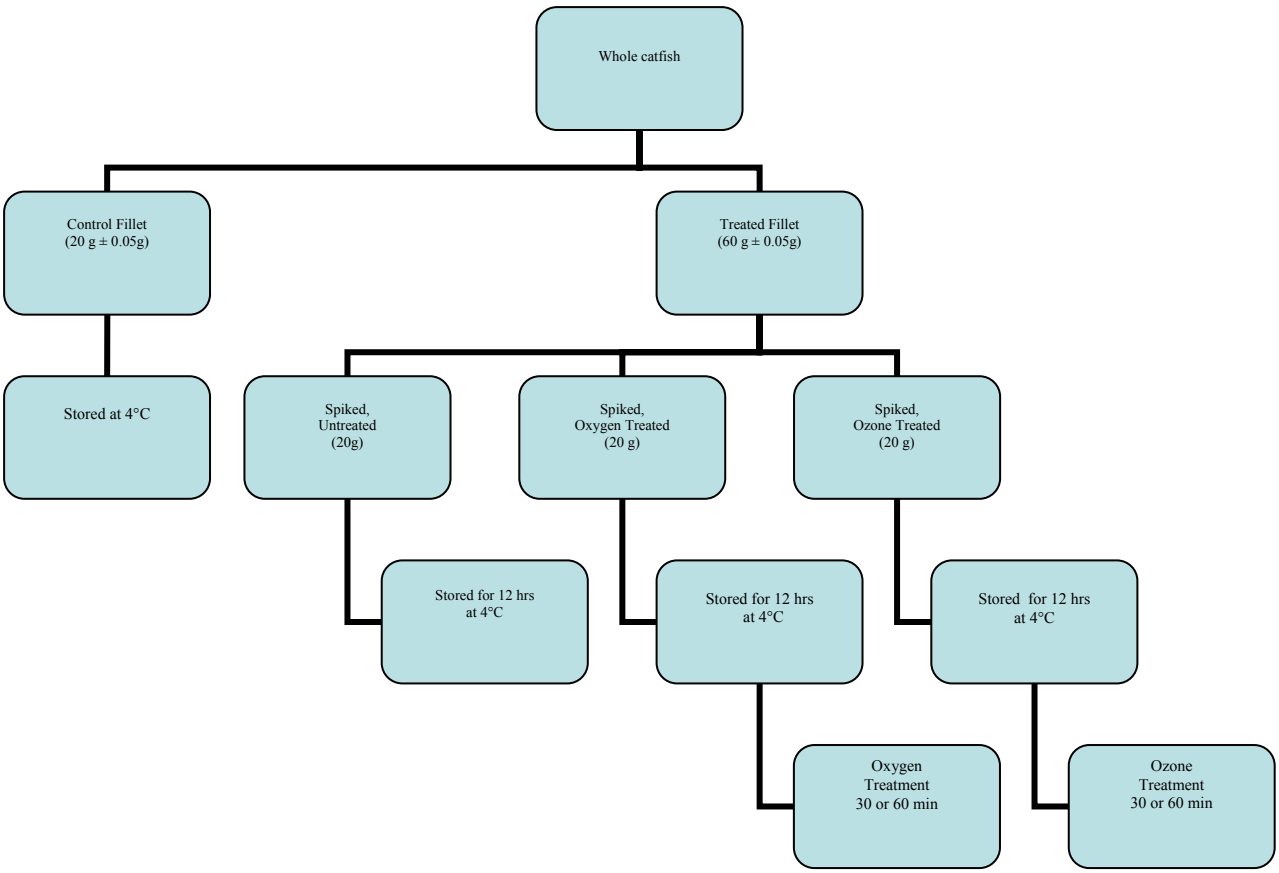

Figure 1 – Flowchart of Sample Preparation for Microwave Extraction

Samples being treated with oxygen or ozone were placed directly into a large plastic weigh boat and a plastic Ziploc bag labeled with a black permanent marker. The sample was then treated with oxygen or ozone gas (no off-flavors added). Samples spiked with off-flavors and treated with either oxygen or ozone were stored for at least 12 hours at 4ºC after preparation. The plastic tube that was attached to the oxygen tank (BOC Gases, Murray Hill, NJ) or ozone generator (Lynntech Inc., Lynntech, TX) was placed inside of the plastic bag and zipped closed. The sample remained in contact with the ozone or the oxygen gas for 10, 20 and 30 minutes for quality analyses and for 30 or 60 minutes for off-flavor analyses. Flow rate was set at approximately 175 sccm (standard cubic centimeter per minute) at 70ºF psig (pounds per square inch gauge). The weight percent of ozone was 16.42%, which was determined by first obtaining the absorbance and then using the following equation:

$$\text{Wt\% } O_3 = \frac{\text{Absorbance (254)} * 30}{(24.313 + \text{Absorbance})} \times 100$$

(Lynntech, Inc. 2001)

After treatment the sample was ready for microwave extraction.

Microwave Extractions

This procedure followed the method of Grimm et al. (2000). The Nitrogen tank (Airgas, Radnor, PA) was turned on and the flow meter was set to regulate at 80 psi. Ice was placed into an ice bath to help the distillate cool as it came out. Salt (Fisher Scientific, Hampton, NH) was placed in each vial with a premeasured scoop (3g) in order to help go from the liquid phase to the gas phase. Previously baked caps (Micro liter Analytical, Suwannee, GA) (120 minutes at 120ºC) were labeled with permanent black marker and a blender (Waring, Torrington, CT) was

set up. Once the materials were setup, 20g catfish fillet samples were blended. In between each sample the blender was rinsed with 1M HCl (Fisher Scientific, Hampton, NH), followed with hot water. The 20g homogenized sample was then placed into the glass bottom of the trap and the top was attached and clamped. The entire trap was then placed into a 150mL beaker (Pyrex, Corning, NY) that contained about 20 mL of deionized water. This was to help with the steam distillation that helped remove the analytes (off-flavors) from the less optimal matrix (fish) and placed it into an aqueous matrix (steam that contained the analytes). The trap was inserted into the microwave (Sharp, Mahwah, NJ) and clamped in. The sample was then microwaved for 3 minutes at a power level of 6 (60% power 700 watt oven). When the cycle was completed the distillate was checked to ensure that at least 10 mL was obtained. If not then deionized water in a squirt bottle was used to rinse residue from the glass apparatus until 10 mL was achieved. The 10 mL was then divided into 5 mL aliquots in separate vials (Micro liter Analytical, Suwannee, GA). The sample was then capped and crimped, ensuring that the cap was sealed correctly to avoid off-flavors from escaping. After the samples were completed they were refrigerated until analyzed by GC/MS.

Analysis of Samples

Off-Flavor Analysis

The method in which the samples were analyzed for off-flavors followed that of Grimm et. al 2000. Briefly the samples were heated up to 65ºC and the needle that contained a 2-cm long, divinylbenzene/carboxen/polydimethylsiloxane SPME fiber (Supelco, Inc, Bellefonte, PA) was inserted through the septum and into the headspace gas in the vial. The SPME fiber was then exposed to the headspace for 20 minutes while vigorously being stirred in order to absorb all of the off-flavors. Afterwards the solid phase was retracted back into the needle and inserted

into the injection port of the gas chromatograph (Agilent Technologies, Palo Alto, CA). The analytes were thermally desorbed (250ºC for 3 minutes) from the fiber and transferred onto the head of the capillary GC column. The compounds were then eluted from the column of the GC to the mass spectrometer (Agilent Technologies Palo Alto, CA).The MS was set to scan from m/z 33 to m/z 300, selectively storing ions using SIM (Selected Ion Monitoring). For example, the selective *m/z* values for MIB are 168, 135, and 95. A calibration curves were generated using MIB standards.

Quality Analysis

Color

The purpose of this test was to determine if any changes would be made to the color of the catfish fillets after being treated with oxygen and ozone. All catfish were purchased fresh the morning before the experiment. The catfish fillets were evaluated for L*, a*, b*, chroma, and hue angle using a Minolta Spectrophotometer CM-508d (Minolta Corporation Spectrophotometer, Ramsey, New Jersey). Color measurements for the untreated (control) samples were taken immediately and immediately after treatment for those that were treated. Each fillet was scanned in two different spots due to the differences in thickness.

Moisture and Fat

The purpose of these tests was to determine whether or not treatment with oxygen or ozone gas would alter the moisture and fat content of the catfish fillet. The catfish fillets were measured for moisture and fat simultaneously using a Smart System 5 Microwave Moisture/Solids Analyzer and the Fat Analyzing System 9001-3 (CEM Corporation, Matthews, NC) using option number 8, the raw meat method. Treated samples were treated as discussed earlier, however whole fillets were used for these analyses. The fillets were then blended. Two

aliquots of the same fillet were tested in order to see if there were any variations in moisture and fat due to the difference in fish thickness throughout the fillet. Dimethyl chloride was the solvent used to extract the fat from the samples.

Statistical Analysis

The data analysis was done using the Statistical Analysis System (SAS) software, version 8.0 (SAS Institute, Inc. Cary, NC). The Analysis of Variance (ANOVA) test with Tukey's studentized range (HSD) test was performed in order to determine the effects of ozonation on the reduction of off-flavors in catfish. The treatments were pure oxygen and ozone. The treatment times were 30 and 60 minutes for off-flavor analysis & 10, 20 and 30 minutes for quality analysis. The analysis used was a 2-tailed t-test ($\alpha = 0.05$).

RESULTS AND DISCUSSION

Off-Flavor

No MIB was detected in the unspiked, untreated fish fillets. Oxygen (100%) was used to determine whether the ozone would displace the off-flavors or actually remove them. There was no expectation of the oxygen removing any of the off-flavors. Ozone was however, expected to decrease the amount of off-flavors in the catfish, which it did according to ANOVA ($p \leq 0.0069$), but Tukey's HSD test, due to its conservative nature, could not distinguish differences between untreated and treated samples (Table 1). Treatment with ozone seemed to show an average reduction of 10.88% and 48.59% compared to control for 30 and 60 minute treatments, respectively (Table 1). We assumed that there would be a trend of the longer the treatment the more reduction that we would see however there was no trend whatsoever as far as time was concerned according to Tukey's HSD test.

Previous research has been aimed at removing MIB and GSM from water, as did Nalinakumari (2002). He discussed different processes to remove MIB and GSM from water, such as powder activated carbon, granular activated carbon, ozone and advanced oxidation process. Although free chlorine, potassium permanganate and chlorine dioxide have also been used they are only somewhat effective in the removal of odor and taste and do not prevent growth of algae. Research has shown that with free chlorine and chlorine dioxide at doses as high as 20 mg/L, less than 60% GSM and 35% MIB were removed from water. With potassium permanganate doses of 20 mg/L resulted in less than 10% MIB & GSM removal. However it was shown that ozone was effective in water with DOCs (Dissolved Organic Carbons) of 9 mg/L Ninety five percent removal of MIB & GSM was observed with ozone doses of 7 mg/L. With DOCs of 5 mg/L, 88% removal of MIB & GSM resulted with a 2.5 mg/L ozone dose.

Table 1: MIB (ppb) Content of Catfish Fillets Treated with Oxygen and Ozone

Treatment	Treatment Time (min)	Concentration (ppb)	%Reduction
None	0	$10.031^{a,b} \pm 3.56$	0
Oxygen	30	$15.052^{a} \pm 0.83$	0
Oxygen	60	$6.361^{b} \pm 2.05$	0
Ozone	30	$8.939^{a,b} \pm 1.31$	10.88
Ozone	60	$5.157^{b} \pm 1.16$	48.59

Means within a column with different letters are statistically different $\alpha = 0.05$, $n = 3$.

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MIB, GSM, and microcystin are resistant to conventional water treatment processes in drinking water (Ho 2004). These processes can lyse the cyanobacterial cells which results in a release of the metabolites in to the water. A combination of ozone and granular activated carbon has been proven to be effective in removing those metabolites from drinking water. Powder activated carbon (PAC) is used in Australia but it is very costly, granular activated carbon (GAC) is more economic and has a long life, have greater adsorptive capacity, easy process control, more efficient use of carbon and the ability to regenerate carbon from reuse. GACs are also an ideal habitat for bacterial growth because of the large surface area which provides additional removal mechanism of biological degradation. The only downfall is that NOMs (natural organic matter) in water can interfere with the adsorption of target compounds using GAC. In 1998, PAC was reported to have failed to remove increased levels of MIB and GSM in the Great Lakes in the US and in 2004 a study showed PAC could be bound up during coagulation decreasing the ability to remove MIB (Ho 2004). A similar study done by Kim et al in 1999, conducted a study and concluded that conventional water treatment followed by ozone/GAC was the most effective in MIB & GSM removal. Therefore ozone in combination of GAC is effective but ozone has to be followed by GAC.

Previous research proves that in order to have effective removal of MIB and GSM from water it is important to have organic material present. Since fish are organic we thought that MIB would be removed fairly easily; and since it was not we believe that ozone needs to be coupled with some other method due to the fact that the ozonation step is ineffective for absolute destruction of MIB and GSM in fish. This could be why we did not see more of a reduction in the levels of MIB.

Color

There was no effect of time on color with oxygen treatment at any time. However there were significant differences from the control for the ozone treated samples, but there is no trend to be seen for time. When looking at the ANOVA all treated samples were significantly different ($p \leq 0.0001$).

L*-values (lightness) ranged from 32.40 to 52.70 (Table 2). The ozone treated samples had significantly lower L*-values and were therefore darker than the control and the 10 and 20 minute oxygen treated samples (Table 2). However 30 minute oxygen also had significantly lower L*-values.

Color *a-values ranged from -1.50 to 7.10. The ozone treated samples had a significantly lower a*-value and were therefore greener than the control and the 10 and 20 minute oxygen samples (Table 2). The 30 minute oxygen treated samples had values that were higher (more red) than the control and other two oxygen samples.

b*-values ranged from -1.36 to 7.68. The control and 10 and 20 minute oxygen treated samples were bluer than the other samples which were more yellow. Chroma values ranged from 4.39 to 10.50. Chroma evaluates the purity of a color. There was no significant difference in chroma, except for the 30 minute oxygen which meant that it had the highest purity. Hue

values ranged from 79.23 to 341.93. Hue is the most dominant wavelength or shade. The hue angle was highest in the control and 10 and 20 minute oxygen treated samples, while the 30 minute oxygen was the lowest. All ozone treated samples had significantly lower hue angle values than the control.

In previous experiments it has been shown that ozone gas has had a whitening or bleaching effect and in others no effect. Ozone has a short half-life and is a strong oxidizer that has decoloration & deodorization effects (Jiang et. al. 1998). Due to this we would expect for the L value (lightness) to increase which would go from darker to lighter. Instead we saw darkening of samples which was shown by Nadas et al. (2003) who observed a darkening effect in strawberries stored in ozone atmosphere. They found opposite effects on chroma and hue values than ours. Sheldon & Brown (1986) found no significant difference in the loss of skin color of beef carcasses was seen when exposed to ozone. Kim et al. (1999) showed no significant difference in carcass color of beef contaminated with fecal matter from the control after being treated with <0.6 μg/L of ozone.

Chen et al (1997) successfully decolored horse mackerel mince washed with ozone within 10-20 minutes. The flow rate for 30 minutes was 10L/min giving the water a final ozone concentration of 2.1mg/L. Although treatment conditions were similar we did not see complete discoloration of the samples. Hoke et al. (2000) observed color values of L* -61.35, a* -1.87 and b* -9.48 for catfish frame mince. Our fillet samples treated with ozone had similar b* values, but were lighter and more green than the catfish frame mince.

Table 2 -Color Analysis of Channel Catfish Fillets After Oxygen and Ozone Treatment

Untreated					Treated					
L*	**a***	**b***	**chroma**	**hue angle**	**Treatment**	**L***	**a***	**b***	**chroma**	**Hue angle**
50.984 ±3.54	4.316 ±1.23	-1.143 ±0.64	4.495 ±1.24	344.743 ±8.21	**Control**	50.223[a] ±4.22	4.104[b] ±1.27	-1.570[d] ±0.55	4.429[b] ±1.25	337.916[a] ±9.80
50.286 ±6.08	4.913 ±1.55	-1.673 ±0.57	5.210 ±1.58	340.638 ±5.69	**10 min O2**	49.910[a] ±6.85	4.993[b] ±1.31	-1.609[d] ±0.59	5.269[b] ±1.34	341.933[a] ±5.60
51.528 ±1.94	4.009 ±0.90	-1.515 ±0.40	4.354 ±0.79	338.425 ±8.83	**20 min O2**	52.703[a] ±2.67	4.126[b] ±0.71	-1.360[d] ±0.59	4.399[b] ±0.57	341.083[a] ±10.02
33.621 ±2.39	6.866 ±2.46	7.109 ±1.21	9.949 ±2.46	47.295 ±7.52	**30 min O2**	32.411[c] ±1.85	7.104[a] ±2.18	7.688[a] ±1.11	10.504[a] ±2.26	48.266[d] ±5.40
40.895 ±1.89	0.860 ±1.25	3.496 ±1.59	3.830 ±1.43	70.891 ±26.75	**10 minO3**	43.344[b] ±3.21	-1.503[d] ±0.46	5.191[b,c] ±1.51	5.426[b] ±1.34	107.746[b] ±8.96
41.294 ±2.08	0.625 ±1.26	3.773 ±1.19	4.006 ±1.18	78.928 ±17.94	**20 minO3**	43.201[b] ±3.79	-1.356[d] ±0.89	4.975[c] ±1.35	5.263[b] ±1.15	106.709[b] ±12.61
31.713 ±2.31	8.201 ±2.40	8.509 ±0.68	11.929 ±1.79	46.975 ±8.28	**30 minO3**	38.811[b] ±0.91	1.320[c] ±1.08	6.806[a,b] ±1.59	6.008[b] ±2.46	79.234[c] ±9.56

Untreated = control with no treatment for each treated sample, Treated = treatment with 30 or 60 minutes of oxygen or ozone

Means within a column with different letters are statistically different $\alpha = 0.05$, $n = 8$.

L = visual lightness, white/black. a = luminous reflectance, red/green. b = luminous reflectance, yellow/blue

Moisture and Fat

Untreated fillets were used as controls for these experiments. The moisture content that we observed for the controls ranged from 75.15% to 78.92%. The moisture content of the treated samples ranged from 75.14% to 78.76% (Table 3). There were no major differences in moisture content. In a study by Forrester et al. (2002) moisture content increased significantly for all treatments especially with water or 0.5% citric acid and as acid increased the expressible moisture content increased because acid affects water holding capacity. The fillets soaked up moisture due to the addition of liquid. In our study moisture remained constant because we did not add any liquid.

Different fish may have different fat contents; therefore it would be hard to compare the controls to the treated samples. The fat content in the treated samples ranged from 4.04% to 7.79%. ANOVA showed that there were significant differences in both moisture and fat, but there were minimal differences seen in the Tukey grouping (Table 3). This was due to the fact that Tukey test is very conservative and differences are rarely seen. There was overlapping of the groups for the fat content, but no trends were observed.

Fat content has been shown to be variable in fish. In 2001, Robinson et al. studied the nutrient characteristics of pond raised channel catfish. After analyzing 50 market sized fish during May, October and February they determined that there was little variation in the fish from season to season, except for the fact that the fish from the fall contained higher levels of fat than those of spring & winter. They observed that the fat content varied from fish to fish, as did we. They attributed the variation to genetics, diet or feed intake. The fat content ranged from 1.9-10.9g per 100g of fish which is 1.9 -10.9% and the average mean was 5.4% with a standard deviation of 0.3. The moisture content ranged from 70.9-81.0g per 100g i.e. 70.9-81.0% and

averaged 77.3%. Interestingly in 1998, Dionigi et al. did a study in which the fat contents were as low as 4.45% but were as high as 30.45%. In our study there was a significant difference in the 30 minute oxygen and the 30 minute ozone samples from the control (Table 4), however no trends were seen for the effect of time.

Forrester et al. (2002) showed an increase in the fat content which was thought to be an indirect result of soluble protein loss since fat could not have been gained. Although in our research we found that ozone did not have any effect on fat, due to variation in fat levels between fish, contrasting studies have shown the opposite effect by decreasing the fat content. One method used in recycling water was to bubble ozone through in order to remove fat. Ozone has also been used in milk to oxidize major milk components. Ozone decreased fat content in condensates (80-230mg/L) by 96-98% and completely eliminated turbidity (Loorits et al 1975). There may have been oxidation of fat in our samples but due to potential variability between fish, but we were unable to identify any significant change.

Table 3: Percent Moisture Content of Catfish Fillets Treated with Oxygen and Ozone

Control (%)	Treatment	Treatment Time (min)	Treated (%)
78.088 ±1.41	None	0	75.230[a]±3.96
78.921±0.99	Oxygen	10	78.501 [a]±1.80
78.774±0.84	Oxygen	20	78.764 [a]±1.65
75.515±1.47	Oxygen	30	76.525 [a]±0.64
77.129±2.80	Ozone	10	75.994 [a]±3.21
76.016±1.16	Ozone	20	76.931 [a]±1.61
75.155±3.19	Ozone	30	75.145 [a]±3.25

Untreated = control with no treatment for each treated sample, Treated = treatment with 30 or 60 minutes of oxygen or ozone

Means within a column with different letters are statistically different $\alpha = 0.05$, $n = 8$.

Four fish were analyzed, two measurements were taken for each fish.

Table 4: Percent Fat Content of Catfish Fillets Treated with Oxygen and Ozone

Control (%)	Treatment	Treatment Time (min)	Treated (%)
6.413±0.67	None	0	6.986 [a,b]±1.10
7.460±3.14	Oxygen	10	7.795 [a]±3.92
6.938±0.66	Oxygen	20	7.410 [a]±0.94
4.325±0.54	Oxygen	30	4.048 [b]±0.58
6.583±2.04	Ozone	10	7.280 [a,b]±2.18
6.222±1.22	Ozone	20	6.481 [a,b]±1.62
4.190±1.96	Ozone	30	4.109[b]±2.45

Untreated = control with no treatment for each treated sample, Treated = treatment with 30 or 60 minutes of oxygen or ozone

Means within a column with different letters are statistically different $\alpha = 0.05$, $n = 8$.

Four fish were analyzed, two measurements were taken for each fish

CONCLUSION

During the course of this research it was shown that samples treated with oxygen had no significant effect on the reduction of off-flavors and treatment with ozone did as determined by ANOVA. It was also shown that moisture and fat content were not significantly affected by treatment with ozone, whereas color was. Some of the controls for these experiments already had variation that would make it extremely difficult to detect any changes and therefore no trends were seen with time of treatment. Also the fact that each fish was different to begin with made it more difficult to accurately see any significant differences using Tukey's HSD test. The fat and moisture content in fish is variable and no difference was able to be detected.

In the future catfish with lower levels of MIB should be evaluated as well as those that contain geosmin and a mixture of both. Also more replication needs to be done in order to see some type of trend. Further, researchers should consider using catfish that are naturally off-flavor. Also, research to compare the effectiveness of direct treatment with ozone gas versus the treatment with ozonated water would be very beneficial.

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APPENDIX 1

Off-Flavor SAS Output

---------- treatment=ox0 ----------

The MEANS Procedure

Analysis Variable : mib

Mean	Std Dev	N
10.03	3.56	6

---------- treatment=ox30 ----------

Analysis Variable : mib

Mean	Std Dev	N
15.05	0.83	3

---------- treatment=ox60 ----------

Analysis Variable : mib

Mean	Std Dev	N
6.36	2.05	3

---------- treatment=oz0 ----------

Analysis Variable : mib

Mean	Std Dev	N
10.03	3.56	6

---------- treatment=oz30 ----------

The MEANS Procedure

Analysis Variable : mib

Mean	Std Dev	N
8.94	1.31	3

---------- treatment=oz60 ----------

Analysis Variable : mib

Mean	Std Dev	N
5.16	1.16	3

---------- treat=Ozone ----------

The ANOVA Procedure

Class Level Information

Class	Levels	Values
treatment	3	oz0 oz30 oz60

Number of Observations Read 12
Number of Observations Used 12

---------- treat=Ozone ----------

The ANOVA Procedure

Dependent Variable: mib

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	48.1464642	24.0732321	3.12	0.0936
Error	9	69.4878278	7.7208698		
Corrected Total	11	117.6342921			

R-Square	Coeff Var	Root MSE	mib Mean
0.409289	32.53929	2.778645	8.539354

Source	DF	Anova SS	Mean Square	F Value	Pr > F
treatment	2	48.14646424	24.07323212	3.12	0.0936

---------- treat=Ozone ----------

The ANOVA Procedure

Tukey's Studentized Range (HSD) Test for mib

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	9
Error Mean Square	7.72087
Critical Value of Studentized Range	3.94850
Minimum Significant Difference	5.7825
Harmonic Mean of Cell Sizes	3.6

NOTE: Cell sizes are not equal.

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	treatment
A	10.031	6	oz0
A			
A	8.939	3	oz30
A			
A	5.157	3	oz60

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---------- treat=oxygen ----------

The ANOVA Procedure

Class Level Information

Class	Levels	Values
treatment	3	ox0 ox30 ox60

Number of Observations Read	12
Number of Observations Used	12

The SAS System

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---------- treat=oxygen ----------

The ANOVA Procedure

Dependent Variable: mib

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	114.6819469	57.3409735	7.06	0.0143
Error	9	73.1282187	8.1253576		
Corrected Total	11	187.8101656			

R-Square	Coeff Var	Root MSE	mib Mean
0.610627	27.49162	2.850501	10.36862

Source	DF	Anova SS	Mean Square	F Value	Pr > F
treatment	2	114.6819469	57.3409735	7.06	0.0143

The SAS System

15:16 Monday, October 3, 2005 8

---------- treat=oxygen ----------

The ANOVA Procedure

Tukey's Studentized Range (HSD) Test for mib

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	9
Error Mean Square	8.125358
Critical Value of Studentized Range	3.94850
Minimum Significant Difference	5.932
Harmonic Mean of Cell Sizes	3.6

NOTE: Cell sizes are not equal.

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	treatment
A	15.052	3	ox30

	A			
B	A	10.031	6	ox0
B				
B		6.361	3	ox60

The ANOVA Procedure

Class Level Information

Class	Levels	Values
treatment	6	ox0 ox30 ox60 oz0 oz30 oz60

Number of Observations Read	24
Number of Observations Used	24

The ANOVA Procedure

Dependent Variable: mib

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	182.9057196	36.5811439	4.62	0.0069
Error	18	142.6160465	7.9231137		
Corrected Total	23	325.5217661			

R-Square	Coeff Var	Root MSE	mib Mean
0.561885	29.77371	2.814803	9.453987

Source	DF	Anova SS	Mean Square	F Value	Pr > F
treatment	5	182.9057196	36.5811439	4.62	0.0069

The ANOVA Procedure

Tukey's Studentized Range (HSD) Test for mib

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	18
Error Mean Square	7.923114
Critical Value of Studentized Range	4.49442
Minimum Significant Difference	6.6676
Harmonic Mean of Cell Sizes	3.6

NOTE: Cell sizes are not equal.

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	treatment
A	15.052	3	ox30
A			
B A	10.031	6	ox0
B A			
B A	10.031	6	oz0

```
B     A
B     A          8.939       3     oz30
B
B                6.361       3     ox60
B
B                5.157       3     oz60
```

APPENDIX 2

Off-Flavor SAS Program

```
dm 'log;clear;output;clear';
data one;
input treat $  treatment  $  mib;
datalines;
oxygen      ox0   14.04852062
oxygen      ox0   11.59329803
oxygen      ox0   13.88771044
oxygen      ox0   6.838733343
oxygen      ox0   6.560841405
oxygen      ox0   7.255836707
oxygen      ox30  14.34925029
oxygen      ox30  15.96488037
oxygen      ox30  14.84233893
oxygen      ox60  8.048986976
oxygen      ox60  6.946895908
oxygen      ox60  4.086155201
Ozone oz0   14.04852062
Ozone oz0   11.59329803
Ozone oz0   13.88771044
Ozone oz0   6.838733343
Ozone oz0   6.560841405
Ozone oz0   7.255836707
Ozone oz30  10.4371239
Ozone oz30  8.031139388
Ozone oz30  8.348043136
Ozone oz60  3.820440365
Ozone oz60  5.804436094
Ozone oz60  5.846119747
;
proc sort;by treatment;
proc means mean std n maxdec=2;by treatment;
var mib;
proc sort; by treat;
proc anova; by treat;
class treatment;
model mib = treatment;
means treatment/tukey lines;
proc anova;
class treatment;
model mib = treatment;
means treatment/tukey lines;
run;
```

APPENDIX 3

Color SAS Output

---------- treat=10minO2 ----------

The MEANS Procedure

Variable	t Value	Pr > |t|
Ldiff	0.57	0.5868
Adiff	-0.18	0.8630
Bdiff	-0.41	0.6925
Cdiff	-0.13	0.8970
Hdiff	-0.55	0.6017

---------- treat=10minO3 ----------

Variable	t Value	Pr > |t|
Ldiff	-2.92	0.0225
Adiff	4.90	0.0018
Bdiff	-1.96	0.0911
Cdiff	-1.99	0.0864
Hdiff	-3.85	0.0063

---------- treat=20minO2 ----------

Variable	t Value	Pr > |t|
Ldiff	-1.71	0.1303
Adiff	-0.38	0.7176
Bdiff	-0.57	0.5852
Cdiff	-0.19	0.8533
Hdiff	-0.55	0.5977

---------- treat=20minO3 ----------

The MEANS Procedure

Variable	t Value	Pr > |t|
Ldiff	-2.27	0.0575
Adiff	3.82	0.0065
Bdiff	-2.04	0.0811
Cdiff	-2.54	0.0386
Hdiff	-3.46	0.0106

---------- treat=30minO2 ----------

Variable	t Value	Pr > |t|
Ldiff	0.97	0.3623
Adiff	-0.16	0.8751
Bdiff	-0.84	0.4292
Cdiff	-0.38	0.7170
Hdiff	-0.24	0.8138

---------- treat=30minO3 ----------

Variable	t Value	Pr > |t|
Ldiff	-9.19	<.0001
Adiff	6.78	0.0003
Bdiff	2.81	0.0262
Cdiff	4.76	0.0021
Hdiff	-7.16	0.0002

---------- treat=NoTreatm ----------

The MEANS Procedure

Variable	t Value	Pr > |t|
Ldiff	1.02	0.3396
Adiff	0.58	0.5791
Bdiff	1.40	0.2047
Cdiff	0.18	0.8640
Hdiff	1.80	0.1144

---------- treat=10minO2 ----------

The MEANS Procedure

Variable	Mean	Std Dev	N
Lcontrol	50.29	6.08	8
Acontrol	4.91	1.55	8
Bcontrol	-1.67	0.57	8
Ccontrol	5.21	1.58	8
Hcontrol	340.64	5.69	8
Ltreat	49.91	6.85	8
Atreat	4.99	1.31	8
Btreat	-1.61	0.59	8
Ctreat	5.27	1.34	8
Htreat	341.93	5.60	8

---------- treat=10minO3 ----------

Variable	Mean	Std Dev	N
Lcontrol	40.90	1.89	8
Acontrol	0.86	1.25	8
Bcontrol	3.50	1.59	8
Ccontrol	3.84	1.43	8
Hcontrol	70.89	26.75	8
Ltreat	43.34	3.21	8
Atreat	-1.50	0.46	8
Btreat	5.19	1.51	8
Ctreat	5.43	1.34	8
Htreat	107.75	8.96	8

---------- treat=20minO2 ----------

Variable	Mean	Std Dev	N
Lcontrol	51.53	1.94	8
Acontrol	4.01	0.90	8
Bcontrol	-1.52	0.40	8
Ccontrol	4.35	0.79	8
Hcontrol	338.43	8.83	8
Ltreat	52.70	2.67	8
Atreat	4.13	0.71	8

---------- treat=20minO2 ----------

The MEANS Procedure

Variable	Mean	Std Dev	N
Btreat	-1.36	0.59	8
Ctreat	4.40	0.57	8
Htreat	341.08	10.02	8

---------- treat=20minO3 ----------

Variable	Mean	Std Dev	N
Lcontrol	41.29	2.08	8
Acontrol	0.63	1.26	8
Bcontrol	3.77	1.19	8
Ccontrol	4.01	1.18	8
Hcontrol	78.93	17.94	8
Ltreat	43.20	3.79	8
Atreat	-1.36	0.89	8
Btreat	4.98	1.35	8
Ctreat	5.26	1.15	8
Htreat	106.71	12.61	8

---------- treat=30minO2 ----------

Variable	Mean	Std Dev	N
Lcontrol	33.62	2.39	8
Acontrol	6.87	2.46	8
Bcontrol	7.11	1.21	8
Ccontrol	9.95	2.46	8
Hcontrol	47.30	7.52	8
Ltreat	32.41	1.85	8
Atreat	7.10	2.18	8
Btreat	7.69	1.11	8
Ctreat	10.50	2.26	8
Htreat	48.27	5.40	8

---------- treat=30minO3 ----------

The MEANS Procedure

Variable	Mean	Std Dev	N
Lcontrol	31.71	2.31	8
Acontrol	8.20	2.40	8
Bcontrol	8.51	0.68	8
Ccontrol	11.93	1.79	8
Hcontrol	46.98	8.28	8
Ltreat	38.81	0.91	8
Atreat	1.32	1.08	8
Btreat	6.81	1.59	8
Ctreat	6.01	2.46	8
Htreat	79.23	9.56	8

---------- treat=NoTreatm ----------

Variable	Mean	Std Dev	N
Lcontrol	50.98	3.54	8
Acontrol	4.32	1.23	8
Bcontrol	-1.14	0.64	8
Ccontrol	4.50	1.24	8
Hcontrol	344.74	8.21	8
Ltreat	50.22	4.22	8

Atreat	4.10	1.27	8
Btreat	-1.57	0.55	8
Ctreat	4.43	1.25	8
Htreat	337.92	9.80	8

ffffffffffffffffffffffffffffffffffffffffffffffffffff

The ANOVA Procedure

Class Level Information

Class	Levels	Values
treat	7	10minO2 10minO3 20minO2 20minO3 30minO2 30minO3 NoTreatm

Number of observations 56

The ANOVA Procedure

Dependent Variable: Ltreat

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	2485.635671	414.272612	28.78	<.0001
Error	49	705.424150	14.396411		
Corrected Total	55	3191.059821			

R-Square	Coeff Var	Root MSE	Ltreat Mean
0.778937	8.551065	3.794260	44.37179

Source	DF	Anova SS	Mean Square	F Value	Pr > F
treat	6	2485.635671	414.272612	28.78	<.0001

The ANOVA Procedure

Dependent Variable: Atreat

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	517.4064679	86.2344113	56.10	<.0001
Error	49	75.3156875	1.5370548		
Corrected Total	55	592.7221554			

R-Square	Coeff Var	Root MSE	Atreat Mean
0.872933	46.18967	1.239780	2.684107

Source	DF	Anova SS	Mean Square	F Value	Pr > F
treat	6	517.4064679	86.2344113	56.10	<.0001

The ANOVA Procedure

Dependent Variable: Btreat

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F

Model	6	849.5935429	141.5989238	111.88	<.0001
Error	49	62.0150500	1.2656133		
Corrected Total	55	911.6085929			

R-Square	Coeff Var	Root MSE	Btreat Mean
0.931972	39.13511	1.124995	2.874643

Source	DF	Anova SS	Mean Square	F Value	Pr > F
treat	6	849.5935429	141.5989238	111.88	<.0001

The ANOVA Procedure

Dependent Variable: Ctreat

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	213.2297464	35.5382911	13.85	<.0001
Error	49	125.7215375	2.5657457		
Corrected Total	55	338.9512839			

R-Square	Coeff Var	Root MSE	Ctreat Mean
0.629087	27.15152	1.601795	5.899464

Source	DF	Anova SS	Mean Square	F Value	Pr > F
treat	6	213.2297464	35.5382911	13.85	<.0001

The ANOVA Procedure

Dependent Variable: Htreat

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	909558.8187	151593.1365	1805.84	<.0001
Error	49	4113.3619	83.9462		
Corrected Total	55	913672.1807			

R-Square	Coeff Var	Root MSE	Htreat Mean
0.995498	4.705858	9.162214	194.6980

Source	DF	Anova SS	Mean Square	F Value	Pr > F
treat	6	909558.8187	151593.1365	1805.84	<.0001

The ANOVA Procedure

Tukey's Studentized Range (HSD) Test for Ltreat

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha 0.05

```
Error Degrees of Freedom                 49
Error Mean Square                 14.39641
Critical Value of Studentized Range  4.34735
Minimum Significant Difference        5.8319


 Means with the same letter are not significantly different.

Tukey Grouping          Mean      N    treat

             A        52.703      8    20minO2
             A
             A        50.223      8    NoTreatm
             A
             A        49.910      8    10minO2

             B        43.344      8    10minO3
             B
             B        43.201      8    20minO3
             B
             B        38.811      8    30minO3

             C        32.411      8    30minO2
```


The ANOVA Procedure

Tukey's Studentized Range (HSD) Test for Atreat

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

```
Alpha                                 0.05
Error Degrees of Freedom                49
Error Mean Square                 1.537055
Critical Value of Studentized Range  4.34735
Minimum Significant Difference        1.9056


 Means with the same letter are not significantly different.

Tukey Grouping          Mean      N    treat

             A        7.1038      8    30minO2

             B        4.9938      8    10minO2
             B
             B        4.1263      8    20minO2
             B
             B        4.1038      8    NoTreatm

             C        1.3200      8    30minO3

             D       -1.3563      8    20minO3
             D
             D       -1.5025      8    10minO3
```


The ANOVA Procedure

Tukey's Studentized Range (HSD) Test for Btreat

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

```
Alpha                                 0.05
Error Degrees of Freedom                49
Error Mean Square                 1.265613
Critical Value of Studentized Range  4.34735
Minimum Significant Difference        1.7291


Means with the same letter are not significantly different.
```

Tukey Grouping	Mean	N	treat
A	7.6888	8	30minO2
A			
B A	6.8063	8	30minO3
B			
B C	5.1913	8	10minO3
C			
C	4.9750	8	20minO3
D	-1.3600	8	20minO2
D			
D	-1.5700	8	NoTreatm
D			
D	-1.6088	8	10minO2

The ANOVA Procedure

Tukey's Studentized Range (HSD) Test for Ctreat

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	49
Error Mean Square	2.565746
Critical Value of Studentized Range	4.34735
Minimum Significant Difference	2.462

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	treat
A	10.5038	8	30minO2
B	6.0075	8	30minO3
B			
B	5.4263	8	10minO3
B			
B	5.2688	8	10minO2
B			
B	5.2625	8	20minO3
B			
B	4.4288	8	NoTreatm
B			
B	4.3988	8	20minO2

The ANOVA Procedure

Tukey's Studentized Range (HSD) Test for Htreat

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	49
Error Mean Square	83.94616
Critical Value of Studentized Range	4.34735
Minimum Significant Difference	14.083

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	treat
A	341.933	8	10minO2
A			
A	341.083	8	20minO2
A			
A	337.916	8	NoTreatm
B	107.746	8	10minO3

B			
B	106.709	8	20minO3
C	79.234	8	30minO3
D	48.266	8	30minO2

APPENDIX 4

Color SAS Program

```
dm 'log;clear;output;clear';
data one;
input treat $ fishno $ Lcontrol Acontrol Bcontrol Ccontrol
Hcontrol Ltreat Atreat Btreat Ctreat Htreat;
Ldiff = Lcontrol-Ltreat;
Adiff = Acontrol-Atreat;
Bdiff = Bcontrol-Btreat;
Cdiff = Ccontrol-Ctreat;
Hdiff = Hcontrol-Htreat;
datalines;
NoTreatment 1a    49.9  5.9   -0.57 5.92  354.45      48.63 5.02  -1.74 5.31
      340.91
NoTreatment 1b    47.91 5.24  -2.17 5.67  337.49      49.75 4.47  -1.42 4.69
      342.41
NoTreatment 2a    49.83 3.47  -0.83 3.51  346.51      47.12 4.98  -1.49 5.2
      343.36
NoTreatment 2b    49.55 3.4   -1.62 3.76  334.55      47.49 4.56  -1.56 4.82
      341.13
NoTreatment 3a    50.94 4.43  -0.5  4.45  353.51      46.85 4.43  -2.57 5.12
      329.86
NoTreatment 3b    47    5.9   -1.68 6.13  344.06      48.17 5.01  -1.82 5.33
      339.99
NoTreatment 4a    56.45 3.36  -0.47 3.4   352.02      57.37 2.88  -0.59 2.94
      348.37
NoTreatment 4b    56.29 2.83  -1.3  3.12  335.35      56.4  1.48  -1.37 2.02
      317.3
10minO2     5a    54.8  3.95  -1.2  4.13  343.15      55.43 4.62  -0.64 4.66
      352.15
10minO2     5b    55.66 2.99  -1.47 3.33  333.75      54.53 4.17  -1.43 4.41
      341.11
10minO2     6a    54.46 5.28  -0.97 5.37  349.57      55.3  3.22  -1.27 3.46
      338.54
10minO2     6b    56.07 3.02  -1.18 3.24  338.7 55.48 4.17  -1.52 4.44
      339.95
10minO2     7a    50.34 5.77  -1.61 5.99  344.41      48.37 7.32  -1.7  7.51
      346.88
10minO2     7b    47.81 6.54  -2.24 6.91  341.07      50.99 5.88  -2.71 6.48
      335.25
10minO2     8a    42.33 7.16  -2.28 7.52  342.32      39.65 6     -1.67 6.23
      344.46
10minO2     8b    40.82 4.59  -2.43 5.19  332.13      39.53 4.57  -1.93 4.96
      337.12
20minO2     9a    52.3  4.9   -1.93 5.26  338.43      55.75 4.53  -0.81 4.61
      349.84
20minO2     9b    52.82 4.18  -1.8  4.55  336.68      52.88 3.08  -2.22 3.8
      324.2
20minO2     10a   51.27 4.49  -1.41 4.93  344.29      52.47 4.67  -1.57 4.93
      341.46
20minO2     10b   50.83 3.96  -1.04 4.1   345.32      55.54 4.23  -0.89 4.32
      348.06
20minO2     11a   49.52 2.46  -1.8  3.05  323.73      50.62 4.46  -0.49 4.48
      353.68
```

```
20minO2     11b   48.28 5.07  -0.81 5.14  350.9 47.54 4.82  -1.84 5.16
      339.07
20minO2     12a   54.08 4.05  -1.55 4.34  339.04      53.93 4.26  -1.34 4.47
      342.57
20minO2     12b   53.12 2.96  -1.78 3.46  329.01      52.89 2.96  -1.72 3.42
      329.78
30minO2     13a   36.01 4.09  5.45  6.81  53.1  31.67 8.08  8.63  11.82 46.88
30minO2     13b   35.83 4.96  5.56  7.45  48.29 29.96 9.73  8.7   13.05 41.82
30minO2     14a   34.1  9.85  7.73  12.52 38.12 35.59 5.38  6.64  8.54  50.99
30minO2     14b   36.44 3.51  6.76  7.61  62.53 30.47 8.91  8.43  12.27 43.41
30minO2     15a   30.65 9.68  9.03  13.24 43.01 32.14 6.76  7.74  10.28 48.87
30minO2     15b   31.32 7.54  7.46  10.61 44.73 32.55 9.08  8.67  12.55 43.67
30minO2     16a   30.98 8.45  7.96  11.61 43.3  32.65 5.17  6.84  8.58  52.93
30minO2     16b   33.64 6.85  6.92  9.74  45.28 34.26 3.72  5.86  6.94  57.56
10minO3     17a   42.98 -1.16 4.57  4.72  104.26      46.78 -2.02 4.11  4.58
      116.14
10minO3     17b   40.23 1.6   4.79  5.05  71.49 45.69 -1.57 5.81  6.02
      105.15
10minO3     18a   39.7  1.56  3.52  3.85  66.04 39.35 -1.39 4.75  4.95
      106.31
10minO3     18b   39.53 1.49  0.42  1.54  15.96 38.76 -1.05 4.48  4.6
      103.15
10minO3     19a   42.91 0.41  3.08  3.1   82.45 43.09 -0.83 8.07  8.17  95.86
10minO3     19b   43.5  0.74  2.09  2.21  70.47 47.47 -1.41 6.35  6.19  102.8
10minO3     20a   39.32 -0.47 4.67  4.69  95.74 42.78 -1.51 4.76  5
      107.62
10minO3     20b   38.99 2.71  4.83  5.53  60.72 42.83 -2.24 3.2   3.9
      124.94
20minO3     21a   42.84 -0.24 5.81  5.82  92.34 47.14 -2.68 3.77  4.62
      125.42
20minO3     21b   44.99 -1.73 4.51  4.83  110.98      48.34 -0.74 7.86  7.89
      95.4
20minO3     22a   39.73 0.51  2.07  2.13  76.14 43.08 -0.86 5.21  5.28  99.38
20minO3     22b   39.02 1.84  2.51  3.11  53.75 41.93 -2.17 3.69  4.28  120.4
20minO3     23a   42.34 0.15  3.11  3.11  87.27 46.15 -1.28 4.28  4.46
      106.63
20minO3     23b   41.92 0.81  3.86  3.95  78.17 42.33 -2.03 4.31  4.77
      115.18
20minO3     24a   40.26 1.53  4.07  4.35  69.4  38.34 -1.14 5.45  5.57
      101.79
20minO3     24b   39.25 2.13  4.24  4.75  63.37 38.3  0.05  5.23  5.23  89.47
30minO3     25a   30.03 5.91  8.07  10    53.75 37.86 2.64  6.67  7.18  68.42
30minO3     25b   30.31 8.47  7.75  11.48 42.48 37.72 1.98  8.09  8.33  76.23
30minO3     26a   36.41 6.25  9.76  11.58 57.37 39.69 2.52  8.62  0.98  73.73
30minO3     26b   33.79 5.55  7.72  9.51  54.29 38.61 0.39  8.18  8.19  87.26
30minO3     27a   30.49 11.42 8.39  14.17 36.3  38.16 1.77  4.22  4.58  67.26
30minO3     27b   29.6  9.5   8.79  12.94 42.76 40.13 -0.42 4.9   4.92  94.95
30minO3     28a   31.49 11.49 8.69  14.41 37.09 38.7  0.84  6.36  6.42  82.48
30minO3     28b   31.58 7.02  8.9   11.34 51.76 39.62 0.84  7.41  7.46  83.54
;
proc sort;by treat;
proc means t prt;by treat;
var Ldiff Adiff Bdiff Cdiff Hdiff;
proc sort; by treat;
proc means mean std n maxdec=2;by treat;
var Lcontrol Acontrol Bcontrol Ccontrol Hcontrol Ltreat Atreat Btreat Ctreat
Htreat;
```

```
proc anova;
class treat;
model Ltreat Atreat Btreat Ctreat Htreat = treat;
means treat/tukey lines;
run;
```

APPENDIX 5

Moisture and Fat SAS Output

---------- treat=None0 ----------

The MEANS Procedure

Variable	t Value	Pr > |t|
Moistdiff	1.89	0.1007
Fatdiff	-2.42	0.0458

---------- treat=Ox10 ----------

Variable	t Value	Pr > |t|
Moistdiff	0.72	0.4922
Fatdiff	-0.24	0.8142

---------- treat=Ox20 ----------

Variable	t Value	Pr > |t|
Moistdiff	0.02	0.9858
Fatdiff	-2.79	0.0269

---------- treat=Ox30 ----------

Variable	t Value	Pr > |t|
Moistdiff	-2.01	0.0849
Fatdiff	1.11	0.3025

---------- treat=Oz10 ----------

The MEANS Procedure

Variable	t Value	Pr > |t|
Moistdiff	1.81	0.1131
Fatdiff	-3.02	0.0193

---------- treat=Oz20 ----------

Variable	t Value	Pr > |t|
Moistdiff	-2.55	0.0380
Fatdiff	-0.26	0.8002

---------- treat=Oz30 ----------

Variable	t Value	Pr > |t|
Moistdiff	0.03	0.9783
Fatdiff	0.24	0.8173

---------- treat=None0 ----------

The MEANS Procedure

Variable	Mean	Std Dev	N

```
Mcontrol           78.09            1.41     8
Mtreat             75.23            3.96     8
Fcontrol            6.45            0.67     8
Ftreat              6.99            1.10     8
ffffffffffffffffffffffffffffffffffffffffffffff
```

---------- treat=Ox10 ----------

```
Variable            Mean         Std Dev     N
ffffffffffffffffffffffffffffffffffffffffffffff
Mcontrol           78.92            0.99     8
Mtreat             78.50            1.80     8
Fcontrol            7.65            3.14     8
Ftreat              7.80            3.92     8
ffffffffffffffffffffffffffffffffffffffffffffff
```

---------- treat=Ox20 ----------

```
Variable            Mean         Std Dev     N
ffffffffffffffffffffffffffffffffffffffffffffff
Mcontrol           78.77            0.84     8
Mtreat             78.76            1.65     8
Fcontrol            6.87            0.66     8
Ftreat              7.41            0.94     8
ffffffffffffffffffffffffffffffffffffffffffffff
```

---------- treat=Ox30 ----------

```
Variable            Mean         Std Dev     N
ffffffffffffffffffffffffffffffffffffffffffffff
Mcontrol           75.52            1.47     8
Mtreat             76.53            0.64     8
Fcontrol            4.32            0.54     8
Ftreat              4.05            0.58     8
ffffffffffffffffffffffffffffffffffffffffffffff
```

---------- treat=Oz10 ----------

The MEANS Procedure

```
Variable            Mean         Std Dev     N
ffffffffffffffffffffffffffffffffffffffffffffff
Mcontrol           77.13            2.80     8
Mtreat             75.99            3.21     8
Fcontrol            6.47            2.04     8
Ftreat              7.28            2.18     8
ffffffffffffffffffffffffffffffffffffffffffffff
```

---------- treat=Oz20 ----------

```
Variable            Mean         Std Dev     N
ffffffffffffffffffffffffffffffffffffffffffffff
Mcontrol           76.02            1.16     8
Mtreat             76.93            1.61     8
Fcontrol            6.31            1.22     8
Ftreat              6.48            1.62     8
ffffffffffffffffffffffffffffffffffffffffffffff
```

---------- treat=Oz30 ----------

```
Variable            Mean         Std Dev     N
ffffffffffffffffffffffffffffffffffffffffffffff
Mcontrol           75.16            3.19     8
Mtreat             75.15            3.25     8
Fcontrol            4.17            1.96     8
Ftreat              4.11            2.45     8
ffffffffffffffffffffffffffffffffffffffffffffff
```

The ANOVA Procedure

Class Level Information

Class	Levels	Values
treat	7	None0 Ox10 Ox20 Ox30 Oz10 Oz20 Oz30

Number of observations 56

The ANOVA Procedure

Dependent Variable: Mtreat

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	101.2817929	16.8802988	2.60	0.0290
Error	49	318.6785500	6.5036439		
Corrected Total	55	419.9603429			

R-Square	Coeff Var	Root MSE	Mtreat Mean
0.241170	3.323758	2.550224	76.72714

Source	DF	Anova SS	Mean Square	F Value	Pr > F
treat	6	101.2817929	16.8802988	2.60	0.0290

The ANOVA Procedure

Dependent Variable: Ftreat

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	118.4531000	19.7421833	4.44	0.0012
Error	49	218.0935125	4.4508880		
Corrected Total	55	336.5466125			

R-Square	Coeff Var	Root MSE	Ftreat Mean
0.351966	33.48086	2.109713	6.301250

Source	DF	Anova SS	Mean Square	F Value	Pr > F
treat	6	118.4531000	19.7421833	4.44	0.0012

The ANOVA Procedure

Tukey's Studentized Range (HSD) Test for Mtreat

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	49
Error Mean Square	6.503644
Critical Value of Studentized Range	4.34735
Minimum Significant Difference	3.9197

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	treat
A	78.764	8	Ox20
A A	78.501	8	Ox10
A A	76.931	8	Oz20
A A	76.525	8	Ox30
A A	75.994	8	Oz10
A A	75.230	8	None0
A			

A	75.145	8	Oz30

The ANOVA Procedure

Tukey's Studentized Range (HSD) Test for Ftreat

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	49
Error Mean Square	4.450888
Critical Value of Studentized Range	4.34735
Minimum Significant Difference	3.2427

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	treat
A	7.795	8	Ox10
A			
A	7.410	8	Ox20
A			
B A	7.280	8	Oz10
B A			
B A	6.986	8	None0
B A			
B A	6.481	8	Oz20
B			
B	4.109	8	Oz30
B			
B	4.048	8	Ox30

The ANOVA Procedure

t Tests (LSD) for Mtreat

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	49
Error Mean Square	6.503644
Critical Value of t	2.00958
Least Significant Difference	2.5624

Means with the same letter are not significantly different.

t Grouping	Mean	N	treat
A	78.764	8	Ox20
A			
B A	78.501	8	Ox10
B A			
B A C	76.931	8	Oz20
B A C			
B A C	76.525	8	Ox30
B C			
B C	75.994	8	Oz10
C			
C	75.230	8	None0
C			
C	75.145	8	Oz30

The ANOVA Procedure

t Tests (LSD) for Ftreat

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	49
Error Mean Square	4.450888
Critical Value of t	2.00958
Least Significant Difference	2.1198

Means with the same letter are not significantly different.

t Grouping	Mean	N	treat
A	7.795	8	Ox10
A			
A	7.410	8	Ox20
A			
A	7.280	8	Oz10
A			
A	6.986	8	None0
A			
A	6.481	8	Oz20
B	4.109	8	Oz30
B			
B	4.048	8	Ox30

APPENDIX 6

Moisture and Fat SAS Program

```
dm 'log;clear;output;clear';
data one;
input treat $ section Mcontrol Mtreat Fcontrol Ftreat;
Moistdiff = Mcontrol-Mtreat;
Fatdiff = Fcontrol-Ftreat;
datalines;
None0 1     76.83 70.58 7.23  8.61
None0 1     76.72 78.15 6.72  7.28
None0 1     80.17 72.87 6.13  5.64
None0 1     79.72 78.89 5.83  6.63
None0 2     77.85 69.21 7.56  8.26
None0 2     78.53 77.63 6.34  7.42
None0 2     78.55 74.99 6.04  5.78
None0 2     76.33 79.52 5.71  6.27
Ox10  1     80.66 80.15 12.35 14.55
Ox10  1     77.43 76.76 5.41  6.66
Ox10  1     79.18 75.28 8.82  6.4
Ox10  1     78.85 77.69 4.8   4.34
Ox10  2     79.44 79.86 11.75 13.42
Ox10  2     79.33 80.37 5.69  6.02
Ox10  2     77.97 79.27 8.12  6.21
Ox10  2     78.51 78.63 4.28  4.76
Ox20  1     80.54 80.92 5.94  6.18
Ox20  1     78.01 80.24 7.81  8.68
Ox20  1     78.85 78.04 6.59  8.1
Ox20  1     78.42 75.65 6.84  7.17
Ox20  2     79.21 79.45 6.08  6.38
Ox20  2     78.92 78.41 7.59  8.27
Ox20  2     77.9  79.51 7.05  7.83
Ox20  2     78.34 77.89 7.03  6.67
Ox30  1     75.21 76.92 4.11  3.6
Ox30  1     73.19 75.78 4.81  4.96
Ox30  1     77.23 76.27 3.59  4
Ox30  1     75.2  77.61 4.77  3.48
Ox30  2     75.47 76.63 4.09  3.71
Ox30  2     74.1  75.69 4.74  4.87
Ox30  2     77.54 76.32 3.62  4.13
Ox30  2     76.18 76.98 4.85  3.63
Oz10  1     78.83 80.59 4.83  4.24
Oz10  1     73.99 72.67 8.16  9.49
Oz10  1     80.14 76.35 4.39  6.33
Oz10  1     74.6  73.58 8.07  9.11
Oz10  2     79.28 80.12 4.11  4.63
Oz10  2     74.59 72.98 8.61  9.28
Oz10  2     80.5  77.82 5.02  6.2
Oz10  2     75.1  73.84 8.59  8.96
Oz20  1     77.59 79.57 5.92  4.02
Oz20  1     76.85 76.34 5.49  7.24
Oz20  1     74.64 76.01 7.97  6.5
Oz20  1     75.26 75.45 6.23  8.02
Oz20  2     76.85 79.31 5.29  4.15
Oz20  2     76.95 77.01 4.87  6.84
```

```
Oz20  2     75.2  75.78 8.24  6.68
Oz20  2     74.79 75.98 6.49  8.4
Oz30  1     70.53 71.4  3.17  2.27
Oz30  1     78.91 79.86 1.71  1.33
Oz30  1     75.38 74.29 5.49  6.39
Oz30  1     76.1  75.06 6.19  6.4
Oz30  2     70.64 71.36 3.22  2.34
Oz30  2     78.72 79.81 1.62  1.41
Oz30  2     74.65 74.17 5.68  6.36
Oz30  2     76.31 75.21 6.24  6.37
;
proc sort;by treat;
proc means t prt;by treat;
var moistdiff fatdiff;
proc sort; by treat;
proc means mean std n maxdec=2;by treat;
var Mcontrol Mtreat Fcontrol Ftreat;
proc anova;
class treat;
model Mtreat Ftreat = treat;
means treat/tukey lines;
means treat/LSD lines;
run;
```

VITA

The author was born in the Netherlands, Holland, on August 7, 1977. She completed her diploma at Center High School, Sacramento, California, in 1995. She attended Xavier University of Louisiana, New Orleans, Louisiana in 1995. In 2000, she completed her Bachelor of Science degree in biology. She returned to Sacramento and worked in the Quality Assurance department at Severn Trent Laboratories until she was accepted into the master's program in the Department of Food Science. In 2001, she worked temporarily in the Quality Assurance laboratory at Folgers Coffee Company until she began attending Louisiana State University and began research on ozone degradation of off-flavors in catfish. During the course of her studies she worked full-time at ITS Caleb Brett (Metairie, Louisiana) as a Chemist and more recently at Louisiana State University Health Science Center (New Orleans, Louisiana) doing cancer research. She is currently a candidate for the Master of Science in the Department of Food Science, Louisiana State University, which will be presented December 2005.