EFFECT OF FILTERED WOOD SMOKE PROCESSING ON SPOILAGE BACTERIA, PATHOGENIC BACTERIA AND SENSORY CHARACTERISTICS OF YELLOWFIN TUNA

By

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To my loving parents, my brother and especially my fiancée Gogce.

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LIST OF ABBREVATIONS

ANOVA	Analysis of Variance
AS	Artificial Smoke (A mixture of 21 % carbon monoxide, 18 % carbon dioxide, 1.1% oxygen and a balance of nitrogen)
CMVS	Color Machine Vision System
Ctrl	Control Group
FID	Flame Ionization Detector
FS	Filtered Smoke
FSHN	Food Science and Human Nutrition Department University of Florida
GC	Gas Chromatograph
SAS	Statistical Analysis Software

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Smoking is a very old technique for preserving fish, meat and dairy products and for enhancing their flavor. Currently the preservation aspect of “smoking fish” is often ignored since other more effective preservation methods, like freezing and refrigeration, have been developed. Today, most smoking applications target mainly the enhanced flavor aspect of smoking, rather then the increased shelf life of food products.

Filtered smoke processing is a new method that uses the preservation effect of smoking on fish and fish products without major changes in their sensory characteristics, like flavor or texture. The goal of this project was to study the effects of filtered smoke processing on spoilage and pathogenic bacteria, quality aspects of warm water fish species and to optimize the smoke treatment method.

Unlike most preservation techniques like freezing or refrigeration, filtered smoke also enhances the appearance of red muscle products such as tuna or mahi. This work also showed that color of the warm water fish species can be enhanced through filtered smoke treatment, especially in conjunction with refrigeration and freezing.

Fresh tuna steaks were treated with filtered and artificial smoke for 24 and 48 hours and then analyzed for 14 days for *Salmonella* spp. growth, total aerobic bacteria growth and changes in color, especially the redness of the samples. A similar study was conducted where the samples were frozen and stored at -20ºC for 30 days prior to analysis. The first two studies showed that there was nearly no affect of filtered or artificial wood smoke processing on the growth of Salmonella spp. for either the fresh or frozen stored samples. However, there appeared to be an inhibitory effect of both the filtered and the artificial smoke treatment on the growth of aerobic spoilage bacteria during the first 4 days of observation. This effect seemed to be enhanced in the samples that were stored at -20ºC for 30 days. However, these effects suggest no improvement in the shelf life of tuna as caused by any of the treatments. The color analysis confirmed the effectiveness of filtered smoke processing on the preservation of the color properties and appearance of the samples, especially after frozen storage. No significant differences could be seen between the filtered smoke and the artificial smoke treatments in any of the mentioned studies. Moreover in a sensorial taste panel no significant differences could be found based on odor and appearance between filtered smoke and artificial smoke treated tuna. To identify whether a product was treated with filtered or artificial smoke for the purpose of quality assurance, a rapid gas chromatography identification method was developed to quantify the amount of residual carbon monoxide in products and at the same time verify whether a product was treated with filtered smoke or the artificial counterpart by a chromatographic fingerprint.

CHAPTER 1
INTRODUCTION

Smoking fish, meat and dairy products is a very old technique that has been used as a method of long term preservation for over 600 years (Cutting 1961). Until other methods like refrigeration and freezing were developed and established, smoking fish and meat products was one of the most used preservation methods. The advantages of smoking food products are increased shelf life, enhanced flavor, and prevention of insect infestation. Although traditional fish smoking can still be found in practice today, the distinct boundaries of its use have vanished (Dillon and others 1994). In industrial countries, smoking of fish and meat products is used today mainly to enhance flavor and texture (Robinson 1983). Modern forms of smoke processing provide sometimes only little protection against microbial spoilage and especially cold smoked products can spoil as readily as non-smoked foods (Hsu and others 1979).

A new technique that uses filtered and compressed wood smoke to treat fish products by using a low temperature treatment process, attempts to use the preserving aspect of wood smoke on food products without changing their flavor or texture. Although most smoking applications today aim to change and enhance the flavor of a food product where it is accepted by the consumer, generally smoke flavor is not widely accepted. As with other flavor and taste expressions like spiciness, sweetness, rancidity or others, the range of likeability goes all the way from no smoke flavor over mild taste to very strong taste for smoked food products. With filtered smoke applications it is now possible to adjust the level of smoke flavor of a food product while at the same time increasing the antimicrobial effect by concentrating effective components.

This project aims to determine the antimicrobial effects of filtered wood smoke processing on fish products while assessing changes that occur in color, texture, taste and overall likeability. This study will determine the effects of filtered wood smoke treatment on the growth, inhibition

and reduction of *Salmonella* spp. The effects were studied on the natural fauna present when the fish was delivered and on inoculated.

Since the filtered smoke treatment is expected to maintain and enhance color properties, especially related to the red muscle parts, it is important to determine whether a product was treated with filtered smoke or just with industrial carbon monoxide, which is already proven to enhance the color of red muscle products. A part of this study was to develop and improve a rapid gas chromatography identification method, designated to determine whether a product was treated with filtered smoke or carbon monoxide. The method is based on a gas chromatography profile, which shows a combination of several natural gases, like methane, ethane, butane and pentanes, in different concentrations that produce chromatographic fingerprint for smoke treated products.

CHAPTER 2
LITERATURE REVIEW

Smoking of Fish and Seafood Products

Smoking fish and meat products is a very old food processing technique and has been used over a long time as a method of long term preservation of foods (Burgess and Bannerman 1963). Smoked foods are generally better preserved and protected against insects infestation than untreated products. Smoking fish and fish products can be divided into two distinct types, a hot smoking process and a cold smoking process.

Hot smoking involves cooking products during or immediately after the smoke treatment, usually in the same chamber, at temperatures around 70° to 80°C. Traditional hot smoking is actually a three step process. The product is first smoked between 30 and 60 minutes at 30°C to dry and toughen the skin. In general the more dry the surface of the products the more smoke flavor is absorbed into the product. The second step is the actual smoking step where the temperature is increased to about 50°C and the amount of smoke blown into the oven is raised. This is considered the smoking step. The last step is the cooking step where the product is cooked at temperatures between 70° and 90°C for about 1 to 2 hours (Dillon and others 1994).

For cold smoke processing, the difference to hot smoking is the temperature at which the product is smoked and finished. The purpose of cold smoking is to apply a smoky flavor to the product without cooking, so the product may be consumed raw, such as cold smoked salmon, or prepared as desired by the consumer. The products are smoked at temperatures below 30°C with an initial heavy smoke deposit into the chamber that is tapered off toward the end. For some species, like herring and mackerel, the temperature is increased after the smoking step to 40°C so that natural oils can come to the surface to provide the final product with a glossy appearance (Dillon and others 1994).

Filtered Smoke

Filtered Smoke treatment is a new technique to preserve meats and seafood by utilizing the preserving aspects of wood smoke and at the same time minimizing the introduction of flavor and odor giving compounds, which are filtered out of the smoke before it is used in the processing of the food product. The idea to use filtered smokes to preserve color and spoilage of seafood products started when modified atmospheric packaging became commercially available, and carbon monoxide, a main component in natural and filtered wood smoke, was used to enhance and preserve the fresh appearance of seafood (Otwell 2006).

Although the use of industrial carbon monoxide to treat meat and seafood products is still debated in many countries, the use of wood smoke has been acknowledged and accepted as a preservation method by competent health authorities in every nation of the world (Olson 2006). Filtered smoke is the next step in this long history of preservation that utilizes the preserving aspects of wood smoke (e.g. carbon monoxide and carbon dioxide) and minimizes or even eliminates unwanted changes in flavor, texture and taste to the product.

Compared to the traditional hot and cold smoking, with treatment temperature from 30°C (for cold smoking) up to 80°C (for hot smoking), the filtered smoke is applied to the product at 0-5°C. The whole process, as described by Olson (2006), involves the generation of wood smoke by burning wood chips in a smoke chamber without air intake, to ensure the incomplete combustion of the wood, leading to smoke, then run this smoke to a series of filters to remove particles such as tar, ash and soot and finally remove most of the odor and flavor compounds with and active carbon filter. This filtered smoke can be used immediately or stored in compressed gas cylinders for later use. The food products are then prepared for the filtered smoke treatment by filleting and steaking to increase the surface area, and then placed on racks and put into the smoking chamber. The chamber is then evacuated from the remaining air and the

filtered smoke is introduced into the chamber and replaced several times during the treatment, which can take from 2 to 48 hours.

The filtered wood smoked products show great advantages in their retail value, compared to untreated or naturally smoked products, due to the enhancement of their color, texture, odor and taste properties. The color of food products is one of the major aspects most consumers are interested. If the color of a piece of tuna is brown or grey instead of red or pink, as expected from a fresh product, the consumer will most likely reject the product. The carbon monoxide, present in filtered smoke, will bind to muscle heme proteins, which determine the color of the muscle based on their chemical state. Freezing or other long term storage and exposure to air (oxygen) will change the conformation of the heme protein, which results in brown to gray colors of the product. Carbon monoxide will bind to the heme protein and stabilizes it so that the color change due to long term storage and freezing can be prevented and the product looks appealing red and pink (Olson 2006).

The same mechanism also affects odor and taste of the product due to oxidative rancidity of the lipids in the fish muscle. Lipid degradation and the reactions of these degradation products lead to undesirable off-odors and flavors. It is believed that hemoglobin and myoglobin are key prooxidants that cause the oxidation and degradation of lipids that lead to off flavors and odors. By stabilizing the heme proteins with carbon monoxide, the oxidation of lipids and therefore the formation of off flavors and odors can be reduced. As a result, the fish product smells and tastes fresh longer (Kristinsson and others 2006b).

Some research has been done on the effect of carbon monoxide, one of the main components of filtered smoke, on the growth of microorganisms. Several studies have been conducted with various levels of carbon monoxide on red meat and seafood to investigate how

these treatments inhibit the growth of spoilage bacteria. Some recent studies compared the growth of total aerobic bacteria with different treatments, including a control group (no treatment), a 4% CO treatment, a treatment with 100% nitrogen to test the exclusion of oxygen, a treatment with 100% CO, a treatment with filtered smoke and a treatment with 18% CO. The results showed that the greatest reduction in total aerobic bacteria was noticed for the filtered smoke and the 18% CO treatment together with the 100% CO treatment (Kristinsson and others 2007). The 100% nitrogen treatment showed some bacterial count reduction, while the bacterial counts for the 4% CO treatment and the control group were increased. In a long term freezing study, the 18% CO and filtered smoke treatments showed an even higher bacterial count reduction (Kristinsson and others 2006b). Nevertheless, data on how filtered smoke and carbon monoxide affect the microbial flora of meat and seafood during fresh and frozen storage is limited and this area needs to be more investigated.

Bacteria and Other Microorganisms in Fish and Seafood

All fresh fish and fish products contain a natural variety of bacteria and spores (Jahncke and Herman 2001). These bacteria and spores are partially responsible for the spoilage of fresh fish and processed fish products (Gram and Huss 1996). Some might also be a potential hazard for human health because of their pathogenic properties (Jahncke and Herman 2001). The visible evidence of spoilage is usually the growth of molds and slimy bacterial colonies, but spoilage can also change the sensory characteristics of a food product, such as smell (off-odors) and taste (off-flavor) (Gram and Huss 1996). Cold-smoked fish products are usually ready-to-eat food products that have not received sanitizing or stabilizing heat treatment (Gram 2001b). Therefore pathogens and biological hazards are of particular concern for these products. Some potential hazards associated with cold-smoked fish products are: *Listeria monocytogenes*, *Clostridium botulinum* type E, *Salmonella* spp., biogenic amines, and parasites (Jahncke and Herman 2001).

Listeria Monocytogenes

Listeria monocytogenes is a widely distributed Gram-positive, food borne pathogen that naturally occurs in many raw food products. It can grow between 1-45°C and between 0 and 10% NaCl. High levels of *L. monocytogenes* in unheated ready-to-eat food products have been associated with listerioses (McLauchlin 1997). Since cold-smoked fish is a ready-to-eat product it has been linked to sporadic cases of listerioses (Gram 2001b). The traditional way of isolating these organisms involves the use of selective media such as PALCAM agar, blood agar with nalidixic acid agar, Oxford Listeria selective agar (LSA) and enrichment and pre-enrichment broths with incubation at 30°C for 48 h. Since the growth of *L. monocytogenes* may be hindered by other microorganisms, PALCAM agar and Oxford LSA have advantages over other media, because of their ability to reduce the presence of contaminating micro-organisms (Neamatallah and others 2003). To eliminate or control the growth of *L. monocytogenes* a variety of different treatments of raw and finished product have already been approved. Two treatments involve: (1) washing the raw fish with water that contains chlorine, and (2) treatment of the raw fish with calcium hydroxide solution (pH 12). Other treatment options include washing the raw fish with acidified sodium chloride solutions, ozone treatment, steam surface pasteurization and electrochemical brine tank treatments. For the finished product, freezing and addition of approved microbial growth inhibitors are options to stop or control growth of *L. monocytogenes* (Jahncke and others 2004).

Clostridium Botulinum

Clostridium botulinum is the name of a range of Gram-positive, anaerobic, spore forming bacteria that produce the botulinum neurotoxin. These bacteria and their specific neurotoxins have been divided into 7 types (A, B, C, D, E, F and G) based on their antigenic properties. The disease caused by these neurotoxins is called botulism. Generally botulism occurs rarely today.

However, the neurotoxins from *Clostridium botulinum* are still some of the most potent toxins known. For cold-smoked fish products, the Group II of *C. botulinum* and particular strains that produce the type E neurotoxin are a major concern (Gram 2001a).

Salmonella Spp.

Salmonella spp. can be carried by fish and shellfish which show no signs of disease. The contamination of this organism derives from terrestrial sources and fish may serve as a vector for *Salmonella* spp. (Novotny and others 2004) Especially shellfish from sewage-polluted waters seems to be a major problem. Although outbreaks of *Salmonella* spp. have occurred, processed seafood products are usually considered to present a lower risk (Heinitz and Johnson 1998).

Biogenic Amines

Biogenic amines are nitrogen compounds that can be expected in nearly all foods that contain proteins or free amino acids (Shalaby 1996). In particular, histamine and tyramine have been associated with some toxicological characteristics and outbreaks of food poisoning. The presence of biogenic amines above a certain level in non-fermented foods indicates the presence of undesired microbial activity and could therefore be used as an indicator of microbial spoilage. While not all biogenic amines correlate with the growth of spoilage organisms, histamine, putrescine and cadaverine levels usually increase during spoilage of fish and fish products (Santos 1996). Biogenic amines and particularly histamine have been also implicated as the causative agent in a number of scombroid food poisonings. It is also a concern that in cold-smoked products secondary amines such as putrescine and cadaverine can react with nitrite and form carcinogens (Flick and others 2001).

Parasites

Cold-smoked or cold-smoked and dried fish and fish products may contain human pathogenic parasites such as *Anisakis* spp. (a nematode or roundworm), *Diphyllobothrium* spp. (a

cestode or tapeworm) and *Nanophyetus salmincola*. The harvest of parasite free fish in the wild is very difficult. Some aquacultured fish, however, are considered free of parasites, since their diet and environment may be controlled. The most effective way to ensure that viable parasites are not present in cold smoked fish products is to freeze the raw fish prior to the smoking step for a prescribed time that assures destruction of all viable parasites in that fish (Bledsoe and Oria 2001).

Sensory Characteristics of Fresh and Smoked Seafood

For most people, fish and seafood is associated with the typical fishy smell that also surrounds fishing piers and fish markets. However, only few consumers know that this "fishy" smell is already a sign of degradation of the fish and seafood products. Most fresh fish and seafood products have no distinct smell or odor at all when caught and processed very fresh. Fish that has been stored for a couple of days or previously been frozen develops a slight fishy smell due to the oxidation of lipids (Olson 2006). Further degradation of fish and seafood then ultimately leads to very unpleasant fishy and spoiled smell that will tell the consumer that this product is spoiled and no longer consumable.

Odor and flavor is only one of the sensory characteristics consumers rely on when making a decision to buy or prepare fish and seafood. In fact, the most important characteristic is the color of the fish or seafood, since most consumers will make eye contact with the product the first time through a glass display, where the color and overall appearance is the only factor they can base their decision. A fresh red and pink color for most seafood products rich in dark muscle is preferred over the brown and gray color of long stored, untreated products. The third sensory characteristic is the texture and mouth-feel of fresh and processed seafood that determines if a customer is satisfied with the product.

All these sensory characteristics change dramatically when seafood products are processed using the hot or cold smoking technique with natural wood smoke. Hot and cold smoking introduces substantial amounts of flavor compounds into the seafood, which are identified by most consumers as smoky flavor and taste. While a long time ago smoking was discovered to be a good preservation method that also introduces flavor and taste to the products, today mostly the flavor effect is desired since other better preservation techniques are available. Hot smoking of fish and seafood results in a fully cooked product with intense smoky flavor and taste. Some forms of hot smoking also involve an intense drying step which removes so much water out of the product that it becomes shelf stable at room temperature without further preservation. Cold smoking is a less intense form of smoking and allows the smoky flavor and taste to be introduced into the product without cooking it. While the hot smoking process changes the color and texture of the product the cold smoking process leaves the color and texture of the seafood nearly unchanged if done at the right temperature (Burt 1988).

Compared to the traditional smoking processes, filtered smoke and carbon monoxide treatments are performed at refrigeration temperatures and have only minimal impact on the texture of the product. Also the taste and smell are nearly unchanged due to the treatment since carbon monoxide is tasteless and most of the taste and odor giving compounds have been removed from the filtered smoke. However, the treatments may have a long term preservation effect that exceeds the traditional smoking techniques. The taste and smell are not only unchanged, but further preserved for an extended period, since the carbon monoxide, which is also present in filtered smoke inhibits the lipid oxidation which causes off odors and tastes (Kristinsson and others 2006b). The color of fish and seafood products treated with filtered

smoke and carbon monoxide is enhanced and preserved during fresh and frozen storage, which increases the consumer acceptance and likeability (Olson 2006).

CHAPTER 3
OBJECTIVES

The hypothesis for this study is filtered wood smoke processing increases the shelf life and the quality of Yellowfin tuna by inhibiting pathogenic and spoilage bacteria without altering the sensory characteristics of the product. The aim of this experiment was to treat Yellowfin tuna with filtered wood smoke to determine whether spoilage and pathogenic bacteria are inhibited by this treatment, and to observe any significant correlation between the inhibition of spoilage bacteria and the growth and inhibition of pathogenic bacteria.

In the first part of this experiment Yellowfin tuna steaks were treated with filtered wood smoke at 0°C and for time periods of 24 and 48 hours. The growth or inhibition of spoilage and pathogenic bacteria was measured before and after treatment, on frozen storage, and also every second day of refrigerated storage up to a total storage time of two weeks. The second part of this experiment aimed to determine the significant changes in odor and color of the filtered smoke treated products compared to the artificial smoke treated products.

A final objective of this study was to design and validate a quick gas chromatography method to identify whether a product was treated with filtered smoke or just with carbon monoxide. The use of carbon monoxide is currently forbidden in Europe and strictly regulated in many other countries.

CHAPTER 4
PRELIMINARY STUDIES

Filtered wood smoke processing is a recently developed processing technique that is gaining widespread use throughout the globe. Only a few companies produce filtered smoked fish products so far and the market has not been fully developed yet. Since the filtered wood smoke processing is so new, not many studies have been done on this particular subject. Ongoing studies in our laboratory involve the determination of polycyclic aromatic hydrocarbons (PAH) in filtered wood smoke and treated products, developing a rapid analysis to identify products treated with filtered wood smoke and general identification of the major components of filtered wood smoke and their relevance to the process. We have also done work on the effect of filtered smoke on the quality of fish muscle, but limited to few treatment times and only a few properties of fish muscle.

Tuna Microbiology Study

Overall these results indicate that filtered smoke and artificial smoke seem to improve the shelf life of tuna, but more conclusive data over a longer period is required to confirm the hypothesis.

Figure 3-1 shows that colony forming units (CFU) count decreased immediately for all treatments, even for the control group, which might be an indication for a reduction in CFU/g due to the stress of handling the samples. After the treatment the control group, the 100% carbon monoxide and the 100% nitrogen group showed a larger increase in CFU/g, followed by the filtered smoke and the artificial filtered smoke treatments. The artificial smoke treatment showed overall the lowest bacterial count until day 6, while the filtered smoke treatment increased significantly in CFU/g after day 3. While conducting this study cross contamination might have occurred for one of the three monitored samples for the filtered smoke treatment, which might

explain this high value. All other treatments, 100% carbon monoxide, 100% nitrogen and control showed higher bacterial load until day 6 compared to the artificial smoke.

Figure 3-2 shows a similar initial effect for all treatments as seen in Figure 1. The CFU count decreased for all treatments, including the control group, after the treatments ended. Over the next six day the control group and the 100% nitrogen group increased the most in bacterial count. The filtered smoke treatment showed the smallest increase in bacterial load over the next six days, followed by the artificial smoke group and the 100% carbon monoxide group.

Figure 3-3 shows the CFU count for all treatments after 48 hours. The graph shows that there is no initial decrease in CFU count for any of the treatments, which could be because the treatment time of 48 hours allowed the microorganisms to leave the lag phase already during the treatment and so they started growing again. Still the control group, the 100% nitrogen group and the 100% carbon monoxide group showed initial increases in bacterial count followed by the filtered smoke and artificial smoke treatments. After six days all treatments reached a similar bacterial load and showed no difference.

A study was conducted to investigate the effect of carbon monoxide, carbon dioxide, nitrogen and filtered smoke on the overall bacterial growth on tuna steaks over one week. Tuna steaks were cut from a fresh whole tuna loin and tumbled for a minute in a sterile container to ensure equal bacterial load on the surface. Two steaks for each treatment were then chosen and placed into a decontaminated gas tight container. The following treatments were applied to the tuna steaks: control (no treatment), carbon monoxide (~100%), 100% nitrogen, filtered smoke, and "artificial filtered smoke" (18% CO, 21% CO_2 and 61% nitrogen). The steaks were held at 4°C for 16, 32 and 48 hours inside the decontaminated containers, which were filled with each gas, Samples were taken from each steak before and immediately after the treatments, as well as 1, 3

and 6 days after the end of the treatments. Each sample was then analyzed for the total bacterial count using “total plate count” Petrifilms from 3M Corp. Figures 3-1 to 3-3 show the average total bacterial count per gram for all treatments after 16, 32 and 48 hours of treatment.

Tuna Color Study

Tuna steaks were cut from one fresh tuna loin and placed into a gastight container where they were treated with carbon monoxide (~100%), 100% nitrogen, filtered smoke and “artificial filtered smoke” (18% CO, 21% CO_2 and balanced with nitrogen) for 16, 32 and 48 hours. The control group was exposed to the surrounding air. The tuna steaks were analyzed with a color vision machine for their color value, according to the L*a*b color system. Pictures were taken before (day -1), after treatment (day 0) and on day 1, 3 and 6 after the treatment and the average color value was determined and calculated by using the color vision machine and color vision software. The average L*-, a*- and b*- values were recorded for each sample. Since the major interest of this study is in the maintenance and enhancement of the red color of the tuna muscle, the a* value representing the redness of the sample as a positive number (negative represents greenness), were compared to determine how the redness of the tuna changes after the treatments. Figures 3-4 to 3-6 show the average a*- values (redness) of the untreated and treated samples over a time period of 6 days after treatment.

As shown above for 16, 24 and 48 hours the control (CTRL) as well as the filtered smoke (FS), the 100% carbon monoxide (100% CO) and the 18% carbon monoxide (18% CO) have increased a*-values immediate after the treatment. Only the sample treated with pure nitrogen (100% N_2) showed an immediate decline in the a*-values after treatment and then continued to decline over the period of 6 day, except for the 48 hour treatment where a slight increase in a*-value can be seen on day 1. Overall the nitrogen treatment seems to have a negative affect on the a*-values, which can be seen immediately after treatment. The control groups show for the 16

and 48 hour treatments increases in a*-values and unchanged a*-values for the 24 hour treatment. From day 1 to day 6 after the treatment the a*-values from the control group declined rapidly for the 16, 32 and 48 hour treatment. The filtered smoke, 100% CO and 18% CO treatments showed a slight increase in a*-values for the 16 and 32 hour treatment and a large increase in a*-values for the 48 hour treatment at day 0. These three treatments show stabilization in a*-values for the 16 hour treatment until day 1 and then a slow decline until day 6. The 32 and 48 hour treatment show stable a*-values until day 3 and then a decline at day 6. Overall the 100% CO treatment showed the highest improvement and stabilization in a*-values after the treatment and over the period of 6 days. The filtered smoke and the 18% CO treatment seemed to stabilize the a*-values nearly as good as the 100% CO treatment, especially over the extended period of 6 days. The lack of data points between these measurements makes it clear that more frequent measurements are needed to describe the color changes due to filtered smoke and carbon monoxide treatments.

Identification of Filtered Smoke Treated Products

A study was conducted to identify gas compounds that were typical for filtered wood smoke. A gas chromatograph from Agilent (6890N) was equipped with a packed column and a nickel catalyst tube to convert carbon monoxide and carbon dioxide into methane. The nickel catalyst was needed to detect carbon monoxide and carbon dioxide with the FID (Flame ionization detector), which are otherwise not detectable. 100% carbon monoxide, filtered wood smoke and unfiltered fresh generated wood smoke were directly injected into the gas chromatograph (GC) and analyzed. The chromatograms showed a large number of peaks for the smoke samples and only one peak for the carbon monoxide sample as expected. Natural gas and refinery gas standards from Scott Specialty Gasses were then analyzed in the GC with the same method as the smoke samples before.

The previous results led to the idea to identify smoke treated products by analyzing gas compounds that are evaporated from smoked products. An experiment was conducted where tuna steaks were treated with 100% carbon monoxide, filtered smoke and natural wood smoke. A portion of 10 g of sample from each treatment was transferred into a 60 ml vial with a gas tight lid with septa inlet. The samples were heated in a water bath at 100°C for 5 minutes. After the heat treatment a sample of 100 µl was taken from the headspace of the vials with a gas tight syringe and injected into the GC. The results show that smoke treated products show two significant peaks that were not present in products that were untreated or treated with carbon monoxide, nitrogen and carbon dioxide. Note that carbon monoxide and carbon dioxide can not be detected directly by using the FID (Flame Ionization Detector), therefore these components will be methanized (transformed into methane) after separation on the column, which can then be detected by the FID. Figures 3-7 to 3-11 show the chromatograms collected from this study.

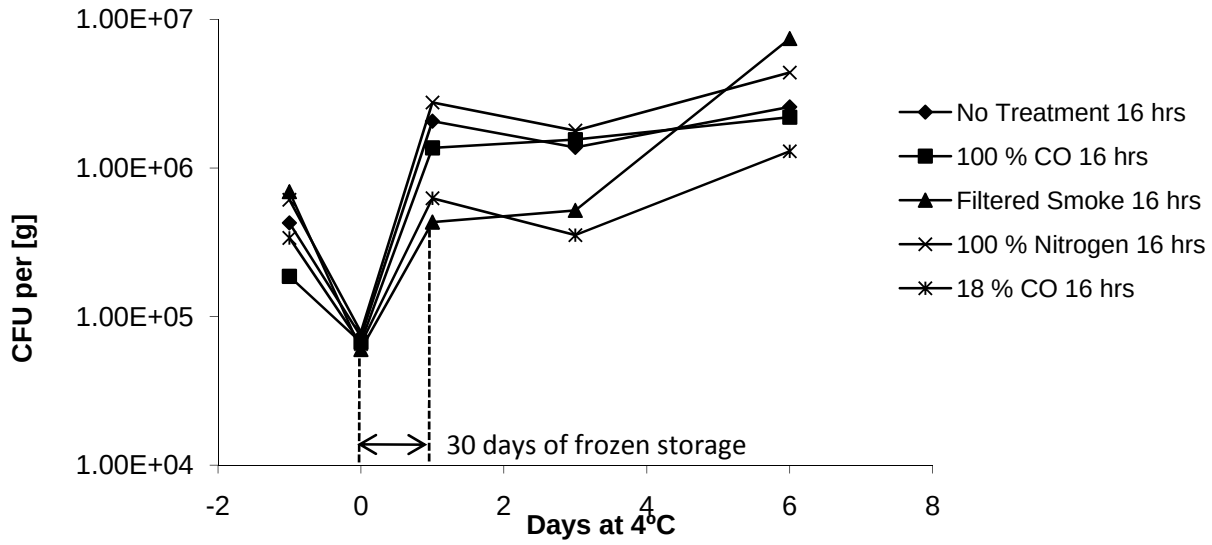

Figure 3-1. Tuna treated for 16 hours with 100% CO, filtered smoke, 100% nitrogen and a mixture of 18% CO with 21% CO_2 balanced with nitrogen. The control group was exposed to the surrounding air only. The graph shows the measurement of colony forming units (CFU) at day -1 (before treatment), day 0 (right after treatment) and then day 1, 3 and 6 after treatment.

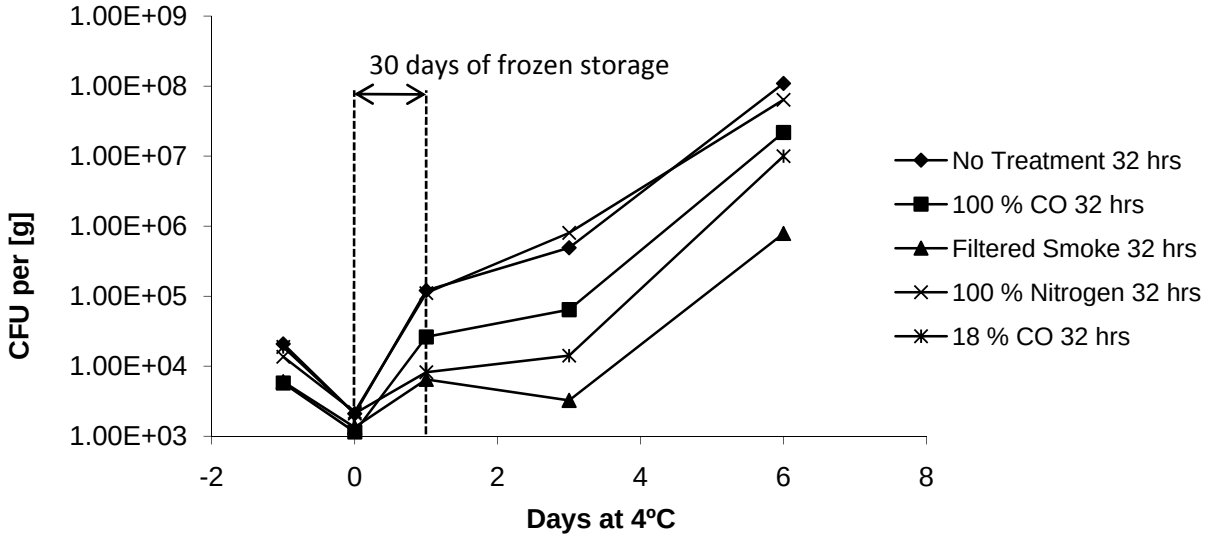

Figure 3-2. Tuna treated for 32 hours with 100% CO, filtered smoke, 100% nitrogen and a mixture of 18% CO with 21% CO_2 balanced with nitrogen. The control group was exposed to the surrounding air only. The graph shows the measurement of colony forming units (CFU) at day -1 (before treatment), day 0 (right after treatment) and then day 1, 3 and 6 after treatment.

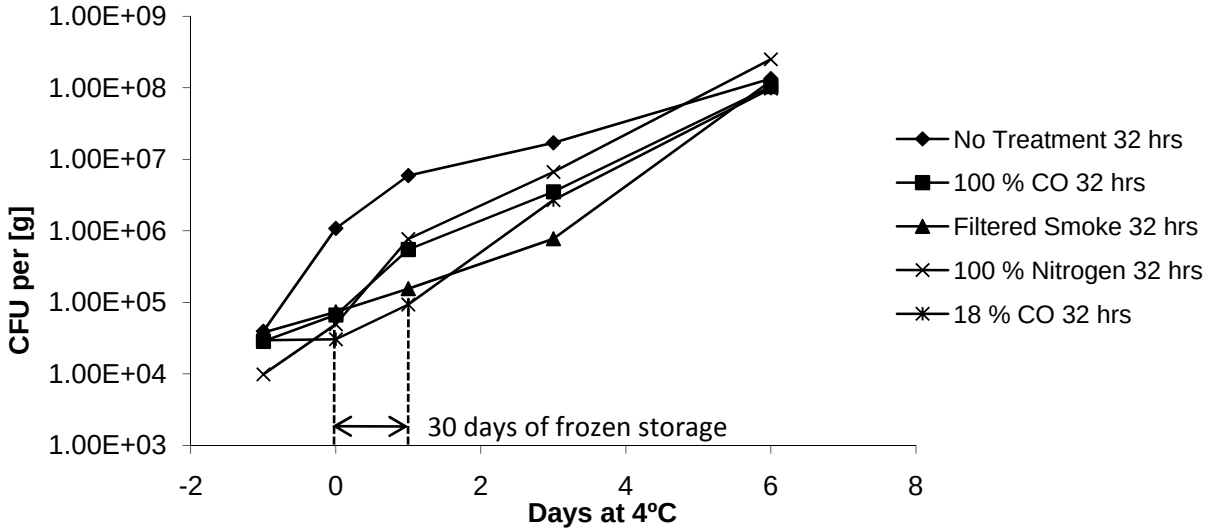

Figure 3-3. Tuna treated for 48 hours with 100% CO, filtered smoke, 100% nitrogen and a mixture of 18% CO with 21% CO_2 balanced with nitrogen. The control group was exposed to the surrounding air only. The graph shows the measurement of colony forming units (CFU) at day -1 (before treatment), day 0 (right after treatment) and then day 1, 3 and 6 after treatment.

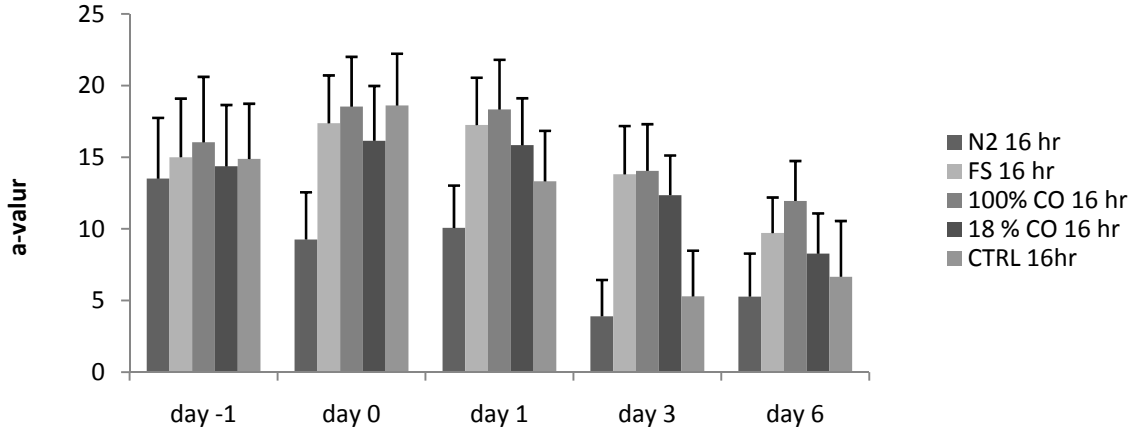

Figure 3-4. Tuna treated for 16 hours with 100% CO, filtered smoke(FS), 100% nitrogen(N_2) and a mixture of 18% CO with 21% CO_2 balanced with nitrogen. The control group (CTRL) was exposed to the surrounding air only. The graph shows the measurement of average a-value at Day -1 (before treatment), Day 0 (right after treatment) and then Day 1, 3 and 6 after treatment.

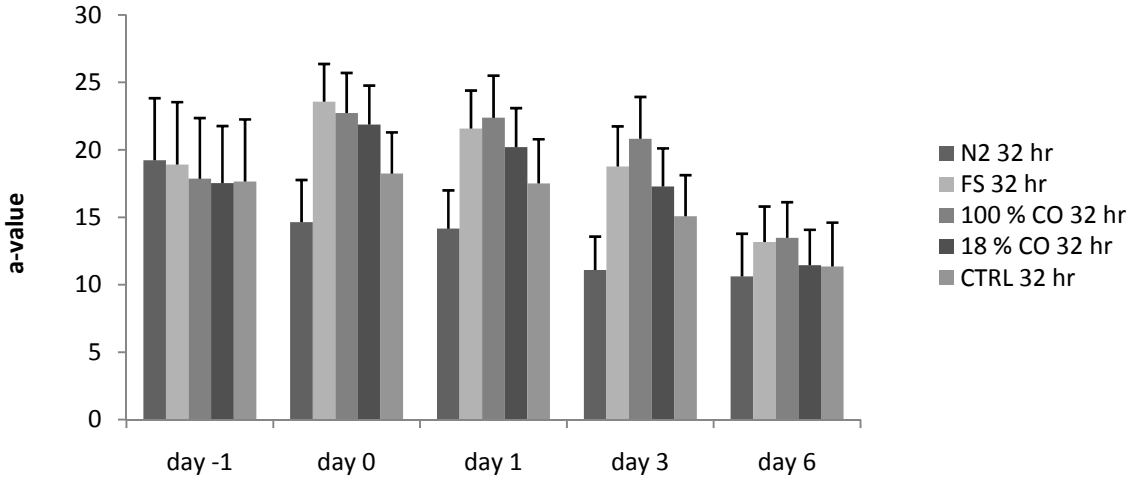

Figure 3-5. Tuna treated for 32 hours with 100% CO, filtered smoke(FS), 100% nitrogen(N_2) and a mixture of 18% CO with 21% CO_2 balanced with nitrogen. The control group (CTRL) was exposed to the surrounding air only. The graph shows the measurement of average a-value at Day -1 (before treatment), Day 0 (right after treatment) and then Day 1, 3 and 6 after treatment.

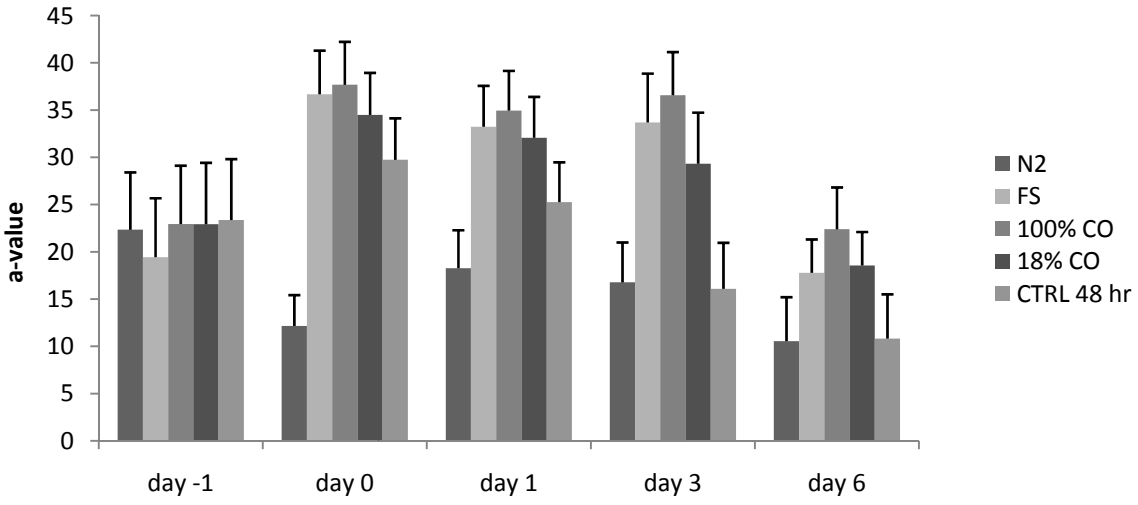

Figure 3-6. Tuna treated for 48 hours with 100% CO, filtered smoke(FS), 100% nitrogen(N_2) and a mixture of 18% CO with 21% CO_2 balanced with nitrogen. The control group (CTRL) was exposed to the surrounding air only. The graph shows the measurement of average a-value at Day -1 (before treatment), Day 0 (right after treatment) and then Day 1, 3 and 6 after treatment.

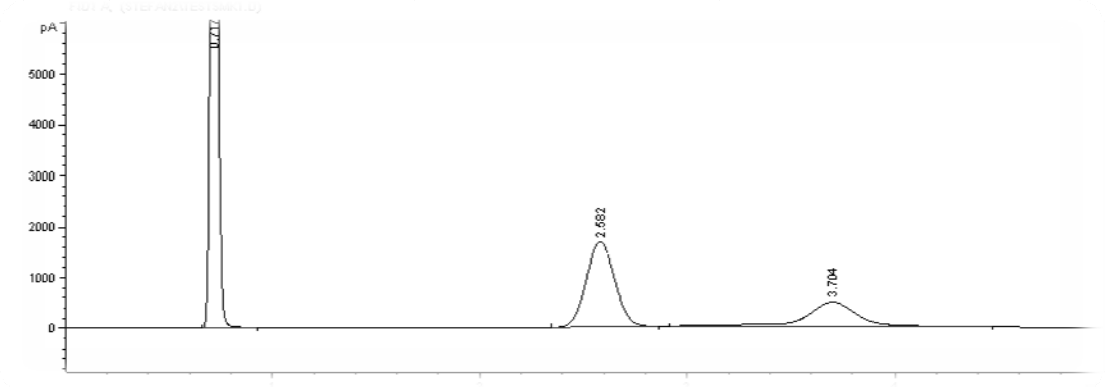

Figure 3-7. Chromatogram of the analysis of Clearsmoke™. The first peak represents carbon monoxide at around 0.7 minutes, the second peak represents methane at around 2.5 minutes and the last peak represents carbon dioxide at around 3.7 minutes

Figure 3-8. Chromatogram of the analysis of industrial carbon monoxide (~100% CO). This peak represents carbon monoxide at around 0.7 minutes. The sample was diluted to 0.1% carbon monoxide in air v/v.

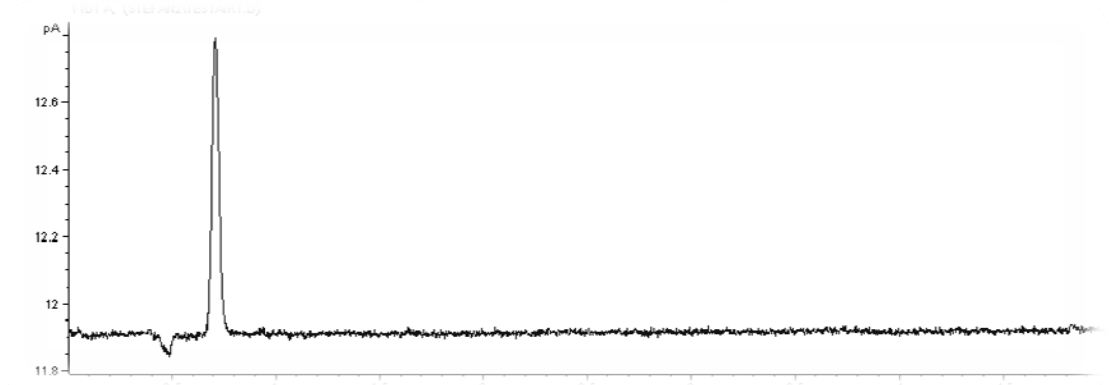

Figure 3-9. Chromatogram of the analysis of atmospheric air. The shown peak represents a trace amount of carbon monoxide residual in the air. Nitrogen and oxygen are not detectable by the FID.

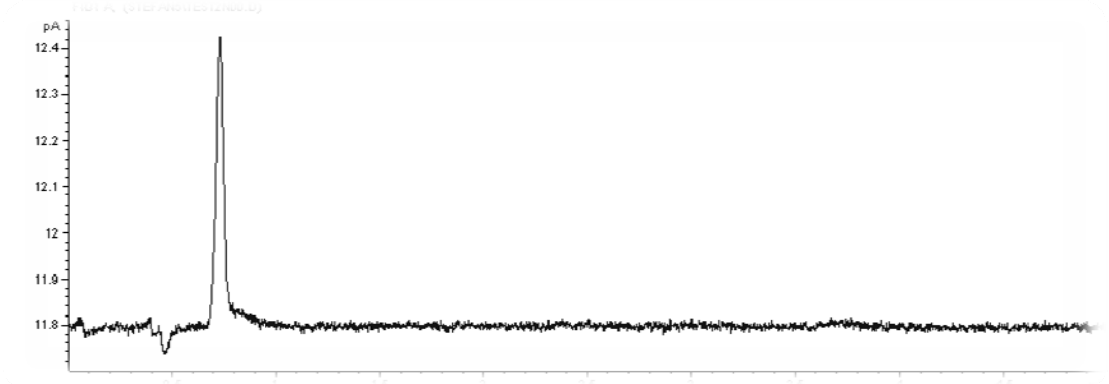

Figure 3-10. Chromatogram of the analysis of nitrogen (N_2). This peak also represents trace amounts of carbon monoxide. Nitrogen and oxygen are not detectable by the FID.

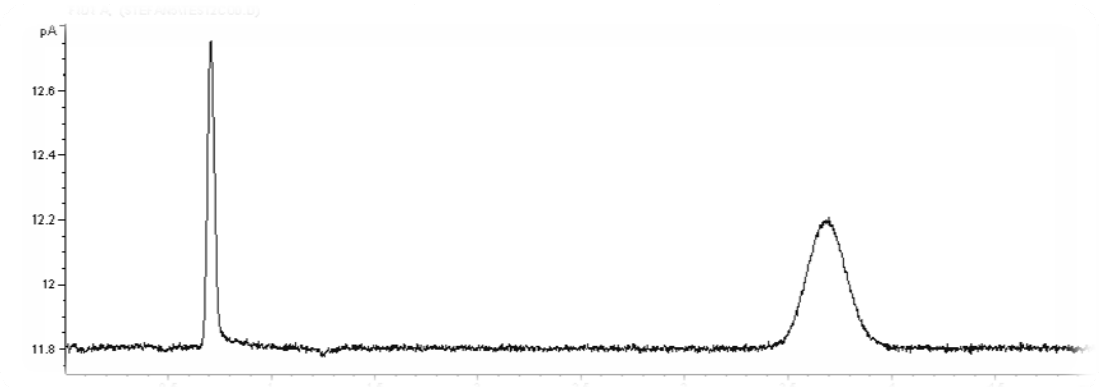

Figure 3-11. Chromatogram of the analysis of carbon dioxide (CO_2) The first peak again show a small amount of carbon monoxide sample and the second peak represents carbon dioxide.

CHAPTER 5
MATERIAL AND METHODS

Fresh and Frozen Storage

This project is mainly divided into two parts, a fresh storage and a frozen storage study. Figure 5-1 shows a scheme for the project plan. For the frozen storage study the samples were prepared as described in "Sample Preparation and Treatments" and each sample was then vacuum sealed in a Foodsaver® Bag, placed in the freezer and then stored for 30 days at -20ºC. Samples for the frozen storage study were thawed out two days prior to the start of the experiments at 4ºC in the cold room. The frozen storage samples were not analyzed during the frozen storage, but rather after the frozen storage in the same manner as the samples for the fresh storage study.

For the fresh storage study the samples were prepared as described in "Sample Preparation and Treatments" and every sample was then transferred into a ZipLoc® bag and stored in the cold room at 4ºC for the remainder of the experiment.

Fresh and frozen storage studies were timed in a manner that fresh and frozen samples could be analyzed together at the end of the frozen storage period. For the *Salmonella*, Total Aerobic Plate Count and the Color-Study, the samples were analyzed before the treatment (day -1), immediately after the treatment (day 0) and then at day 1, day 2 and every other day after that for a total of 14 days. For the frozen storage studies Day 1 represents the day when the thaw out process was completed, while it represents the actual first day after treatment for the fresh storage studies. For the taste panel and GC- experiments only samples representing day 1 after treatment or thaw out were chosen and analyzed.

Sample Preparation and Treatments

For all studies except the taste panel study samples were prepared in duplicates per treatment. For all studies fresh yellowfin tuna was ordered from Save-On Seafood (Tampa, Fl) and was processed and treated immediately upon delivery. For all studies the workplace was cleaned and sanitized with 70% ethanol v/v in water to minimize microbial cross contamination. The fully trimmed tuna loins were cut into 2.5 cm thick steaks, which were again trimmed and then sorted for uniformity, especially for the color and taste panel studies. All samples were then transferred into the prepared Foodsaver bags. From a roll 23 by 28 cm bags were cut and sealed on three sites. The bags were then equipped with a silicon septum valve recovered from PVF gas sampling bags from LabPure® Instruments. Each bag filled with one tuna steak was individually vacuum sealed and then stored on ice until treatment. For the *Salmonella* study the samples were inoculated with *Salmonella enteriditis* as described in the chapter "*Salmonella* study". Each bag was then filled with three liters of the designated treatment atmosphere except for the control which was kept vacuum sealed. The treatment gases were injected directly from the gas cylinders via a hose, equipped with a pressure gun and a needle, thru the septa valve into the sample bags. The amount of gas introduced to each bag was controlled with a gas flow meter from "Alicat Scientific" Model M-50SLPM-D which was set to Air mode since this is the closest representation of the treatment gases in terms of density. The actual volume of each treatment gas was not too important in these experiments, but rather the fact that each bag was filled with the same amount of treatment gas to ensure uniformity among the treatments.

The filtered smoke was delivered in compressed gas cylinders and ready to use for the treatments. The filtered smoke was delivered with a certified analysis stating that it contained 21% carbon monoxide, 18 % carbon dioxide, 55% nitrogen and is balanced with oxygen and other gases. Trace components from the incomplete combustion of wood chips that were not

filtered out were not analyzed or determined. According to the typical treatment times used in the industry it was chosen to determine the effect of a 24 and a 48 hour treatment of filtered smoke on tuna. The second treatment gas that was used in these experiments was a pure mixture of 21 % carbon monoxide, 18 % carbon dioxide, 1.1% oxygen and a balance of nitrogen. This mixture was chosen since it represents the exact composition of the filtered smoke without the trace components that are possibly found in filtered wood smoke. This mixture was called artificial smoke and will be abbreviated as AS. The original filtered smoke will be abbreviated as FS. To compare AS to FS equal treatment times (24 and 48 hours) were chosen for the AS treatments as well. The control group for all experiments consists of tuna steaks which were vacuum-packed and sealed and then kept on ice for 24 hours prior to analysis. Table 4-1 shows an overview over the treatment combinations that were applied to all studies.

Table 5-1. Treatment combinations used in all studies of this project

Label	Treatment Gas	Treatment Time	Storage Conditions
FS24F	Filtered smoke (FS)	24 hours	30 days at -20ºC
FS48F	Filtered smoke (FS)	48 hours	30 days at -20ºC
AS24F	Artificial smoke (AS)	24 hours	30 days at -20ºC
AS48F	Artificial smoke (AS)	48 hours	30 days at -20ºC
CtrlF	No gas treatment	24 hours	30 days at -20ºC
FS24	Filtered smoke (FS)	24 hours	1 day at 4ºC
FS48	Filtered smoke (FS)	48 hours	1 day at 4ºC
AS24	Artificial smoke (AS)	24 hours	1 day at 4ºC
AS48	Artificial smoke (AS)	48 hours	1 day at 4ºC
Ctrl	No gas treatment	24 hours	1 day at 4ºC

Salmonella **Study**

To study the effect of filtered and artificial smoke on the inhibition or growth of *Salmonella* spp. tuna steaks were prepared as described in “Sample preparations and treatments” with 4 steaks per treatment. Two steaks per treatment were inoculated with *Salmonella enteriditis*, which was obtained from the American Type Culture Collection (ATCC® #13076). The culture was prepared and enriched according to the instructions given by the ATCC® in

Nutrient Broth. For each steak 1 ml of culture was spread evenly over the entire steak and the bag was shaken for 1 minute to ensure an even distribution of the culture over the tuna steak. For the *Salmonella* analysis a cube of 10 g was cut from the each inoculated tuna steak in a manner that the cube consists of three surfaces exposed and three internal exposed sites to ensure an even distribution of microorganisms each time the steaks were sampled. The two other steaks were kept non-inoculated to see the effect of the treatments on the naturally existing *Salmonella* spp. flora. These steaks were sampled in the same manner as the inoculated samples. The non-inoculated samples were sampled at day -1, day 0, day 1 and day 2 from that point it was decided to abandon the non-inoculated study, since no *Salmonella* growth could be identified. These samples were analyzed a last time at day 14 to ensure that there was actually no *Salmonella* growth and the decision to abandon this study was correct. The inoculated steaks were sampled right after the inoculation but before the treatment (Day -1), directly after the treatment (Day 0) and at Day 1, 2 and then every other day until day 14. During this time the steaks were stored in Ziploc® Bags at 4ºC in a cold room.

Each 10 g samples for each analysis were placed into a sterile Nasco® Whirlpack bag and 90 ml of Hardy Diagnostics® Dilu-LOK II Phosphate buffer with magnesium chloride (buffer solution) added to the sample. The sample was squeezed and mixed by hand inside the bag with the buffer solution. Three Dilutions were prepared by pipetting 10 ml of the sample solution into 90 ml of sterile buffer solution. More Dilutions were prepared if the results from the previous day indicated that the first three dilutions would have to numerous to count microorganisms. Then 0.2 ml of each dilution were plated onto two preplated XLD-Agar Plates from Biomérieux Industries® (0.1 ml per plate) and spread with a Lazy L spreader over the entire plate to ensure uniform growth. Always the last three dilutions were plated and counted. All plates were

incubated at 35ºC for 24 hours. To determine the amount of Colony Forming Units (CFU) per ml of solution all yellow colonies with black centers were counted by hand for each plate. Which dilutions were prepared and plated was decided on the basis of the results from the previous day. Most of the Salmonella study was performed under a sterile hood.

Total Aerobic Plate Count Study

To study the effect of filtered and artificial smoke on the inhibition or growth of all naturally present aerobic bacteria, tuna steaks were prepared as described in " Sample preparations and methods" with 2 steaks per treatment. During preparation and treatment all samples were handled aseptically to avoid cross contamination. All sample steaks were stored in Ziploc® Bags at 4ºC during the entirety of the 14 day shelf life study. For the fresh storage study the steaks were sampled before treatment (Day -1), directly after treatment (Day 0) and at Day 1, 2 and then every other day until day 14. For the frozen storage study the steaks were sampled before treatment (Day -1), directly after treatment (Day 0) and then after the two day defrosting process (Day 1) and at day 2 and every other day until day 14. To determine the amount of CFU per g of tuna at any given sampling time, a 10 g cube consisting of three surface-exposed and three inside-exposed sites was cut from each steak and placed aseptically into a sterile Nasco® Whirlpack bag. The bag was then filled with 90 ml of sterile premade Dilu-LOK II Butterfields buffer from Hardy Diagnostics® and the sample cube was squeezed and mixed inside the bag with the buffer by hand for 1 minute. The sample solution in the bag is then considered the first dilution. Further dilutions were prepared with the same buffer solution by transferring 9 ml of the sterile buffer solution and 1 ml of the sample solution aseptically into a sterilized culture tube. More Dilutions were prepared if the results from the previous day indicated that the first three dilutions would have to numerous to count microorganisms. For each dilution and sample 2 ml

were plated onto two Petrifilm™ Plates for “Aerobic Plate Count” from 3M® Microbiology Products with 1 ml of solution per plate. Always the last three dilutions were plated and counted. All plates were then incubated at 34.5ºC for 48 hours. Each plate was counted by hand to determine the amount of CFU per gram of sample. Which dilutions were prepared and plated was determined based on the results from the previous day.

Color Analysis

The effect of filtered and artificial smoke on the color of tuna during 14 days of storage at 4ºC was determined by a digital Color Machine Vision System (CMVS). The CMVS was used according to the procedure described by Balaban and Luzuriaga (2001). The CMVS can report the average L*-(lightness), a*-(redness) and b*-(yellowness) values for each sample. For this experiment the main focus was on the changes in the average a*- values since the redness of tuna is one of the main quality criterion for the industry and especially the consumer. Pictures were taken before treatment (Day -1), direct after treatment (Day 0) and at day 1 (after thaw out period for the frozen storage study), day 2 and then every second day until day 14. The samples were placed in a light box and top lighting with two fluorescent lights each to simulate illumination by noonday summer sun (D65 illumination). The door remained closed while images were capturedto assure uniformity of light inside and to minimize the effect of outside light. Images were captured using a camera (Nikon D200 Digital Camera, Nikon Corp., Japan) located inside the chamber mounted to face the bottom of the light box. The Nikon D200 Settings used are described in Table 5-2. A red reference tile was laid into each picture to compensate for changes in light and camera settings in the computer analysis of the pictures.

Sensory Taste Panel Analysis

To determine if there were any human detectable differences between a filtered and an artificial smoked tuna steak in appearance and smell, a sensory taste panel was conducted with

60 random untrained panelists. The taste panel analysis was conducted on two different days, with the first day focusing on the odor differences among the samples and on the second day the color differences were analyzed. Only the 48 hour treatments with filtered smoke and artificial smoke for the frozen and the fresh storage study were compared, since it was known that these treatments would give the most odor and color differences. Four triangle tests were conducted to determine if there were any detectable differences in odor or color between 48 hour treated filtered and artificial smoked tuna after frozen (30 days at -20ºC) and fresh (1 day at 4ºC) storage. The design, test and analysis of this study were conducted in the FSHN taste panel facility with the use of the Compusense® software program. The detailed design for each triangle test can be found in Appendix A "Taste Panel Design Sheets". Each panelist who identified the odd sample as the different sample was asked to write down any comments he might have to describe the difference. Tables B-1, B-2, B-3 and B-4 show a summery of all comments given by the panelists.

Rapid Gas Chromatography Identification Method

The main components of filtered and artificial smoke are carbon monoxide, carbon dioxide and nitrogen, as well as a small amount of oxygen. However, filtered smoke contains more then just these components. A GC method which was mainly developed to quantify the amount of carbon monoxide in red muscle food might also have the potential to determine whether a product was treated with real filtered wood smoke or just a combination of 4 industrial gases that we call artificial smoke. The first step was to determine which components can be identified in filtered smoke that are not present in artificial smoke with the simple GC method developed for carbon monoxide quantification. An Agilent Technologies 6890N Network GC System, equipped with a Flame Ionization Detector (FID), a Supelco 80/100 Porapak Q Column (1.82 m long) and a hydrogen aided Nickel Catalyst (to convert carbon monoxide and carbon dioxide into

methane and make it detectable for the FID) was used for this study. The settings for the rapid identification procedure are shown in Table 5-3. PVF Gas sampling bags from LabPure® Laboratory Instruments were used to collect the pure gasses and I-Chem Economy 100 series 60 ml glass vials with a Teflon-fluorocarbon-resin/silicone septa lid were used for gas dilutions and sampling. The glass vials were flushed with nitrogen prior to sampling to minimize any contamination from the surrounding air. To identify the gas components in filtered smoke its chromatogram was compared with the chromatogram of the "Refinery Gas Test Sample" from Agilent Technologies. After the all peaks from the filtered smoke chromatogram had been identified all these gases were ordered in high purities to create standard curves (for quantification) for each of them. Ethylene (99.5% purity) and ethane (99.0% purity) were obtained from Scott Specialty Gasses. Methane (Ultra High Purity Grade), carbon monoxide (CP-Grade) and carbon dioxide (CP-Grade) were obtained from Airgas®. Standard curves for each of these gasses were produced by diluting each gas several times and injecting different amounts of each dilution into the GC. The areas under the corresponding peaks were then plotted against the actual quantity of gas injected. After a linear regression analysis an equation was obtained for each gas that allows their quantification by peak area.

Two different samples of filtered smoke, the artificial smoke and a filtered smoke, which was obtained from another source (filtered smoke B), were analyzed with the GC for their content of each of the individual gasses.

The final step was to identify whether a piece of tuna was treated with filtered or artificial wood smoke and to quantify the amount of carbon monoxide per gram of muscle tissue. For this analysis 10 g of sample from each treatment and each storage study were minced and transferred quickly into the 60 ml glass vials and the vials were sealed. The samples were then heated at

100ºC for five minutes and then cooled down to room temperature for another five minutes. A quantity of 100 µl of the headspace atmosphere from each vial was then injected into the GC and analyzed for the previously mentioned gas components. Since the artificial smoke does not contain any methane, ethylene or ethane, it would be unlikely for the tuna to absorb these gases and release them into the headspace of the vial as it would do with a sample treated with filtered smoke. Therefore the presence of these gases in the headspace over a sample would indicate a treatment with filtered smoke.

Statistical Analysis

For the *Salmonella*, total aerobic plate count and color-studies the data for the fresh and the frozen storage was analyzed separately. Analysis of Variance (ANOVA) was used to determine significant differences between all treatments at all sampling times. When significant differences among the sample means were detected Tukey's Studentized Range (HSD) test was used to do a pair wise comparison between these sample means. The level of significance for all of the ANOVA and Tukey's test were set to 5 % or 0.05.

Simple linear regression analysis was used to determine the standard curve equations for the GC analysis of carbon monoxide, carbon dioxide, methane, ethylene and ethane. The Statistical Analysis Software (SAS) and Microsoft Excel were used for the analysis of the data.

Table 5-2. Nikon D200 Settings

Setting	Specification
Device	Nikon D200
Lens	VR 18-200 mm F 3.5-5.6 G
Focal length	36 mm
Sensitivity	ISO 100
Optimize image	Custom
High ISO NR	Off
Exposure mode	Manual
Metering mode	Multi-pattern
Shutter speed and aperture	1/3s-F/11
Exposure compensation (in camera)	0 EV
Focus mode	AF-S
Long exposure NR	Off
Exposure compensation (by capture NX)	0 EV
Sharpening	Auto
Tone compensation	Auto
Color mode	Model
Saturation	Normal
Hue adjustment	0
White balance	Direct sunlight

Table 5-3. GC-Settings for the rapid identification method

Parameters	Settings
Injection Temperature	100ºC
Carrier Gas and Flow rate	Helium at 26.9 ml/min (splitless)
Nickel Catalyst Temperature	375ºC
Oven Temperature	30ºC isotherm
Runtime	5 minutes
Detector Temperature	250ºC
Column	80/100 Porapak Q packed column (1.82 m)

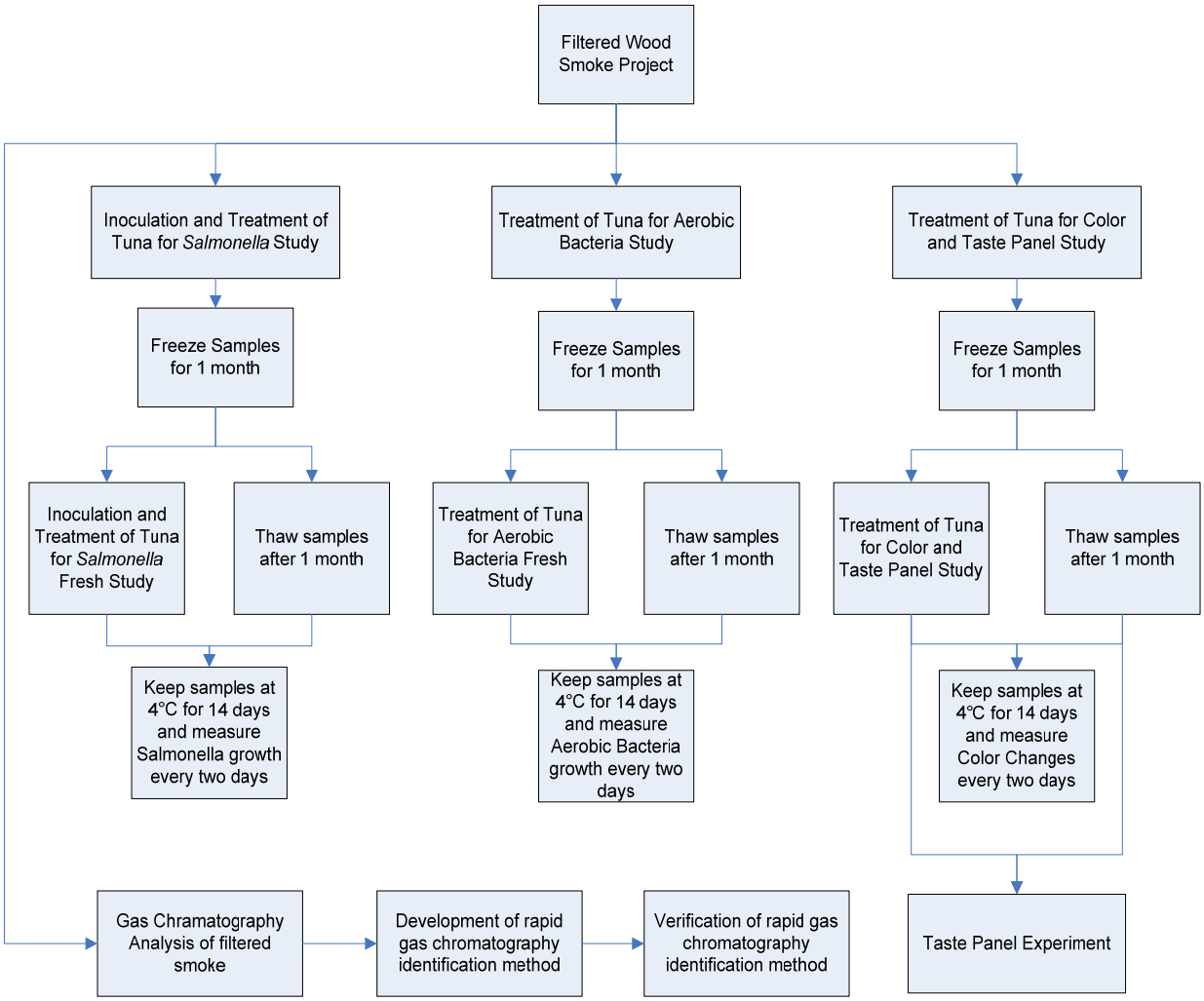

Figure 5-1. Flow diagram of all studies conducted during this project

CHAPTER 6
RESULTS AND DISCUSSION

Salmonella Results

The first part of this project was to investigate what effect a filtered smoke and artificial smoke treatment will have on the growth of *Salmonella enteriditis* on Yellowfin tuna after fresh and frozen storage. It should be noted at this point that it was planned to follow the *Salmonella* growth over a period of 14 days for inoculated and non-inoculated samples. *Salmonella* spp. is the leading cause of several food poisoning related diseases on humans (Scott 1996) and *Salmonella enteriditis* is one of the most aggressive species (Butt and others 2004). *Salmonella enteriditis* was therefore obtained from the American Type Culture Collection (ATCC). The non-inoculated samples showed absolutely no *Salmonella* growth during the first 4 days of the study for all samples and all treatments. It was therefore decided to abandon the analysis of the non-inoculated samples and keep them under the same conditions as the inoculated samples to analyze them a last time at day 14. When no growth of *Salmonella* was detected on the non-inoculated samples at day 14, it was decided not to include any collected data on the non-inoculated samples since there was obviously nothing to report. Although no *Salmonella* spp. were found on the non-inoculated samples, several studies still report incidences with *Salmonella* spp. in raw and cold smoked seafood, where the chance of a *Salmonella* incident is usually higher in imported seafood (Heinitz and Johnson 1998; Heinitz and others 2000).

The focus was laid on the analysis of the growth of *Salmonella* spp. on the inoculated samples after filtered smoke and artificial smoke treatments for the fresh and frozen studies, since *Salmonella* species were detected in several smoked fish samples from different countries (Heinitz and Johnson 1998; Fell and others 2000). Figures 6-1 and 6-2 show the average amount of Colony Forming Units (CFU) for each treatment at any measured time point for the fresh and

frozen studies, respectively. For both the fresh and the frozen study, Analysis of Variance (ANOVA) tests were conducted for every observed day to detect any significant differences among the average CFU count for all treatments. Tables 6-1 and 6-2 show the results of the ANOVA tests as well as the results of the Tukey pair wise comparisons, which were conducted when a significant difference was detected by an ANOVA test.

Fresh Storage Study

As Table 6-1 for the fresh study shows, a significant difference among the sample means could only be detected at day 4 of the study. However, the data for Tukey's test shows that there is no significant difference among all treatments except for the 48 hour artificial smoke treatment, which is significantly different from all other treatments except the 24 hour filtered smoke treatment. It is very likely that this difference is based on a random effect and an experimental error than an effect of the 48 hour artificial smoke treatment. It should be noted here that to conserve time and resources for all experiments duplicate samples were prepared and analyzed. All statistical analysis is based on the analysis of duplicate samples. Nevertheless it can be clearly seen that none of the applied treatments seems to have a major effect on the growth or inhibition of growth of *Salmonella* spp. It is of course well observed in Figure 6-1 that from the day of inoculation during the complete time of observation, the growth of *Salmonella* spp. was inhibited and the count of *Salmonella* CFU decreased. A possible explanation for this effect might be the competition of the *Salmonella* bacteria with the natural existing microbial flora present on the tuna samples (Revolledo and others 2003; Liao 2007). Therefore it is suggested that not so much any specific treatment, artificial or filtered smoke, inhibited the growth of the *Salmonella* spp. but rather the growth of naturaly existing microorganisms present on the tuna samples. Another observation supporting these findings is the fact that no *Salmonella* growth was detected in the non-inoculated samples. Heinitz and others (2000) reported a 10%

chance of *Salmonella* spp on raw imported seafood and only a 2.8% chance on raw domestic seafood based on samples taken by USDA field laboratories over a period of 9 years.

Frozen Storage Study

The results for the frozen storage study were quite similar to the fresh storage study, as shown in Figure 6-2. There were no significant differences among the treatment means for all observed days as shown in Table 6-2, which shows the p-values of the ANOVA tests that were conducted to detect any significant differences among the sample means for each observed day.

It seems that in Figure 6-2 compared to the fresh storage study there was some growth of *Salmonell*a spp. observed between the inoculation and the end of the treatments, but this can be explained by a minor experimental flaw that was eliminated immediately after the first measurement. When the steaks were inoculated with *Salmonella enteriditis*, the culture was kept in a buffer solution and 1 ml of this buffer solution was spread over the entire surface of the sample steak as evenly as possible. A sample for analysis was then taken immediately, as described in "Materials and Methods", before the sample was vacuum packed for the treatment. It was later discovered that the vacuum packing actually contributes to the distribution of the culture on the sample through the force of atmospheric pressure on the outside of the packaging material. For the fresh storage study all samples were vacuum packed after the inoculation and then reopened and sampled before the treatment to improve the even distribution of the culture. Since in fact all samples were vacuum packed before any treatment was applied to the samples there should have been no further impacts on the outcome of this analysis.

It can therefore be concluded that filtered smoke and artificial smoke processing have no immediate effect on the growth or inhibition of *Salmonella* spp. whether the sample were stored frozen or kept at 4ºC.

Although it was not observed in this experiment, the inhibition or growth of *Salmonella* spp. can be highly influenced by the natural microbial flora present on the product (Liao 2007).

Table 6-1. ANOVA results for *Salmonella* spp. for the fresh storage study.

Days	-1	0	1	2	4	6	8	10	12	14
p-Value	0.79	0.05	0.09	0.14	0.01	0.51	0.46	0.30	0.73	0.47
Ctrl	a	a	a	a	a	a	a	a	a	a
FS 24	a	a	a	a	ab	a	a	a	a	a
FS 48	a	a	a	a	a	a	a	a	a	a
AS 24	a	a	a	a	a	a	a	a	a	a
AS 48	a	a	a	a	b	a	a	a	a	a

P-Values smaller then 0.05 indicate a significant difference among the average CFU counts for the specific treatments. Treatment means with the same letter are not significantly different from each other

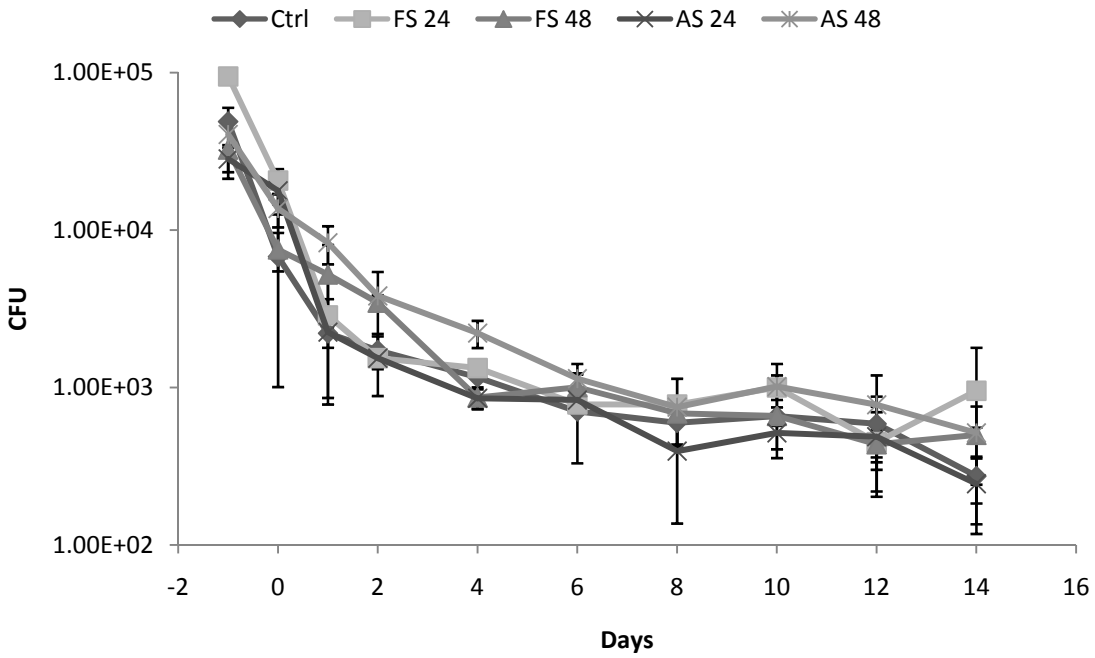

Figure 6-1. Average amount of CFU/10g of tuna for *Salmonella* spp. for the fresh storage study. The graph shows the average CFU/10g of tuna at day -1 (before any treatment was applied), day 0 (right after the treatment) and day 1 though day 14. The samples were kept at 4ºC during the entire time of observation. All samples were inoculated with *Salmonella enteriditis* before the day -1 measurement. The samples were treated with filtered smoke for 24 (FS24) and 48 (FS48) hours and with artificial smoke for 24 (AS24) and 48 (AS48) hours. The control samples (Ctrl) remained untreated.

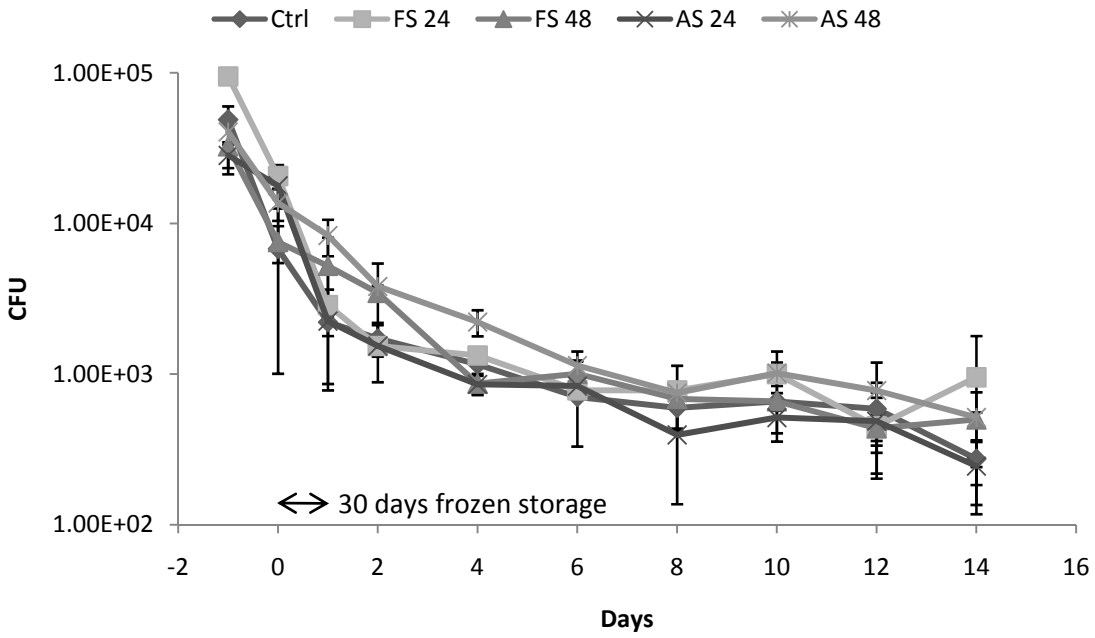

Figure 6-2. Average amount of CFU/10g of tuna for *Salmonella* spp. for the frozen storage study. The graph shows the average CFU/10g of tuna at day -1 (before any treatment was applied), day 0 (right after the treatment) and day 1 though day 14. The samples were frozen for 30 days at -20ºC between day 0 and day 1.The samples were then kept at 4ºC during the remaining time of observation. All samples were inoculated with *Salmonella enteriditis* before the day -1 measurement. The samples were treated with filtered smoke for 24 (FS24) and 48 (FS48) hours and with artificial smoke for 24 (AS24) and 48 (AS48) hours. The control samples (Ctrl) remained untreated.

Table 6-2. ANOVA results for *Salmonella* spp. for the frozen storage study.

Days	-1	0	1	2	4	6	8	10	12	14
p-Value	0.05	0.78	0.20	0.75	0.72	0.16	0.28	0.41	0.75	0.81
Ctrl	a	a	a	a	a	a	a	a	a	a
FS 24	a	a	a	a	a	a	a	a	a	a
FS 48	a	a	a	a	a	a	a	a	a	a
AS 24	a	a	a	a	a	a	a	a	a	a
AS 48	a	a	a	a	a	a	a	a	a	a

P-values smaller then 0.05 indicate a significant difference among the average CFU counts for the specific treatments. Treatment means with the same letter are not significantly different from each other

Total Aerobic Plate Count

In the early days of natural wood smoke processing the main purpose was to preserve meats and seafood for a prolonged period. Although other factors like the heat treatment and the dehydration of the product (Burt 1988), which accompanied the smoke treatment, are affecting the microbial flora present on the product, it was suggested that certain components, present in natural wood smoke might inhibit the growth of spoilage bacteria and enhance their shelf life (Kristinsson and others 2006b). This study therefore focuses on the effect of filtered wood smoke on the growth or inhibition of aerobic bacteria naturally present on Yellowfin tuna. Earlier studies already suggested that filtered wood smoke processing inhibits the growth of aerobic bacteria during the first 6 days of storage (Danyali 2004). In this study the effect of filtered wood smoke and artificial wood smoke processing for 24 and 48 hours on fresh and frozen tuna was analyzed over a period of 14 days of storage at 4ºC. The frozen tuna was treated prior to subjecting it to 30 days of frozen storage. Figures 6-3 and 6-4 show the results of the average CFU count for all treatments at all observed time points for the fresh and frozen storage studies, respectively. For each single time point, ANOVA tests were conducted to detect any significant differences among the sample means of the treated and untreated products. When significant differences were detected Tukey's pair wise comparison tests were conducted to determine exactly which treatments are significantly different at this time point. Table 6-3 and 6-4 show the p-values of the ANOVA tests for each time point as well as the results of the pair wise comparison for the fresh and frozen studies respectively.

Fresh Storage Study

Results of the fresh storage study show that no significant differences could be detected for most of the observed days, except day 0 (immediately after treatment) and day 2. The p-value of the ANOVA tests for day 1 shows that no significant difference could be detected at a

significance level of 0.05, but there was a significant difference at a level of 0.09 and higher. As described before, all experiments were conducted in duplicates. Therefore one outlier can influence the outcome. Still it has to be observed that at day 1 the control group showed a much higher CFU count than all other groups. These findings are similar to the results described by Kristinsson and others (2006b). It can be said that all treatments seem to suppress the growth of aerobic bacteria during the first 4 days of observation. Total aerobic bacterial count then suddenly stopped increasing at day 4 and reached an equal level for all treatments, including the control group. From day 4 to day 14 there were no significant differences detectable between the control group and any of the treatments. The reduced CFU count at day 4 represents the end of the lag phase of a typical bacterial growth curve (Creager and others 1990), followed by the log or exponential phase (Tortora and others 1992) from day 4 to day 6 and the stationary phase from day 8 to day 14. The end of the stationary phase can not be seen here since no measurements were taken after day 14. Although no significant differences could be detected after day 4 it seems like there is a small second lag phase for the sample treated with filtered and artificial smoke at day 8 compared to the control group, however the lack of significance and of data surrounding this event makes these findings inconclusive. Overall it can be said that the filtered and artificial smoke treated samples appear to be better protected against aerobic bacteria growth during the first three days of storage at 4ºC after the treatment. However no significant improvement on shelf life can be reported after the exponential growth of the organisms was initiated. There is also no evidence in this study suggesting any significant difference between the filtered and artificial smoke treatments or a prolonged 48 hour treatment compared to the 24 hour treatment for both treatment gasses.

Frozen Storage Study

The results for the frozen storage study showed that there are no significant differences among the sample means for day -1 (before treatment), day 0 (after treatment) and day 1, day 10, day 12 and ay 14. At day 1, this is the first sampling day after the 30 day frozen storage period, all samples seem to have the same CFU count regardless of their treatment. This can be explained by the fact that so shortly after the thaw out process only few viable cells could be sampled and survived the stress of freezing and thawing (Speck and Ray 1977; Bhaduri and Cottrell 2004). However, already at day 2 it can be observed that CFU count for the control group is significantly higher then the CFU count for all other treatments. It should be noted that, although not statistically significant, the CFU count for the control group seems already to be higher directly after the treatment and before the samples were placed into frozen storage at day 0. Furthermore should be noted that at day 0 it seems that the 48 hour filtered smoke and artificial smoke treatments inhibit the growth of aerobic bacteria more effectively then the 24 hour treatments, respectively. From day 4 to day 6 the same behavior is observed as compared to the fresh storage study, where day 4 marks the end of the lag phase of the bacterial growth and the beginning of the log or exponential phase. From day 6 to day 14 a more or less stable stationary phase is observed. The slight increase in CFU count for all samples during the last 8 days could suggest that there are still microorganisms recovering from the 30 day frozen storage (Speck and Ray 1977). Nevertheless the control group, although not longer statistically significant, has higher CFU counts than the treatment groups at all times from day 6 to 14. The statistical analysis shows that for most of the time points no significant difference could be detected between filtered and artificial smoke processed samples, whether they were treated for 24 or 48 hours. These findings again suggest that a 48 hour treatment does not improve the overall results compared to a 24 hour treatment. Also, the artificial smoke treatment seems to

affect the growth of aerobic bacteria in a similar way as filtered smoke treatment contrasted to the results from other studies which suggest an advantage of the filtered smoke treatment over the artificial smoke treatment (Danyali 2004; Kristinsson and others 2007).

Overall, both the filtered and the artificial smoke treatment, show an effect on the growth of aerobic bacteria on tuna, especially before the exponential growth phase is initiated. Furthermore the combined effect of freezing and filtered or artificial smoke seems to prolong the shelf life of tuna fish after thawing by inhibiting aerobic bacterial growth during the first 6 days after thawing. Similar findings have been reported by Rawles and others (1996) and Demir and others (2004)

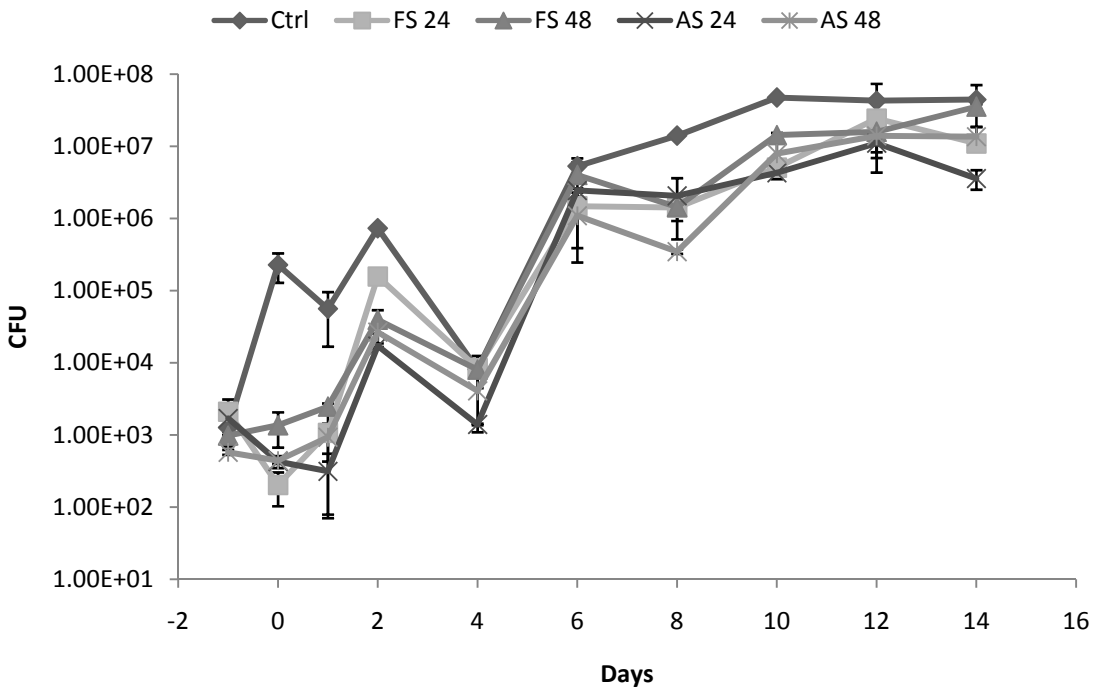

Figure 6-3. Average amount of CFU/10g of tuna for aerobic plate count for the fresh storage study. The graph shows the average CFU/10g of tuna at day -1 (before any treatment was applied), day 0 (right after the treatment) and day 1 though day 14. The samples were kept at 4ºC during the entire time of observation. The samples were treated with filtered smoke for 24 (FS24) and 48 (FS48) hours and with artificial smoke for 24 (AS24) and 48 (AS48) hours. The control samples (Ctrl) remained untreated.

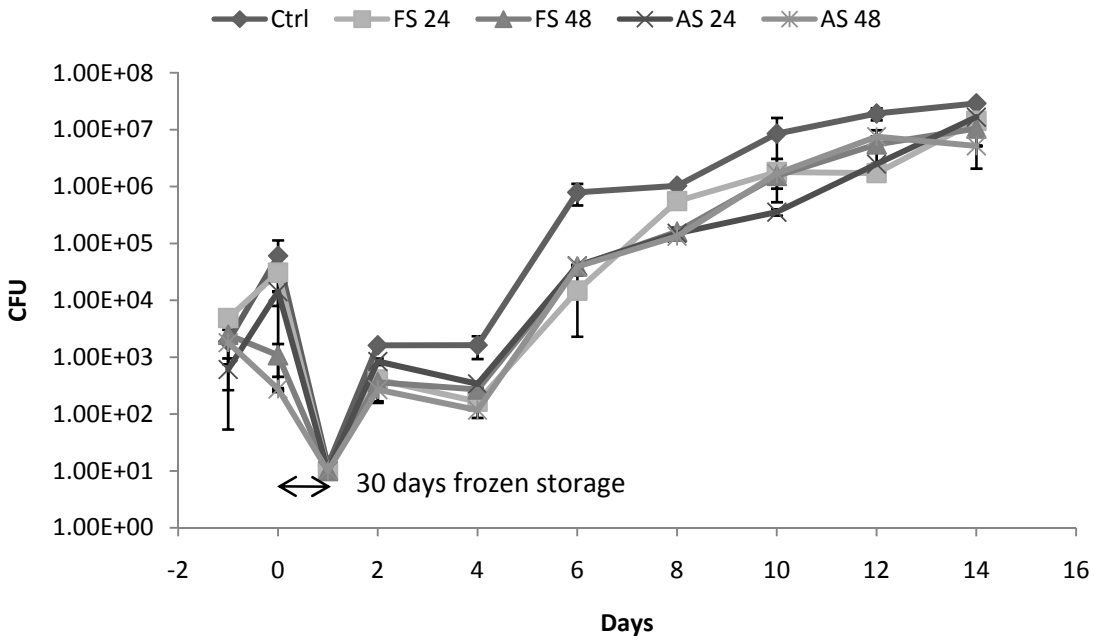

Figure 6-4. Average amount of CFU/10g of tuna for aerobic plate count for the fresh storage study. The graph shows the average CFU/10g of tuna at day -1 (before any treatment was applied), day 0 (right after the treatment) and day 1 though day 14. The samples were frozen for 30 days at -20ºC between day 0 and day 1.The samples were then kept at 4ºC during the remaining time of observation. The samples were treated with filtered smoke for 24 (FS24) and 48 (FS48) hours and with artificial smoke for 24 (AS24) and 48 (AS48) hours. The control samples (Ctrl) remained untreated.

Table 6-3. ANOVA results for aerobic plate count for the fresh storage study.

Days	-1	0	1	2	4	6	8	10	12	14
p-Value	0.85	>0.01	0.08	>0.01	0.86	0.47	0.52	0.52	0.39	0.11
Ctrl	a	a	a	a	a	a	a	a	a	a
FS 24	a	b	a	b	a	a	a	a	a	a
FS 48	a	b	a	c	a	a	a	a	a	a
AS 24	a	b	a	bc	a	a	a	a	a	a
AS 48	a	b	a	c	a	a	a	a	a	a

P-values smaller then 0.05 indicate a significant difference among the average CFU counts for the specific treatments. Treatment means with the same letter are not significantly different from each other

Table 6-4. ANOVA results for aerobic plate count for the frozen storage study.

Days	-1	0	1	2	4	6	8	10	12	14
p-Value	0.72	0.28	0.49	>0.01	0.02	0.01	>0.01	0.29	0.06	0.12
Ctrl	a	a	a	a	a	a	a	a	a	a
FS 24	a	a	a	b	b	b	b	a	a	a
FS 48	a	a	a	ab	b	b	c	a	a	a
AS 24	a	a	a	b	b	b	c	a	a	a
AS 48	a	a	a	b	b	b	c	a	a	a

P-values smaller then 0.05 indicate a significant difference among the average CFU counts for the specific treatments. Treatment means with the same letter are not significantly different from each other

Color Analysis

The appearance and color of fresh and frozen seafood is one of the major characteristics consumers use to judge the quality of a product. Most of the time the consumer is not able to taste or smell the actual product since it is either vacuum packed and frozen or it is laid out on a tray behind the counter where the consumer can only visually examine the product. Therefore a fresh color and appearance are of major importance. For fresh Yellowfin tuna a bright red to dark red color is desired for consumer acceptance. Carbon monoxide processing and modified atmospheric packaging have been known and used for a while to preserve and enhance the color properties and especially the redness of fish and seafood products (Mancini and Hunt 2005; Sorheim and others 2006). For this experiment a Color Machine Vision System was used to analyze the color and color changes of the samples during this study. Since the degree of redness (a*-value) is one of the most important quality indicators in red muscle seafood, such as tuna, the average a*-value of each sample was determined for each treatment at any time point. Figures 6-5 and 6-6 show the average a*-values for each treatment measured over a period of 14 days for the fresh and frozen study, respectively. ANOVA tests were conducted to detect any significant differences among the treatment means for each observed day of the fresh and frozen storage studies. Table 6-5 and 6-6 show the results of the ANOVA tests as well as the results of the Tukey's pair wise comparisons, which were conducted to determine which sample means where

different from each other. Reading the table column by column, same letters assigned to sample means show there are no significant differences among these means. The L*-values (lightness) and b*-values (yellowness) were also analyzed and are shown in Figures 6-7 to 6-10. Kristinsson and others (2006a) reported that in several studies the redness of the fish muscle was highly influenced by carbon monoxide but not the lightness (L*-values) and the yellowness (b*-values).

Fresh Storage Study

Table 6-5 shows that the ANOVA tests detected significant differences among the sample means for all observed days of the fresh storage study. It can also be seen that for all observed days, except the first day before any treatment were applied to the samples, the control group means are always significantly different from the other treatment means, according to Tukey's pairwise comparison. Similar to the frozen storage study there are significant differences among the samples before any treatment were applied, which again can be explained by the way sample steaks were visually sorted and assigned to the treatments, as shown in Figure 6-5. In comparison to the frozen storage study however we were starting clearly at the same a*-value level. It also seems that there is a difference between the 24 and 48 hour treatments at this point that can not be explained by the treatments since no treatments were applied at this time. At day 0, directly after the treatments all samples showed an improvement in a*-values, even the control group, which could be explained that all samples were transferred directly into the open Ziploc® bags after the treatment (the control group too) where the are immediately exposed to the oxygen in the air, compared to the frozen study where the samples were kept in the vacuum bags for the frozen storage. Similar to the frozen storage study, the 48 hour treatments for filtered and artificial smoke showed a greater improvement in redness than the 24 hour treatment or the control group. At day 1 after the treatment, the filtered smoke treated samples showed a great improvement in the redness, similar to the frozen storage study, while the artificial smoke treated

samples show no improvement in redness from this point until day 6. Actually, the redness in the artificial smoke treated sample decreased slightly from day 4 to day 6. The a*-values of the filtered smoke treated samples follow nearly the same pattern as recorded for the frozen storage study. The control group also follows a similar pattern in the development of the a*-values as compared to the frozen study, where there can be seen a decline in redness until day 6 and then a slight increase until day 14. The artificial smoke treated samples seem to follow now a similar pattern where a decline in redness can be from day 0 until day 6 and then a slight increase until day 14. Increased overall variation among the sample means, indicated by increased standard deviation values, suggests, similar to the frozen storage study, that more factors than just the treatments start to influence the color development of the samples starting from day 6. So far, no reasonable explanation could be found why the artificial smoke treatments does not show the same effect as the filtered smoke treatment in the fresh storage study. Still, the improvement in redness among the artificial smoke treated samples exceeds the redness of the control group by far and results in fresh and red looking tuna steaks, as shown in Figure 6-6.

The results from the analysis of L*-values (brightness) and b*-values (yellowness) for the fresh storage study were quite similar to the findings of the frozen storage study (Figures 6-7 and 6-9), where the L*- and b*- values follow the pattern of the a*-values and show large increases in brightness and yellowness for all treatments during the first 6 days of observation while the control group stays behind. These findings again confirm that there are changes in the L*- and b*- values affected by the filtered smoke and artificial smoke treatment, contrary to the reports of several other studies (Danyali 2004; Garner 2004; Balaban and others 2005), where little effects on L*- and b*- values were reported.

It should be noted at this point that overall no differences were observed whether a sample was treated for 24 or 48 hours, except when analyzed right after the treatment. It is therefore suggested that a 24 hour treatment of either filtered or artificial smoke should be sufficient to improve the color properties of tuna for fresh or frozen storage.

Frozen Storage Study

As Table 6-6 indicates, for every observed day, except the last day of the frozen storage study there are significant differences among the treatment means. Furthermore Tukey's test results show that for every observed day (except day 14) the control group is significantly different from all other treatments. Surprisingly the control group means are even significantly different from all other treatment means before any treatment was applied to any of the samples. This could be explained by a minor flaw in the way the sample steaks were sorted and assigned to the treatments visually prior to the beginning of this experiment. However, Figure 6-6 shows clearly that in the overall development of redness (a*-values) of the samples the initial measurements are very close to each other with relatively small standard deviations resulting in a highly detectable difference among the samples for the ANOVA test. It is also clearly visible that the value of the standard deviations increased over time, when other factors like microbial spoilage, oxidation and degradation influence the appearance of each sample individually. Therefore, at day 14 no significant differences can be detected among the treatments and the control samples. Immediately after treatment Figure 6-6 indicates that the 48 hour treatment with filtered and artificial smoke results in higher a*-values then the 24 hour treatment, which is confirmed significant by Tukey's test (Table 6-6). This can be explained by the fact that the 48 hour treated samples spent 24 more hours in treatment prior to the analysis and the carbon monoxide had therefore more time to penetrate into the muscle and form the carboxy-myoglobin complex, which is responsible for the redness of the sample. It is also clearly visible that there

are no significant differences between the filtered and artificial smoke treatments. Day 1 represents the first day of analysis after the 30 day frozen storage period, and it is clearly visible that the a*-values, and therefore the redness, of all treatments greatly improved while the control group lacks significantly behind. A small increase can be seen in the control group, possibly to the effect of oxygen exposure of the sample, when the bags where opened for analysis after the thaw-out process. The a*-values for all treatments seem to be stable for a period of about 4 days of storage at 4ºC, from which point they slowly decline. At the end of the 14 day study the differences among the samples are so big, indicated by increased standard deviations, that no significant differences can be detected among any treatment and the control group. The control groups shows a decrease in their a*-value for the first 4 days of the analysis and then a very slow increase, which could be a result of increased spoilage and oxidation, but they never reach the redness as resulted by the treatments. Figure 6-11 shows visual comparison of untreated tuna steaks and filtered smoke treated tuna steaks after 30 days of frozen storage right after thawing. The differences in color and overall appearance are clearly visible. The control group has a dark grayish appearance whereas the filtered smoke treated samples look nice red and fresh. The L*-values (Figure 6-8) show a similar pattern as described for the a*-values above, an effect that was also reported by Balaban and others (2006). The lightness of the 24 hour treated samples (filtered and artificial smoke) increased dramatically after the frozen storage, while the 24 hour treatments increased only half as much in lightness and the control groups stoud unchanged. Also the b*-values (Figure 6-10) show a increase in yellowness for the 48 hour treated samples compared to the 24 hour treated samples and the control group. These findings are quite interesting since Kristinsson and others (2006a) reported that no significant changes have been found in several studies among the L*- and b*-values for carbon monoxide treated fish.

Table 6-5. ANOVA results for the average a*-values for the fresh storage study.

Days	-1	0	1	2	4	6	8	10	12	14
p-Value	>0.01	>0.01	>0.01	>0.01	>0.01	>0.01	>0.01	>0.01	>0.01	>0.01
Ctrl	a	a	a	a	a	a	a	a	a	a
FS 24	b	c	c	c	d	c	bc	b	b	b
FS 48	a	b	b	b	b	b	b	bc	b	b
AS 24	b	d	d	c	d	c	c	c	b	b
AS 48	a	b	b	n	c	b	b	bc	b	b

P-values smaller then 0.05 indicate a significant difference among the average CFU counts for the specific treatments. Treatment means with the same letter are not significantly different from each other

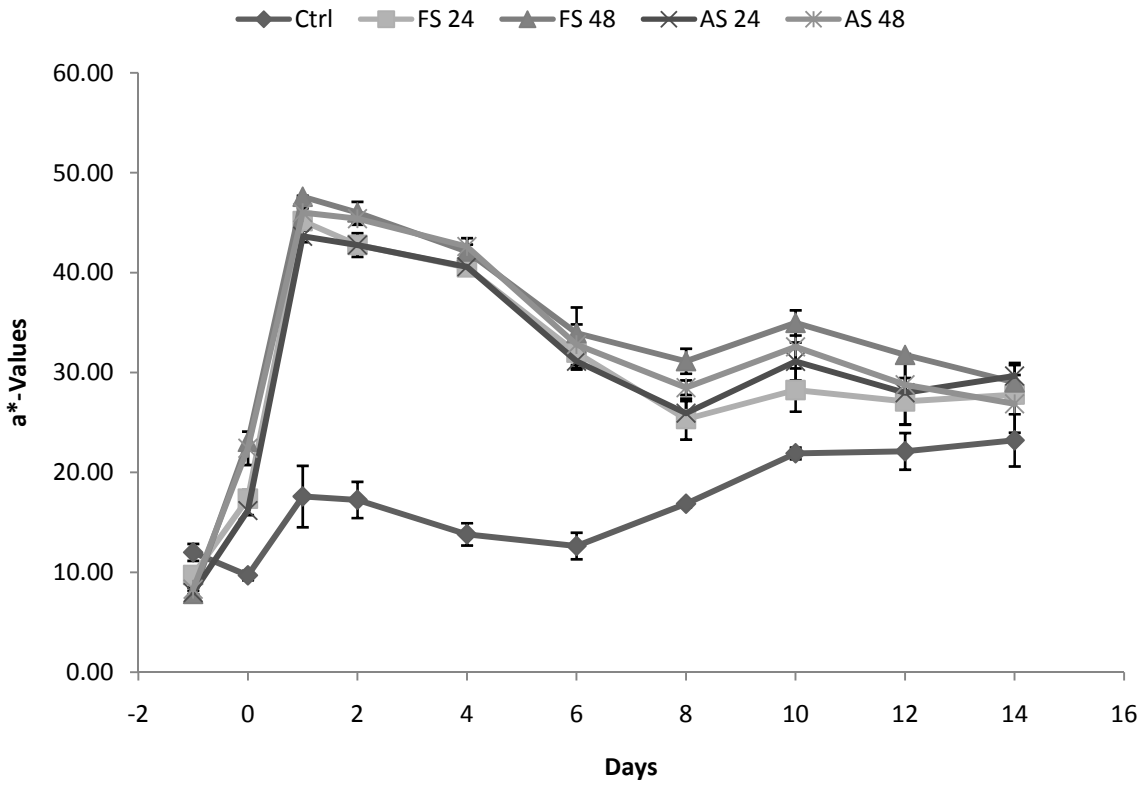

Figure 6-5. Average a*-values of the color of tuna samples over 14 days for the frozen storage study. The graph shows the average a*values of tuna at day -1 (before any treatment was applied), day 0 (right after the treatment) and day 1 though day 14. The samples were kept at 4ºC during the entire time of observation. The samples were treated with filtered smoke for 24 (FS24) and 48 (FS48) hours and with artificial smoke for 24 (AS24) and 48 (AS48) hours. The control samples (Ctrl) remained untreated.

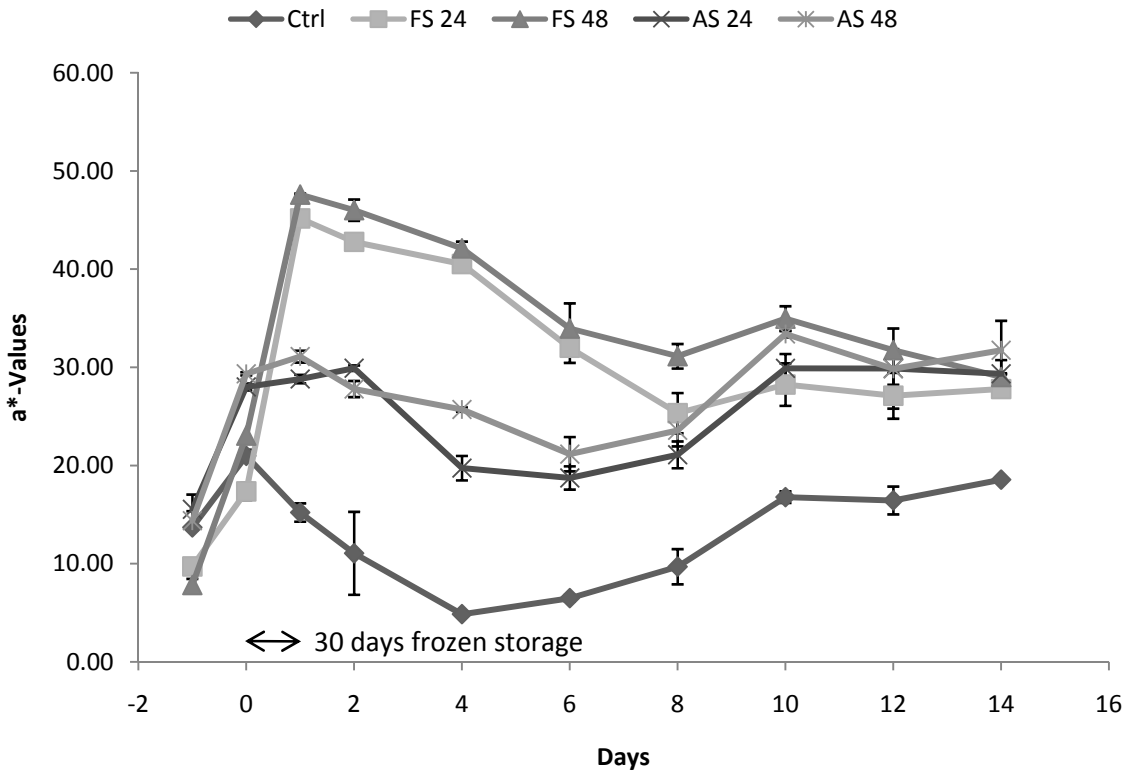

Figure 6-6. Average a*-values of the color of tuna samples over 14 days for the fresh storage study. The graph shows the average a*values of tuna at day -1 (before any treatment was applied), day 0 (right after the treatment) and day 1 though day 14. The samples were frozen for 30 days at -20ºC between day 0 and day 1.The samples were then kept at 4ºC during the remaining time of observation. The samples were treated with filtered smoke for 24 (FS24) and 48 (FS48) hours and with artificial smoke for 24 (AS24) and 48 (AS48) hours. The control samples (Ctrl) remained untreated.

Table 6-6. ANOVA results for the average a*-values for the frozen storage study.

Days	-1	0	1	2	4	6	8	10	12	14
p-Value	>0.01	>0.01	>0.01	>0.01	>0.01	>0.01	>0.01	>0.01	0.05	0.12
Ctrl	a	a	a	a	a	a	a	a	a	a
FS 24	b	b	b	b	b	b	b	ab	ab	a
FS 48	b	b	b	b	b	b	b	bc	ab	a
AS 24	b	c	b	b	b	b	c	bc	b	a
AS 48	b	c	b	b	b	b	bc	c	ab	a

P-values smaller then 0.05 indicate a significant difference among the average CFU counts for the specific treatments. Treatment means with the same letter are not significantly different from each other

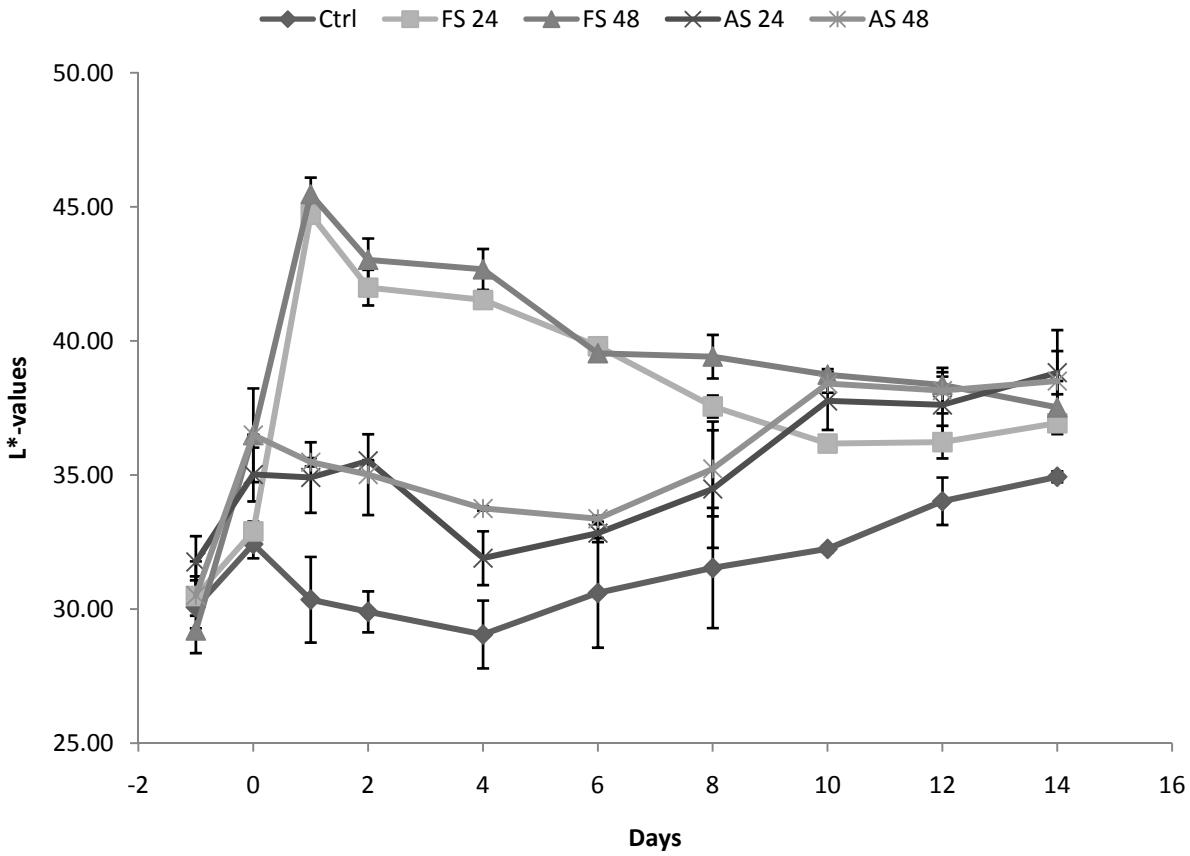

Figure 6-7. Average L*-values of the color of tuna samples over 14 days for the fresh storage study. The graph shows the average a*values of tuna at day -1 (before any treatment was applied), day 0 (right after the treatment) and day 1 though day 14. The samples were kept at 4ºC during the entire time of observation. The samples were treated with filtered smoke for 24 (FS24) and 48 (FS48) hours and with artificial smoke for 24 (AS24) and 48 (AS48) hours. The control samples (Ctrl) remained untreated.

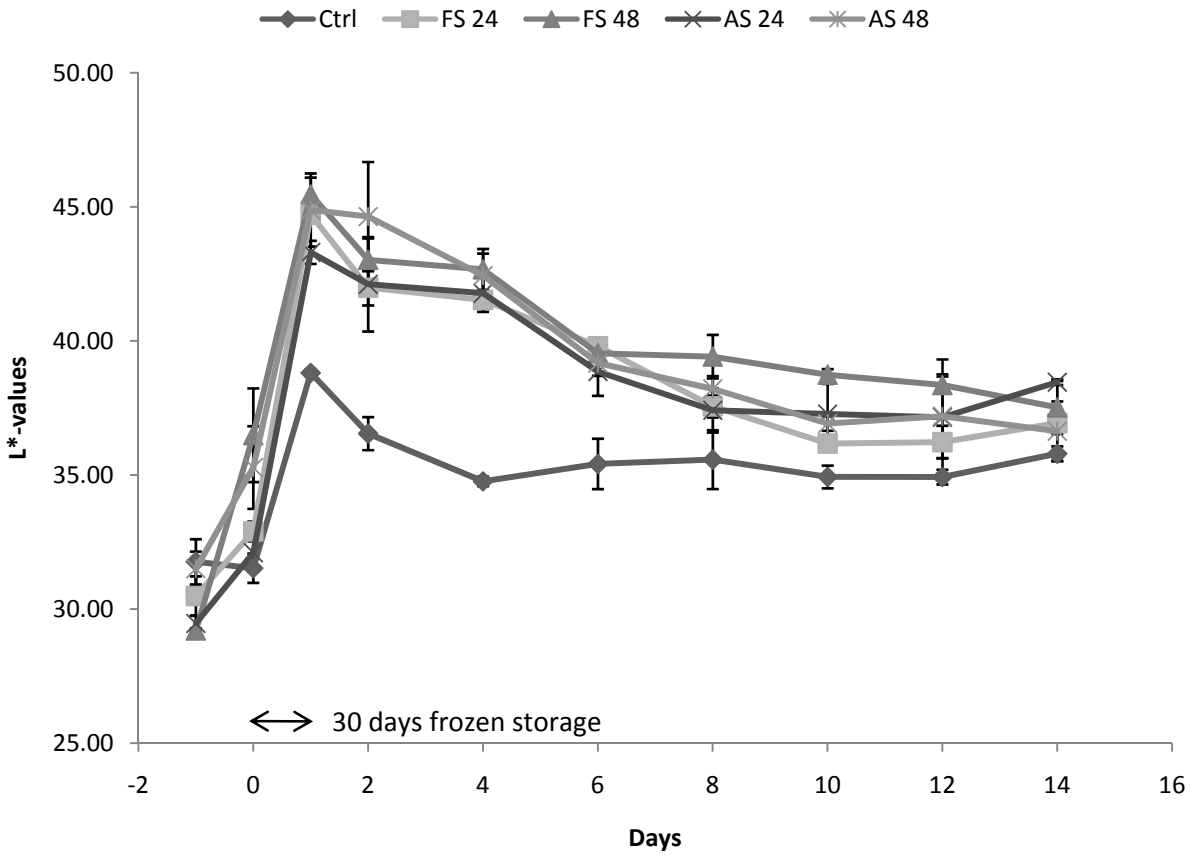

Figure 6-8. Average L*-values of the color of tuna samples over 14 days for the frozen storage study. The graph shows the average a*values of tuna at day -1 (before any treatment was applied), day 0 (right after the treatment) and day 1 though day 14. The samples were frozen for 30 days at -20ºC between day 0 and day 1.The samples were then kept at 4ºC during the remaining time of observation. The samples were treated with filtered smoke for 24 (FS24) and 48 (FS48) hours and with artificial smoke for 24 (AS24) and 48 (AS48) hours. The control samples (Ctrl) remained untreated.

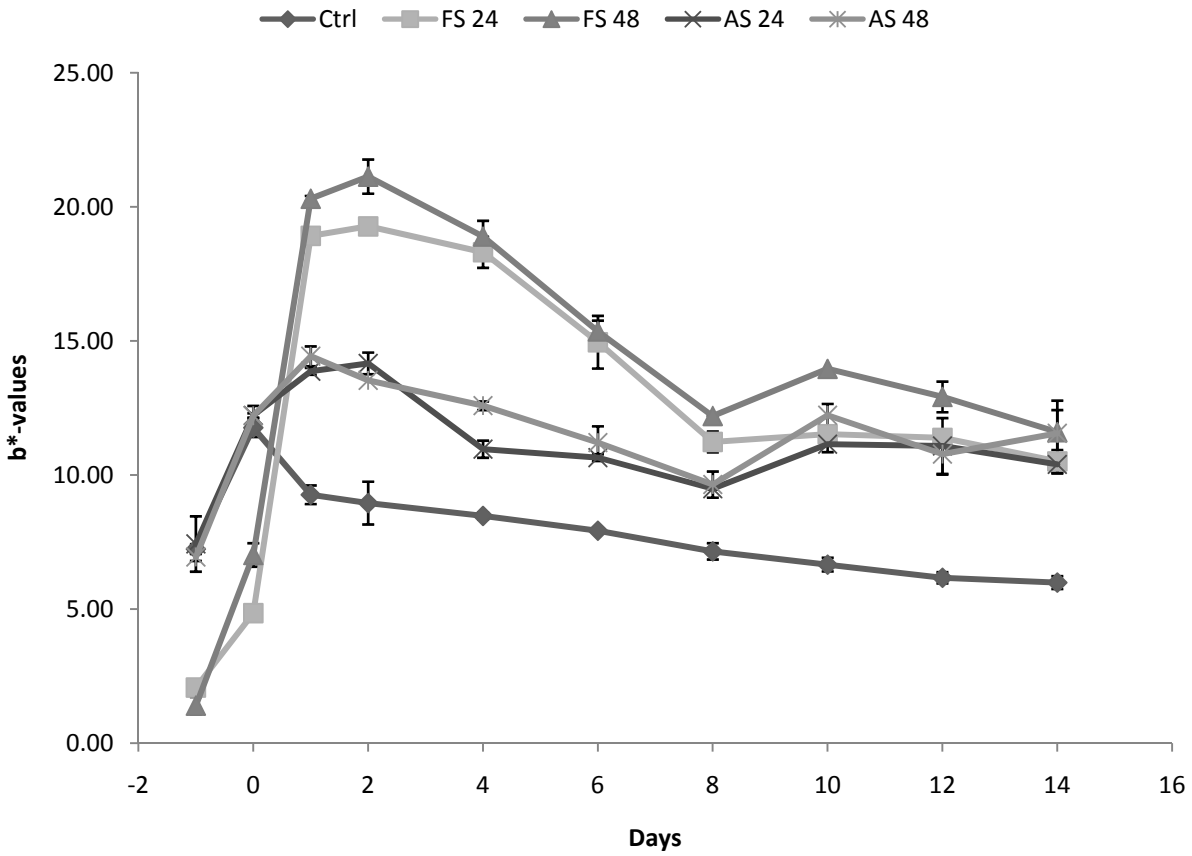

Figure 6-9. Average b*-values of the color of tuna samples over 14 days for the fresh storage study. The graph shows the average a*values of tuna at day -1 (before any treatment was applied), day 0 (right after the treatment) and day 1 though day 14. The samples were kept at 4ºC during the entire time of observation. The samples were treated with filtered smoke for 24 (FS24) and 48 (FS48) hours and with artificial smoke for 24 (AS24) and 48 (AS48) hours. The control samples (Ctrl) remained untreated.

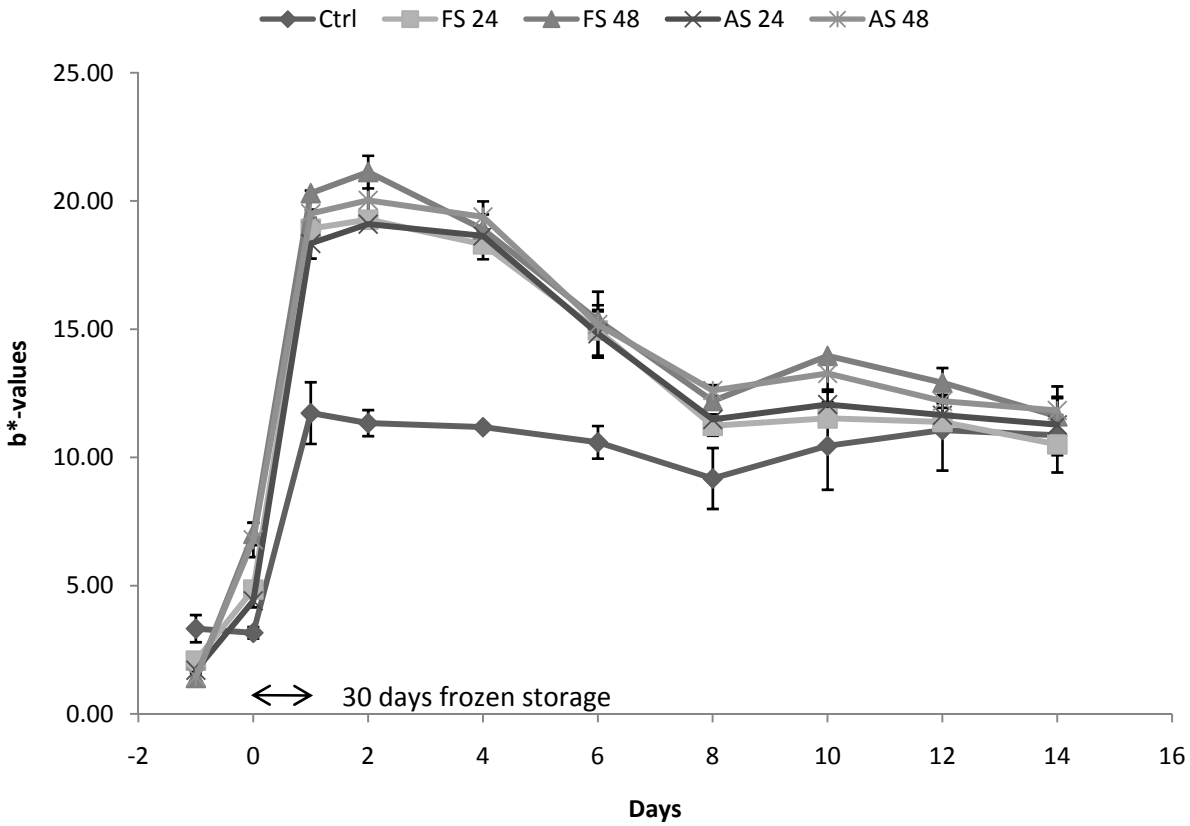

Figure 6-10. Average b*-values of the color of tuna samples over 14 days for the frozen storage study. The graph shows the average a*values of tuna at day -1 (before any treatment was applied), day 0 (right after the treatment) and day 1 though day 14. The samples were frozen for 30 days at -20ºC between day 0 and day 1.The samples were then kept at 4ºC during the remaining time of observation. The samples were treated with filtered smoke for 24 (FS24) and 48 (FS48) hours and with artificial smoke for 24 (AS24) and 48 (AS48) hours. The control samples (Ctrl) remained untreated.

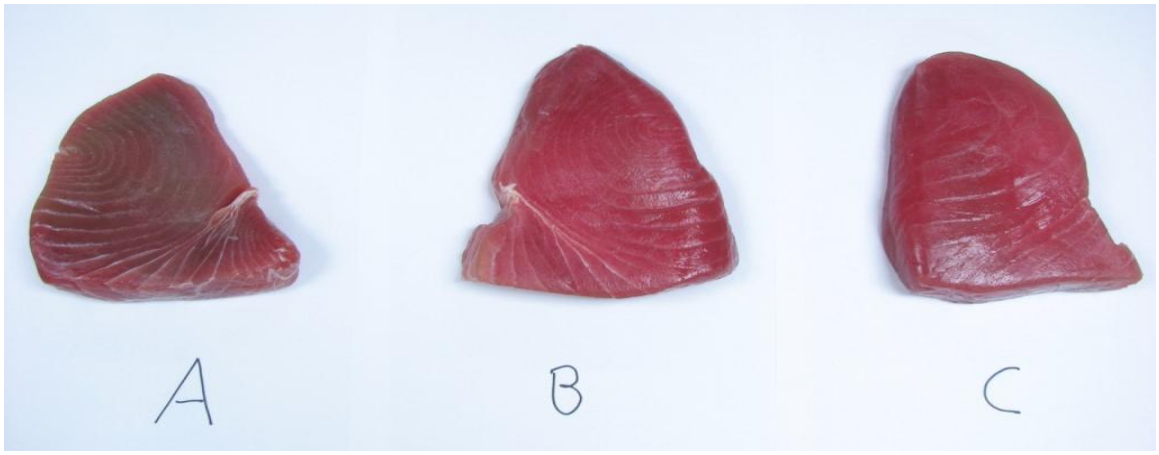

Figure 6-11. Tuna steaks after 30 days of frozen storage at -20ºC. A) Shows the untreated control sample. B) Shows the filtered smoke treated sample. C) Shows the artificial smoke treated sample

Sensory Taste Panel

The sensory characteristics of a product such as appearance, smell and taste are very important factors for consumers to make their decision to buy a product. Since the use of filtered and artificial smoke had similar results in their effect on color and appearance, as well as microbial growth of the samples, the task was now to verify if there are any human detectable differences in smell and appearance among filtered and artificial smoke treated samples. Four triangle tests were conducted and 60 random untrained panelists per test were asked if they could identify the odd sample by odor (first and second triangle tests) and by appearance (thirds and fourth triangle tests). The design, execution and analysis of these triangle tests were conducted with the Compusense® Software. Table 6-7 shows the results of all four triangle tests, where the actual number of correct answers is compared to the number of correct answers necessary to establish a level of significance at 5%. This means that a lower number of correct answers indicate that there are no significant differences detectable in a human sensory panel between the treatments. It can be seen that for all four tests no significant differences have been detected between filtered and artificial smoke treated samples in odor and appearance for the fresh and frozen storage studies. All samples were prepared in the same manner and presented to the panelists immediately after treatment or after thawing. All samples were treated for 48 hours since a longer treatment suggested a greater effect on color and odor from preliminary experiments.

To gain additional information, each panelist who identified the odd sample correctly was asked to note any comments he or she might have about the differences among the samples that led to their decision of choosing the odd sample. Tables A-5 to A-8 in Appendix A show all comments given by the panelists for all four tests. For the color analysis, most of the panelists described the filtered smoke sample to appear darker and more red then the artificial smoke

sample for the fresh storage study, while nearly equal number of panelists describe either the artificial smoked or the filtered smoked sample as lighter in color for the frozen storage study. This confirms in part the findings of the color study, where for the frozen storage study no significant differences were found at day 1 between the filtered and artificial smoke treated samples. The samples for the color studies and the taste panel analysis were obtained separately from each other at different time points and also treated separately and at different times.

Comments regarding the odor triangle tests show that an equal amount of people noticed a stronger smell in either the filtered smoke or the artificial smoke treated samples for both the fresh and frozen storage studies. It is therefore inconclusive whether a particular smell or odor was responsible for these panelists to decide which the odd sample was. It should be noted at this point that only the panelists who identified the odd sample correctly were asked to comment their decision and that there is a possibility that some of these panelists found the odd sample by chance and not based on a real difference. The Compusense® analysis software includes the possibility of a right answer by guessing in their statistical analysis. Also the demographical variety was limited to students from the University of Florida between the ages of 18 to 24 years.

Table 6-7. Results of the four taste panel triangle tests.

Tests	1	2	3	4
Total number of panelists	60	60	60	60
Number of correct answers needed	27	27	27	27
Actual Number of correct answers	23	26	19	19
Significance (p-Value)	0.244	0.068	0.654	0.654

Number 1 represents the odor test with frozen samples, number 2 the odor test with fresh samples, number 3 the color test with frozen samples and number 4 the color test with fresh samples.

GC-Analysis

Carbon monoxide is known to preserve and enhance the color properties of red muscle foods and is used in several applications today. Small quantities of carbon monoxide (> 0.5%) are used today in modified atmospheric packaging to preserve the redness of fresh meats, such as

ground beef (Sorheim and others 1999; Kusmider and others 2002; Hunt and others 2004) and pork sausages (Sorheim and others 2001; Martinez and others 2005; Sorheim and others 2006). A short term treatment with higher percentages of carbon monoxide (up to 100%) is used in the seafood industry to retain the color of certain seafoods prior to frozen storage. However, carbon monoxide is not a brand new component in the process of preserving meats and seafood. Natural wood smoke, produced by the incomplete combustion of wood chips, always contains a certain amount of carbon monoxide, which is applied to the product during the smoking process. During cold smoking, a process where the product is kept at a low temperature (usually below 15ºC) while the smoke is applied, the product is not cooked and therefore retains its color and texture properties on the inside. The outside of the product usually turns into a slight brown to grey color because of solid particles of the smoke that condense on the product during smoking. Filtered wood smoke is a relatively new invention where these particles and most of the odor and flavor components are filtered out and therefore don't condense onto the product. According to the analysis of the manufacturer the main components of the filtered smoke used in this experiment are carbon monoxide (21%), carbon dioxide (18%), oxygen (1.1%) and a balance of nitrogen. However, filtered smoke contains many more components that have not yet been identified. Since the production of filtered wood smoke can be a complicated and costly process, a cheaper alternative is a treatment where the mentioned gas components are mixed together from commercially available gasses and used in the same way as the filtered smoke. For this reason we called this mixture artificial smoke (AS). As the previous studies showed it had a similar effect on the color properties, sensory characteristics and microbial growth as the original filtered smoke. To differentiate whether a product was treated with the original filtered smoke or the artificial smoke mixture, a rapid gas chromatography method was developed that focuses mainly

on the identification and quantification of other gaseous components which are present in filtered smoke but not in artificial smoke.

Identification of Gaseous Components in Filtered and Artificial Smoke

The fist step in the development was to identify the gaseous components in filtered smoke and artificial smoke and develop a standard curve for each of these components with the GC-Method mentioned in the chapter “Materials and Methods”. Filtered smoke was injected into the GC and 5 peaks could be verified with retention times less than five minutes. Two of these peaks (retention times around 0.49 minutes and 1.52 minutes) were known already from previous experiments as carbon monoxide and carbon dioxide respectively. The other three peaks were not identified yet. It was also known from previous experiments that only low molecular hydrocarbons will be eluted from the GC column in less than five minutes. It was therefore decided to use refinery gas standard from Agilent technologies, which is a mixture of low molecular weight hydrocarbons (like methane, ethylene, ethane, propane and so on), to identify the three unknown components found in filtered smoke.

It should be noted at this point that there are most likely many more gaseous components contained in filtered smoke, but with retention times much longer than 30 minutes in this particular GC setup and therefore do not contribute to a “rapid” identification method.

With a comparison of the filtered smoke and the gas standard chromatograms the three unknown components could be identified as methane, ethylene and ethane respectively in the order of their elution.

The second step was now to obtain these five gases found in filtered smoke in the highest purities available and produce standard curves for each of them to quantify them in the filtered smoke, artificial smoke and the treated samples. Figures 6-12 to 6-16 show these standard curves and display the resulting equations that were used to quantify the amount of each gas, based on

and injection volume of 100 µl and their respective peak area. Now the five components found in filtered smoke with this method could be identified and quantified. As a comparison the artificial smoke and a filtered smoke (FS-B), which was obtained from a different company, were analyzed with this method. Table 6-8 shows the percentage of each of these five gasses found in filtered smoke (FS), filtered smoke B (FS-B) and artificial smoke (AS). Since it is was clear that the artificial smoke lacks any methane, ethylene and ethane, the next step was to experiment whether any of these five gasses can also be found absorbed in the actual products.

Table 6-8. Percentage of gaseous components in the treatment gasses.

Treatment Gas	Filtered smoke A	Filtered smoke B	Artificial Smoke
Carbon Monoxide	22.53	11.00	22.20
Methane	9.84	2.85	0.00
Carbon Dioxide	14.32	12.55	19.43
Ethylene	0.94	0.14	0.00
Ethane	0.17	0.20	0.00

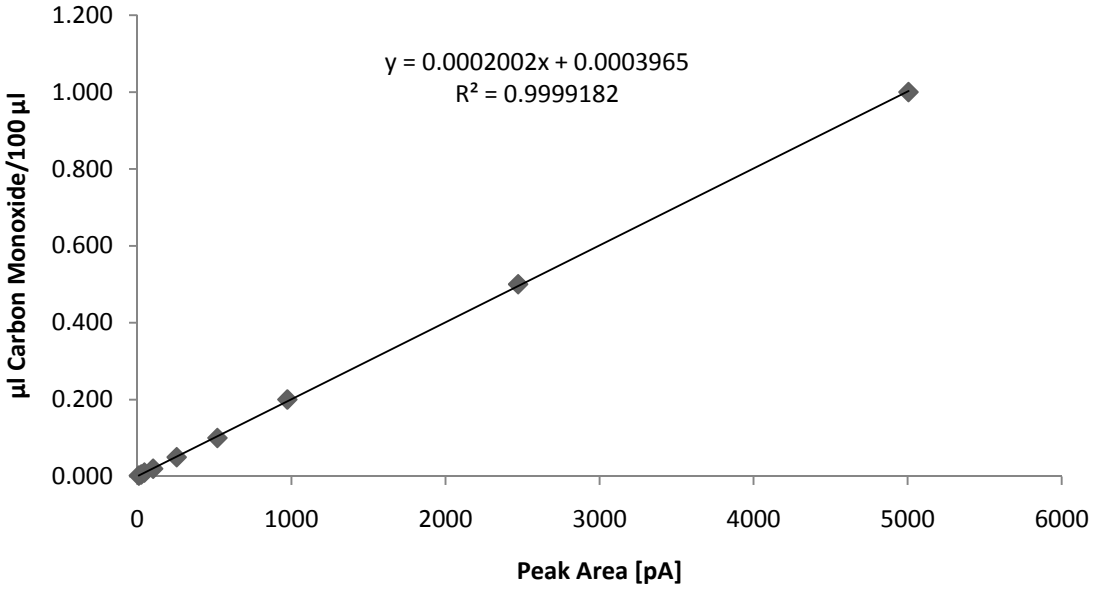

Figure 6-12. Carbon monoxide standard curve. This graph shows the average peak area for the injection of different quantities of carbon monoxide. The displayed equation results from the linear regression of the displayed data can be used to calculate the amount of carbon monoxide in a 100µl sample based on the peak area

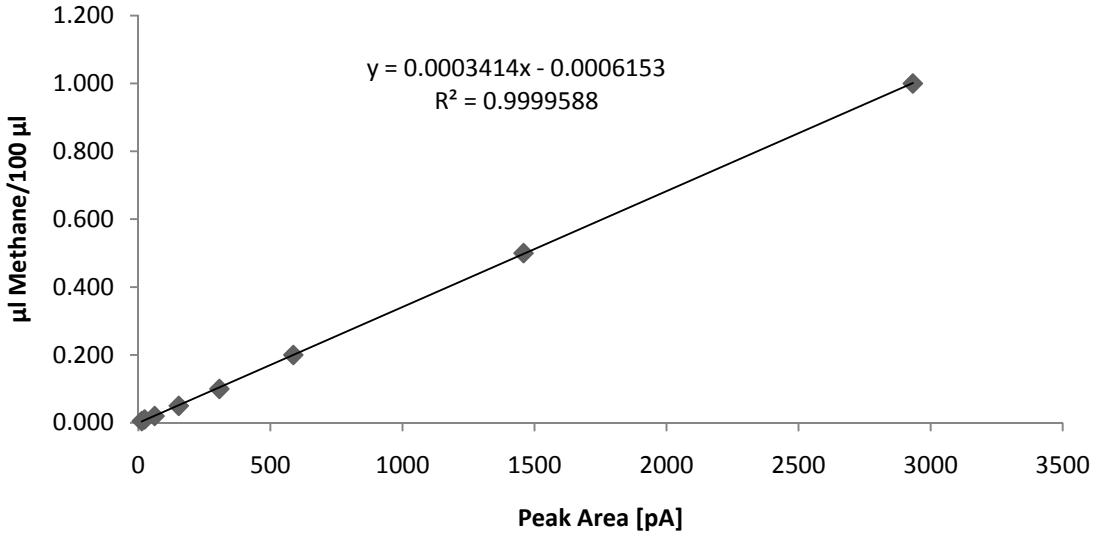

Figure 6-13. Methane standard curve. This graph shows the average peak area for the injection of different quantities of methane. The displayed equation results from the linear regression of the displayed data can be used to calculate the amount of methane in a 100µl sample based on the peak area

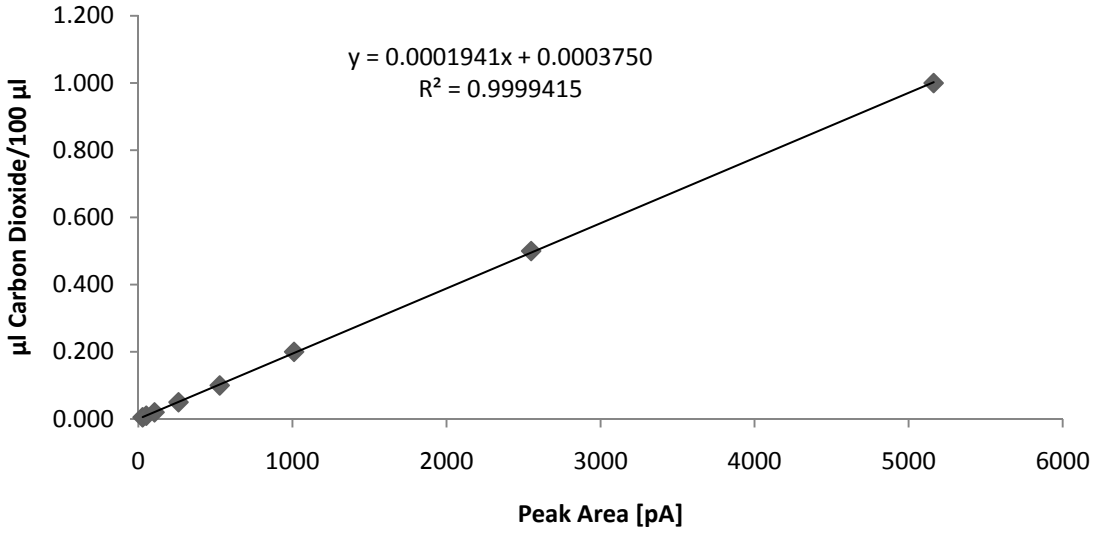

Figure 6-14. Carbon dioxide standard curve. This graph shows the average peak area for the injection of different quantities of carbon dioxide. The displayed equation results from the linear regression of the displayed data can be used to calculate the amount of carbon dioxide in a 100µl sample based on the peak area

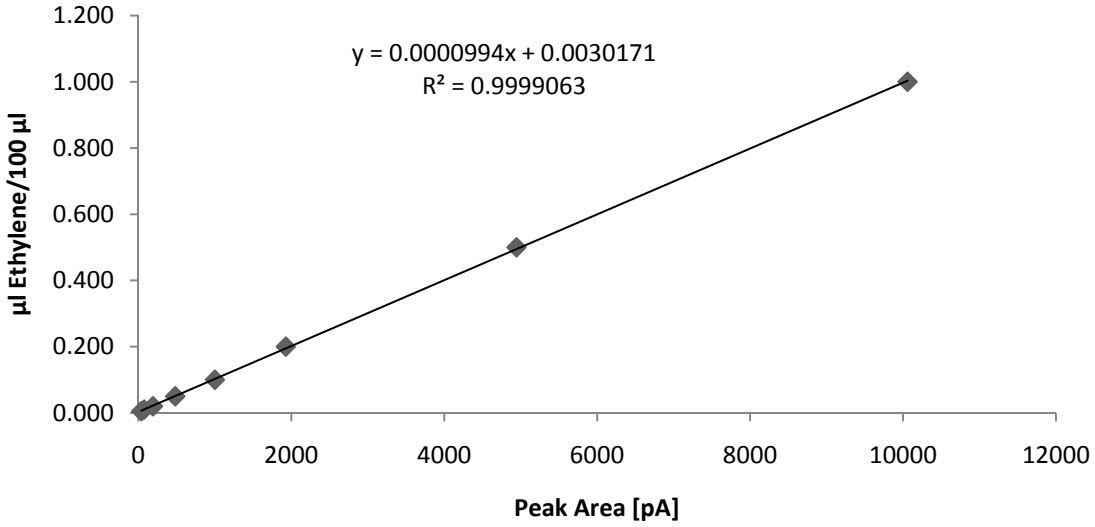

Figure 6-15. Ethylene standard curve. This graph shows the average peak area for the injection of different quantities of ethylene. The displayed equation results from the linear regression of the displayed data can be used to calculate the amount of ethylene in a 100µl sample based on the peak area

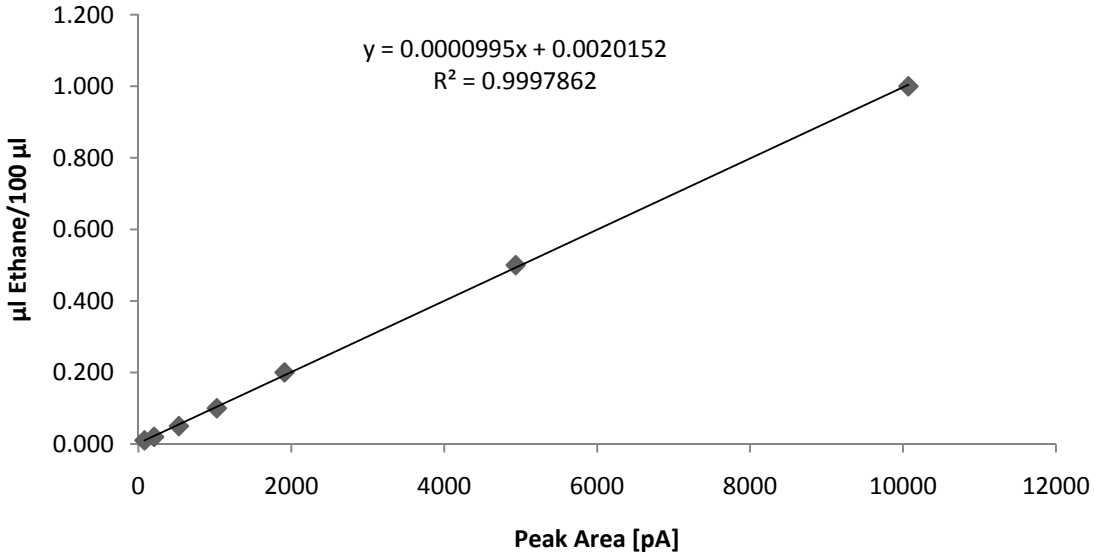

Figure 6-16. Ethane standard curve. This graph shows the average peak area for the injection of different quantities of ethane. The displayed equation results from the linear regression of the displayed data can be used to calculate the amount of ethane in a 100µl sample based on the peak area

Analysis of Treated Samples

The last step in the development of the rapid GC identification method was to find a way to actually determine whether a product was treated with filtered smoke or just a mixture of carbon monoxide and carbon dioxide, called artificial smoke. Previous experiments showed that when a certain amount of treated muscle tissue is heated at 100ºC for about 5 minutes in a sealed vial, hydrocarbons that were absorbed into the tissue during the treatment will get released into the headspace of the vial where the can be sampled with a syringe and then analyzed with the GC. Samples were taken from each treatment at day 2 after treatment and analyzed with the procedure described in "Material and Methods". Figures 6-17 to 6-24 display the chromatograms obtained by this analysis that represent the filtered smoke and artificial smoke treated samples as well as well as the control samples. Figure 6-21 shows the chromatogram of pure filtered smoke, directly analyzed with the GC. By comparing these figures it is obvious that in the headspace from artificial smoke treated samples the peaks for methane, ethylene and ethane are missing, as well as in the control samples. The filtered smoke treated samples show the peaks for carbon monoxide, methane, carbon dioxide and ethylene in the order they were eluted from the column respectively. Only the ethane peak is missing from the filtered smoke treated samples as well. A possible explanation for the missing ethane peak in the filtered smoke treated sample could be that ethane is not as well absorbed as other hydrocarbons or not as easily released by the sample. Nevertheless all chromatograms from the artificial smoke treated samples showed the same results as well as did the filtered smoke treated among each other and the control samples. Finally filtered smoke treated frozen tuna was obtained from a local ethnic grocery store and tested the same way. The chromatogram of this sample is shown in figure 6-20. Note that for all treatments several samples were obtained and analyzed with the GC method. For every sample two chromatograms were obtained and analyzed to ensure the method works accurately.

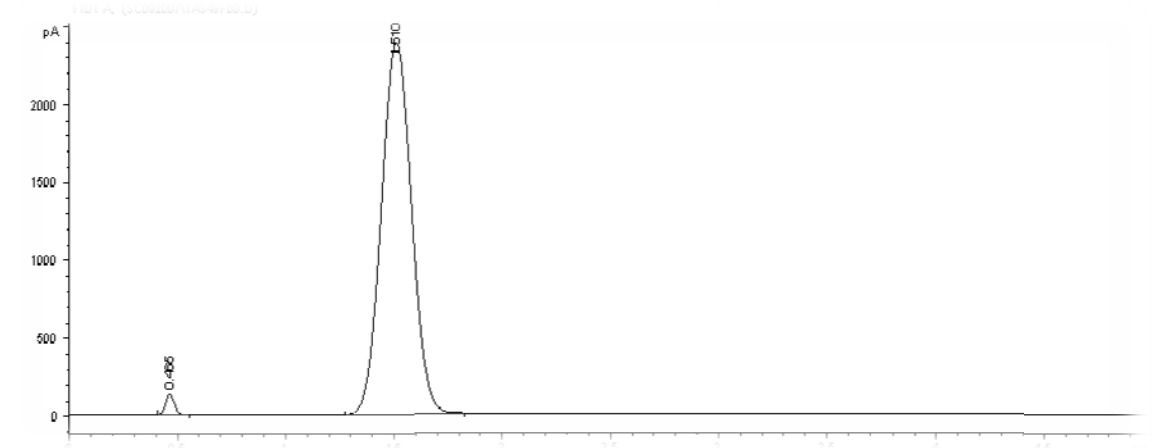

Figure 6-17. Chromatogram of the headspace analysis over a sample treated with artificial smoke. The first peak from left represents carbon monoxide and the second peak represents carbon dioxide. The area under a peak is proportional to the quantity of the representing compound.

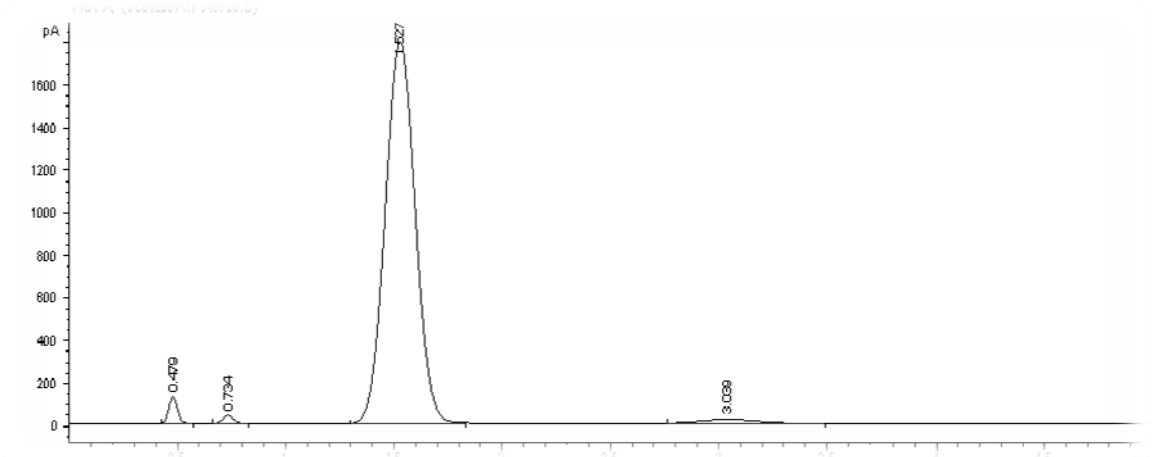

Figure 6-18. Chromatogram of the headspace analysis over a sample treated with filtered smoke. The first peak from left represents carbon monoxide, the second peak represents methane, the third peak represents carbon dioxide and the last peak represents ethylene. The area under a peak is proportional to the quantity of the representing compound.

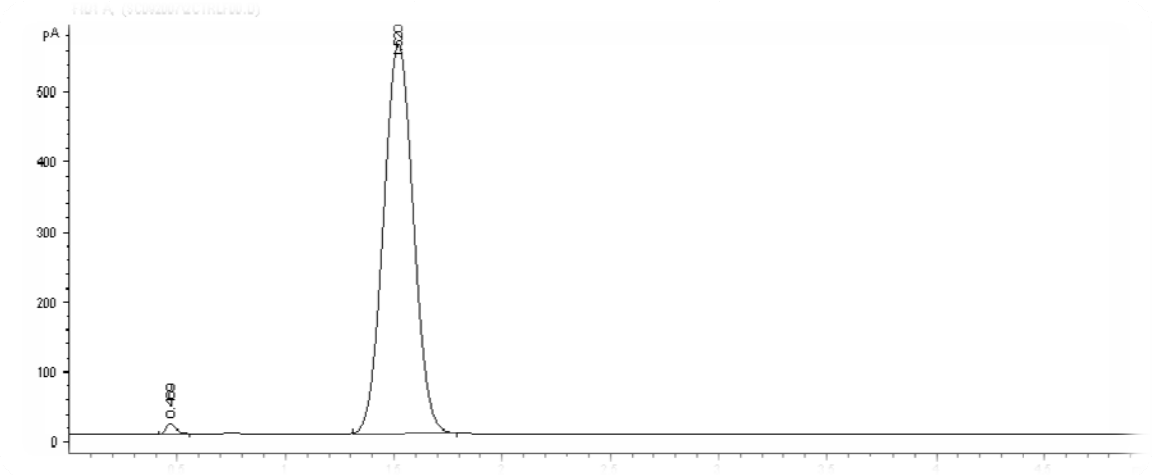

Figure 6-19. Chromatogram of the headspace analysis over an untreated control sample. The first peak from left represents carbon monoxide and the second peak represents carbon dioxide. The area under a peak is proportional to the quantity of the representing compound.

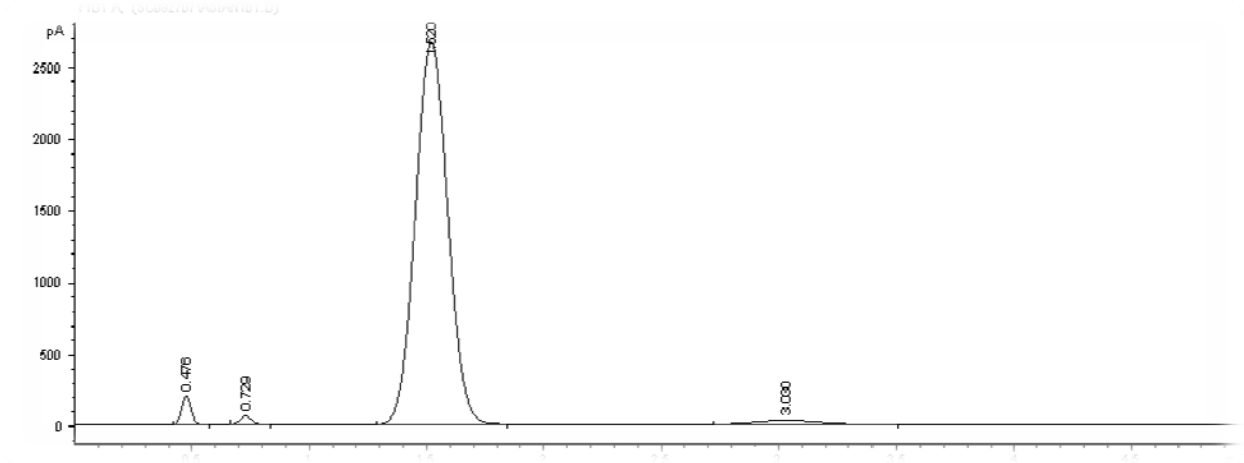

Figure 6-20. Chromatogram of the headspace analysis over a sample, purchased at a local ethnic store. The first peak from left represents carbon monoxide, the second peak represents methane, the third peak represents carbon dioxide and the last peak represents ethylene. The area under a peak is proportional to the quantity of the representing compound.

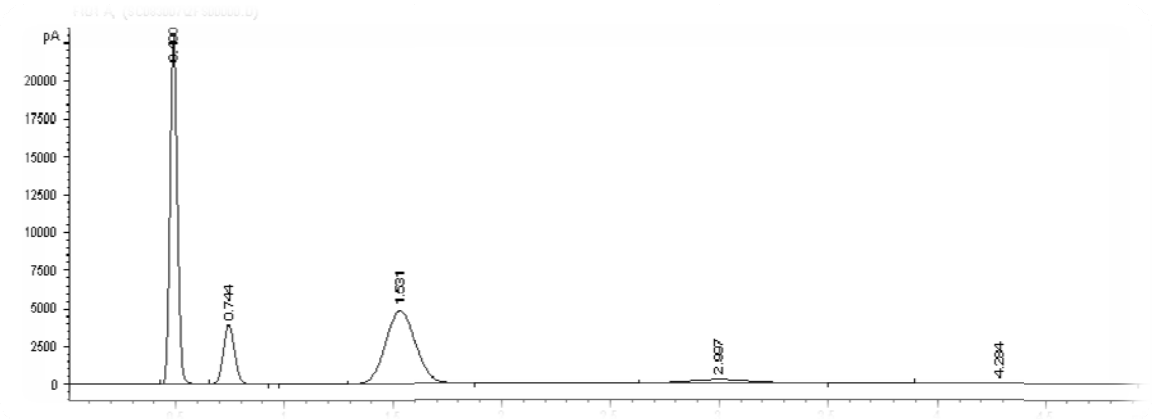

Figure 6-21. Chromatogram of the injection of 50 µl of pure filtered smoke. The first peak from left represents carbon monoxide, the second peak represents methane, the third peak represents carbon dioxide, the fourth peak represents ethylene and the last peak represents ethane. The area under a peak is proportional to the quantity of the representing compound.

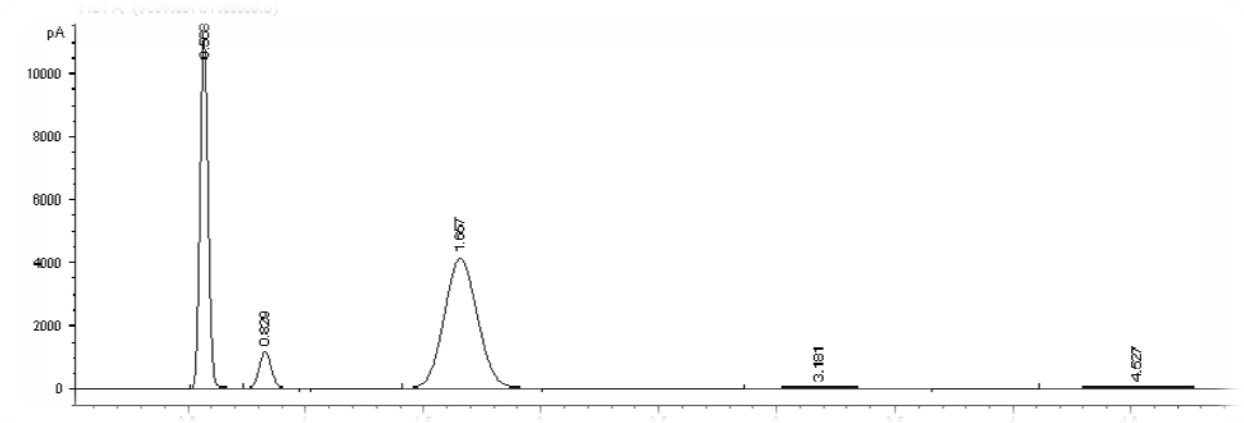

Figure 6-22. Chromatogram of the injection of 50 µl of pure filtered smoke "B". The first peak from left represents carbon monoxide, the second peak represents methane, the third peak represents carbon dioxide, the fourth peak represents ethylene and the last peak represents ethane. The area under a peak is proportional to the quantity of the representing compound.

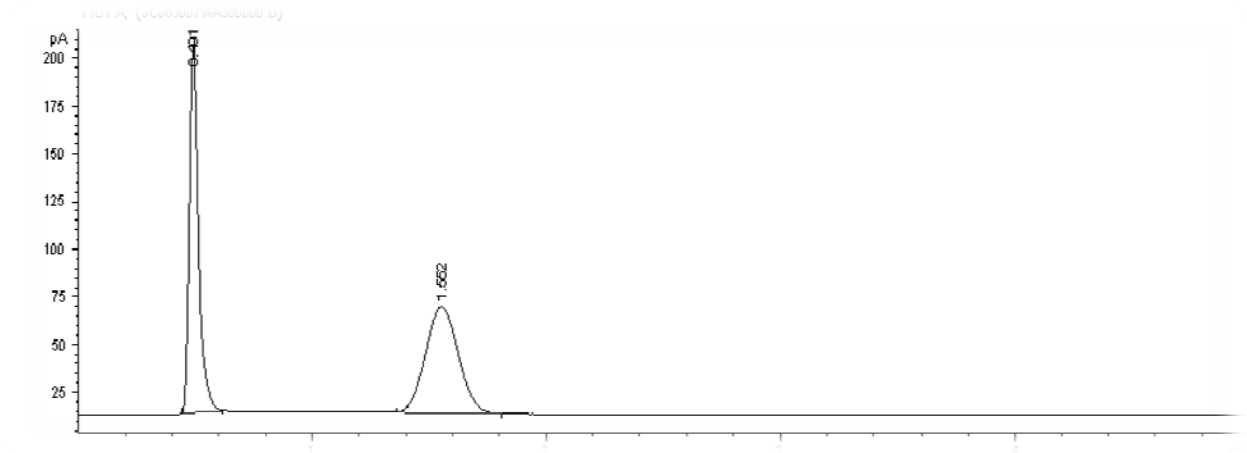

Figure 6-23. Chromatogram of the injection of 50 µl of pure artificial smoke. The first peak from left represents carbon monoxide and the second peak represents carbon dioxide. The area under a peak is proportional to the quantity of the representing compound.

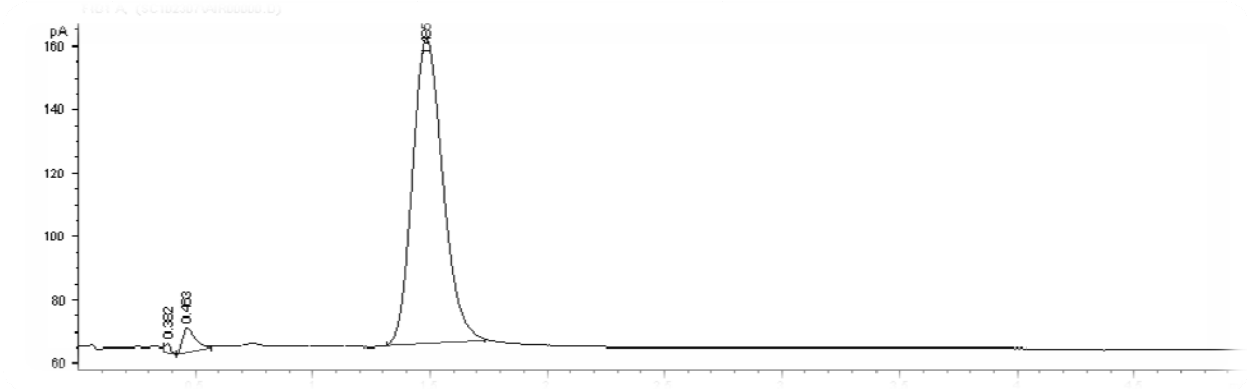

Figure 6-24. Chromatogram of the injection of 50 µl of air sampled from the surrounding lab environment. The first two small peaks from left represent noise from the injection and a very small amount of carbon monoxide. The third peak represents carbon dioxide. The area under a peak is proportional to the quantity of the representing compound.

CHAPTER 7
SUMMARY AND CONCLUSIONS

Smoking of meats and seafood has been a preservation method for a long period of time, although in the recent century it became more a flavoring agent than a necessity in order to preserve foods. From the early days, when the products were mainly cooked and dried, it became a specialized process that induces just the right amount of flavor in sophisticated processes. Today it is possible to smoke products at nearly 0°C and in the right humidity, so that the product does not differ in texture or appearance from a fresh product other then by its odor and taste. However, smoke can do more than that. The next development of smoking food products is called filtered wood smoke processing, a process that uses modern technology to clarify and filter the naturaly produced smoke and high tech processing to apply this filtered smoke to the product at the lowest possible temperature to retain a product that is nearly unchanged in texture and odor compared to its untreated fresh counterpart. From the beginning it was believed that filtered wood smoke has the potential to prolong the shelf life of fresh and frozen muscle foods and to enhance their appearance and color property. Today it is known that the color retention properties of filtered smoke relies on one of the main ingredients in filtered wood smoke, carbon monoxide, a potentialy hazardous gas for humans and animals that replaces the oxygen in the blood and muscle tissue and causes muscle relaxation and asphyxiation. However, just this effect makes it very interesting and attractive for the meat and seafood industry, since the new complex that is formed in this process results in a stable red color in these foods.

Filtered wood smoke is produced by burning wood chips at a high temperature without oxygen to produce smoke that is then filtered and concentrated in compressed gas cylinders. To compare if a mixture of the major gaseous components in filtered smoke would achieve the same

effect, artificial smoke was prepared from industrially available gases and used also in this project.

It is shown during this project that filtered and artificial smoke processing did not affect the growth or inhibition if *Salmonella* spp. as they seem to be more affected by their competitive microbial environment. No *Salmonella* spp. growth was reported at all in non-inoculated samples, which raises the question whether it is a real threat in fresh tuna or not. There was some inhibition effect of filtered and artificial smoke on the growth of aerobic spoilage bacteria during the first 4 to 6 days of storage, however it is suggested by the author that this effect does not enhance the shelf life of the product dramatically in this particular study. However, the combined effect of filtered and artificial smoke processing and subsequent frozen storage at -20ºC leads to a greater inhibition of aerobic spoilage bacteria then was observed in an untreated frozen product. The main effect observed in this study for filtered and artificial smoke processing is their ability to preserve and improve the color properties and appearance of red muscle foods as shown in the color experiment in this project. After the visual observation of a piece of tuna that was frozen in a vacuum bag without any treatment compared to a filtered or artificial smoke treated product it is very clear which product looks more red and fresh. The filtered and artificial smoke treated products have clearly an advantage over control.

During the whole study filtered smoke and artificial smoke treatments were always compared side by side and no significant differences were detected in any of the mentioned studies between filtered and artificial smoke treated samples. A consumer can not differentiate whether a product was treated with filtered or artificial smoke according to the taste panel results, based on either odor or appearance.

All these finding raise the final question what is really the difference between filtered smoke and artificial smoke. Why would we use filtered smoke or the less expensive artificial version? Artificial smoke contains industrial carbon monoxide, which is regulated by many countries in the world in its use as a food ingredient or additive. But filtered smoke, although containing the same carbon monoxide is considered a natural product, based on a long history of food processing with wood smoke in most of these countries. Since it is not obvious just by visual or sensorial inspection whether a product was treated with the original filtered smoke or its artificial counterpart the rapid GC identification method was developed to detect in a simple way whether a product was treated with filtered smoke or just with carbon monoxide. The results show that it is possible to identify a filtered smoke treated product based on the presence of three additional components that are not found in the artificial smoke mixture. The rapid method also allows the quantification of the residual amount of carbon monoxide in the muscle tissue when a standard curve for the quantification of carbon monoxide was established. It has to be acknowledged that an individual, who wants to deliberately misguide the consumer about the products treatment, could do so by adding all the ingredients into the artificial smoke that are tested by the rapid identification method. But this is a question of risk for every consumer and producer.

APPENDIX A
TASTE PANEL DEMOGRAPHICS AND COMMENTS

Table A-1. Comments from the Panelists for the Fresh Odor Triangle Test

Name	Treatment	Comments
Panelist 2	Filtered Smoke	this smell has a stronger smell
Panelist 8	Filtered Smoke	had a stronger aroma than the rest of them.
Panelist 10	Filtered Smoke	Smelled the same as 564\Smelled the same as 262
Panelist 13	Filtered Smoke	least fishy smell
Panelist 17	Filtered Smoke	smells like #546\smells like #262
Panelist 19	Filtered Smoke	less scent
Panelist 24	Filtered Smoke	same as the first one.\It smells odd like it was dead a week ago.
Panelist 25	Filtered Smoke	did not have as strong of a fishy smell as the other two
Panelist 26	Filtered Smoke	musky
Panelist 27	Filtered Smoke	Lighter smell
Panelist 32	Filtered Smoke	smells raw
Panelist 37	Filtered Smoke	not as strong of an odor
Panelist 41	Filtered Smoke	very little scent\little scent
Panelist 44	Filtered Smoke	This sample smells stronger than the other two.
Panelist 46	Filtered Smoke	It smelled very strong as well.\This sample smelled very strong.
Panelist 48	Filtered Smoke	smelled unfresh \, like paint\smelled unfresh, like paint
Panelist 52	Filtered Smoke	Smells like 546\seems the same as 262
Panelist 56	Filtered Smoke	It had a stronger/heavier smell
Panelist 57	Filtered Smoke	strong and smelled fatty
Panelist 59	Filtered Smoke	harsh smelling\more smelly
Panelist 2	Artificial Smoke	this smell is similar to the second one\smells like saltwater
Panelist 8	Artificial Smoke	smelled same as the 927 sample.\it really didn't smell like fish
Panelist 10	Artificial Smoke	Smelled worse (more fishy)
Panelist 12	Artificial Smoke	smells less fishy
Panelist 13	Artificial Smoke	fishy\strongest smell
Panelist 17	Artificial Smoke	sample #927 has a more potent smell, it smells saltier too.
Panelist 25	Artificial Smoke	very strong fishy smell\very strong fishy smell
Panelist 32	Artificial Smoke	smells putrid\bitter scent
Panelist 37	Artificial Smoke	strong smell\strong smell
Panelist 41	Artificial Smoke	more fishy odor
Panelist 43	Artificial Smoke	The same as the previous\Smells fresh out of the sea
Panelist 44	Artificial Smoke	This sample smells similar to 927.\This smells similar to 347.
Panelist 46	Artificial Smoke	The smell of this sample wasn't as strong as the other two.
Panelist 48	Artificial Smoke	smelled fresher, didn’t smell as much like paint
Panelist 52	Artificial Smoke	The scent is less stronger
Panelist 59	Artificial Smoke	has an odor of salmon

Only panelists who identified the odd sample correct were asked to leave comments. Numbers 546 and 262 refer to the filtered smoke treated sample. Numbers 347 and 927 refer to the artificial smoke treated sample. Samples were not previously frozen.

Table A-2. Comments from the Panelists for the Frozen Odor Triangle Test

Name	Treatment	Comments
Panelist 2	Filtered Smoke	smells cooked\smells cooked
Panelist 3	Filtered Smoke	This one was the same as number 466 and did not smell as strong and fishy as number 173\This one and number 882 both smelt fishy but the odor was not as strong and disgusting as number 173
Panelist 5	Filtered Smoke	can't really smell anything
Panelist 8	Filtered Smoke	it also smelled like chicken.\it smelled like chicken.
Panelist 9	Filtered Smoke	882 smelled like 466\It smelled like fish, and t was a bad smell.
Panelist 11	Filtered Smoke	not as strong as the others
Panelist 12	Filtered Smoke	smells less fishy
Panelist 13	Filtered Smoke	more like crab\smells more like crab
Panelist 14	Filtered Smoke	puungent smell like it had gone bad\subtle smell like canned tuna
Panelist 15	Filtered Smoke	similar to 173\scent not as strong
Panelist 18	Filtered Smoke	one of them smells less fishy than the other two.
Panelist 20	Filtered Smoke	this one smells like 466\this one smells very bitter
Panelist 24	Filtered Smoke	It has a distinc odor which can be smelled from other stink fish but it was kind of lighter smell than the first ones.
Panelist 26	Filtered Smoke	smells not as fresh\smells not as fresh
Panelist 29	Filtered Smoke	Smells salty
Panelist 30	Filtered Smoke	Doesnt smell as strong
Panelist 33	Filtered Smoke	same as 466\different smell
Panelist 35	Filtered Smoke	smelled very plain
Panelist 36	Filtered Smoke	stronger odor
Panelist 40	Filtered Smoke	weakest scent
Panelist 43	Filtered Smoke	Same as the first one\It doesnt even smell like tuna
Panelist 47	Filtered Smoke	No fishy smell
Panelist 50	Filtered Smoke	no strong odor\no strong odor
Panelist 53	Filtered Smoke	not sure. there was a slight smell of saltyness.
Panelist 54	Filtered Smoke	it has a lighter smell.
Panelist 59	Filtered Smoke	harsh smell, rot
Panelist 2	Artificial Smoke	smells like it went bad
Panelist 3	Artificial Smoke	This one smelt extra fishy
Panelist 5	Artificial Smoke	has a smell\has a smell
Panelist 8	Artificial Smoke	smelled like there was some sort of chemical on the fish.
Panelist 9	Artificial Smoke	792 did not really have much of a smell, as opposed to the other ones that did have a smell. It did not really smell like fish at all.
Panelist 11	Artificial Smoke	very strong\very strong
Panelist 12	Artificial Smoke	hardly has a smell\hardly smells like anything
Panelist 13	Artificial Smoke	didn't smell that much
Panelist 14	Artificial Smoke	exactly the same as 466
Panelist 15	Artificial Smoke	strongest smell
Panelist 18	Artificial Smoke	smells very fishy\it smells fishy
Panelist 20	Artificial Smoke	this one smells sweeter
Panelist 26	Artificial Smoke	smells very fishy
Panelist 29	Artificial Smoke	Does not have a strong odor\Does not have a strong odor
Panelist 30	Artificial Smoke	smells stronger\smells stronger
Panelist 33	Artificial Smoke	different smell
Panelist 35	Artificial Smoke	barely smelled at all\strong smell
Panelist 36	Artificial Smoke	weaker odor\weaker odor

Table A-2. Continued

Name	Treatment	Comments
Panelist 40	Artificial Smoke	most 'fishy'\mild scent
Panelist 43	Artificial Smoke	Smells rotted.
Panelist 47	Artificial Smoke	fishy smell\fishy smell
Panelist 50	Artificial Smoke	it has more odor
Panelist 53	Artificial Smoke	smelled a little stronger than the other one
Panelist 54	Artificial Smoke	not as strong as 466.\it has more of the typical fish smell.
Panelist 24	Artificial Smoke	Smells not that bad like the second\I couldn't figure out its smell.

Only panelists who identified the odd sample correct were asked to leave comments. Numbers 882 and 446 refer to the filtered smoke treated sample. Numbers 792 and 173 refer to the artificial smoke treated sample. Samples were not previously frozen.

Table A-3. Comments from the Panelists for the Fresh Color Triangle Test

Name	Treatment	Comments
Panelist 6	Filtered Smoke	darker\darker
Panelist 7	Filtered Smoke	Sample 119 had thicker veins than the other two.
Panelist 8	Filtered Smoke	much darker than the other two
Panelist 16	Filtered Smoke	more red\more red
Panelist 23	Filtered Smoke	the lines in it were different.\the lines in it were different.
Panelist 24	Filtered Smoke	dark\dark
Panelist 25	Filtered Smoke	darker
Panelist 27	Filtered Smoke	Aside from seeming harder, sample 119 seems darker as well.
Panelist 29	Filtered Smoke	119 is darker than 511, but just as dark as 730.\730 is darker than 511, but just as dark as 119.
Panelist 33	Filtered Smoke	This sample seems sliced differently than the other two samples.
Panelist 34	Filtered Smoke	This sample looks the best\There are thinner lines on this sample
Panelist 35	Filtered Smoke	Looks exactly like 119.\they are all the same color, but sample 119 and 730 are the same size and have the same amount of fat content and the same sort of lines in them (the grain of the meat).
Panelist 41	Filtered Smoke	This has a darker color.\This has a darker color.
Panelist 44	Filtered Smoke	lighter
Panelist 46	Filtered Smoke	There is a slight indentation in this sample that looks similar to that of sample 730, just not as long.\There is one barely noticeable line going through it. The color is very similar to that of sample 119.
Panelist 47	Filtered Smoke	Identical to 730, darker and more grainy than 378\Identical to 119, darker and more grainy than 378
Panelist 48	Filtered Smoke	Identical to 730.\Identical to 119.
Panelist 51	Filtered Smoke	Didn't have the defined serrations the other two samples had, also seemed lighter.
Panelist 54	Filtered Smoke	Solid red/pink color\Similar to 730 with solid pink/red color
Panelist 58	Filtered Smoke	\
Panelist 6	Artificial Smoke	it's lighter in color
Panelist 7	Artificial Smoke	378 looked liked 511\511 looked like 378
Panelist 8	Artificial Smoke	grainier\grainier and lighter
Panelist 16	Artificial Smoke	pinker
Panelist 23	Artificial Smoke	The lines in it were different.
Panelist 25	Artificial Smoke	lighter\lighter
Panelist 27	Artificial Smoke	Sample 378 appears more tender and soft than 119.\Sample 511 appears more tender than 119.

Table A-3. Continued

Name	Sample	Comments
Panelist 29	Artificial Smoke	it is the only pink piece (the lightest)
Panelist 33	Artificial Smoke	This sample has more uniformity than the other in its appearance.\It has a semi-circle shape line on its top.
Panelist 34	Artificial Smoke	Sample 378 seems to have two white streaks running through it which look like fat and, and therefore are unappetizing.
Panelist 35	Artificial Smoke	This sample is smaller than the other two and has more fat in the meat. You can see it in the grain.
Panelist 41	Artificial Smoke	This sample has a brighter color. Distinguishable.
Panelist 46	Artificial Smoke	this sample has four visible lines going through it diagonally. The color is also slightly darker than sample 730.
Panelist 47	Artificial Smoke	Lighter and less grainy than 730 and 119
Panelist 48	Artificial Smoke	Significantly lighter and pinker than the other samples.
Panelist 51	Artificial Smoke	Had defined serrations/grooves in it.\Had defined serrations/grooves in sample.
Panelist 54	Artificial Smoke	Striped appearance

Only panelists who identified the odd sample correct were asked to leave comments. Numbers 119 and 730 refer to the filtered smoke treated sample. Numbers 378 and 511 refer to the artificial smoke treated sample. Samples were not previously frozen.

Table A-4. Comments from the Panelists for the Frozen Color Triangle Test

Name	Treatment	Comments
Panelist 4	Filtered Smoke	416 has striations on it that none of the other 2 have.
Panelist 17	Filtered Smoke	lighter shade and luminous glow
Panelist 22	Filtered Smoke	it was lighter in color and had less lines
Panelist 23	Filtered Smoke	the lines in it were different
Panelist 26	Filtered Smoke	this samples\ seems layedand the grain is similar to sample 419\this sample is more smooth with less of vertical grain
Panelist 27	Filtered Smoke	416 appears tender and soft, much lighter than 614.\822 appears tender and light.
Panelist 28	Filtered Smoke	this one seems to be more red, a bit darker
Panelist 29	Filtered Smoke	416 is the lightest, it has little white strands in it.
Panelist 33	Filtered Smoke	It seems the same a sample 822.\it seems the same as sample 416.
Panelist 36	Filtered Smoke	416 seems to look sinewy and less like gelatin, but the difference is small
Panelist 46	Filtered Smoke	This piece has indentation in it and the shape is different than the first two. It also has visible white lines..
Panelist 49	Filtered Smoke	a dent on the surface\a dent in the surface
Panelist 50	Filtered Smoke	3 cuts. pinkish\3 cuts and pinkish
Panelist 54	Filtered Smoke	Pretty smooth appearance
Panelist 56	Filtered Smoke	its quite coarse\quite flat with little marks
Panelist 58	Filtered Smoke	this sample had fewer white lines and looked less 'defined' than the typical tuna steak cell structure
Panelist 59	Filtered Smoke	Lighter color, also seems to have less fatty tissue.
Panelist 4	Artificial Smoke	No striations.\No striations.
Panelist 17	Artificial Smoke	looks like the other\resembles 294
Panelist 22	Artificial Smoke	had lines\had lines
Panelist 23	Artificial Smoke	the lines in it were different\the lines in it are different
Panelist 26	Artificial Smoke	the filet seems like the grain is more vertical then horizontal
Panelist 27	Artificial Smoke	614 seems again not as soft and tender as the other samples. It also appears a little bit darker.
Panelist 29	Artificial Smoke	same as 614\same as 294
Panelist 33	Artificial Smoke	IT has whit division lines differently than the other two.
Panelist 36	Artificial Smoke	texture is slightly different, but still similar to 614\very cleanly cut and gelatin like
Panelist 38	Artificial Smoke	This sample does not contain any white areas or lines where the other two samples do. It appears uniform in color and texture.
Panelist 44	Artificial Smoke	lighter
Panelist 46	Artificial Smoke	Square piece that is thinner than 614 but the same color.\Square piece that is pink.
Panelist 49	Artificial Smoke	smooth texture
Panelist 50	Artificial Smoke	more salmon color
Panelist 54	Artificial Smoke	Striped and a bit jagged\White stripes
Panelist 56	Artificial Smoke	its more smoother than the rest
Panelist 58	Artificial Smoke	This one looked about the same compared to sample 614\This sample looked about the same as sample 294
Panelist 59	Artificial Smoke	darker color, same as 614\Darker color, same as 294

Only panelists who identified the odd sample correct were asked to leave comments. Numbers 416 and 822 refer to the filtered smoke treated sample. Numbers 614 and 294 refer to the artificial smoke treated sample. Samples were previously frozen.

Table A-5. Demographic Variance of the Odor Triangle Tests

Age Range	Under 18	18-20	21-24	Over 24	Total
Female	2	33	3	2	40
Male	0	14	6	0	20
Total	2	47	9	2	60

Note: The demographic variance for the fresh and frozen storage odor tests are the same.

Table A-6. Demographic Variance of the Color Triangle Tests

Age Range	Under 18	18-20	21-24	Over 24	Total
Female	1	31	3	1	36
Male	0	15	7	2	24
Total	1	46	10	3	60

Note: The demographic variance for the fresh and frozen storage color tests are the same.

APPENDIX B
COLOR STUDY PICTURES

Figure B-1. Control group images for the frozen storage study captured by the CMVS. a) Image of samples before treatment was applied. b) Image of samples immediately after treatment was applied and ceased (here no treatment – control group). c) Image of samples after 30 days of frozen storage (-20ºC) followed by 14 days of refrigerated storage (4ºC).

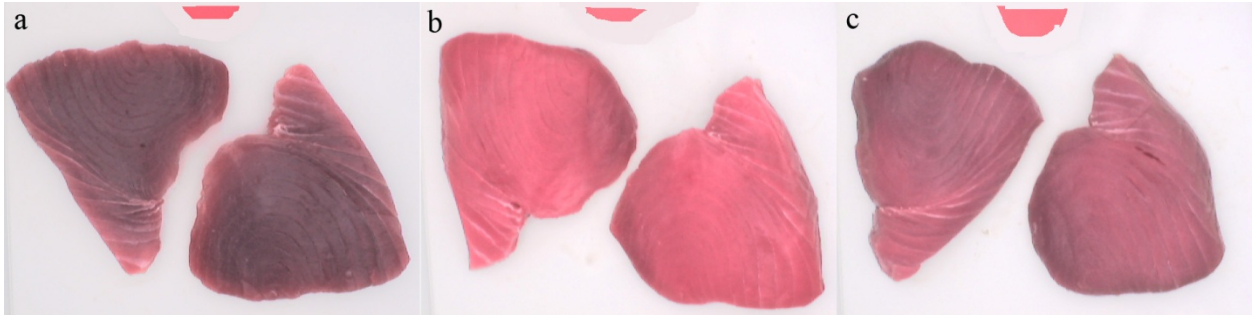

Figure B-2. Filtered smoke (24h-treatment) group images for the frozen storage study captured by the CMVS. a) Image of samples before treatment was applied. b) Image of samples immediately after treatment was applied and ceased. c) Image of samples after 30 days of frozen storage (-20ºC) followed by 14 days of refrigerated storage (4ºC).

Figure B-3. Filtered smoke (48h-treatment) group images for the frozen storage study captured by the CMVS. a) Image of samples before treatment was applied. b) Image of samples immediately after treatment was applied and ceased. c) Image of samples after 30 days of frozen storage (-20ºC) followed by 14 days of refrigerated storage (4ºC).

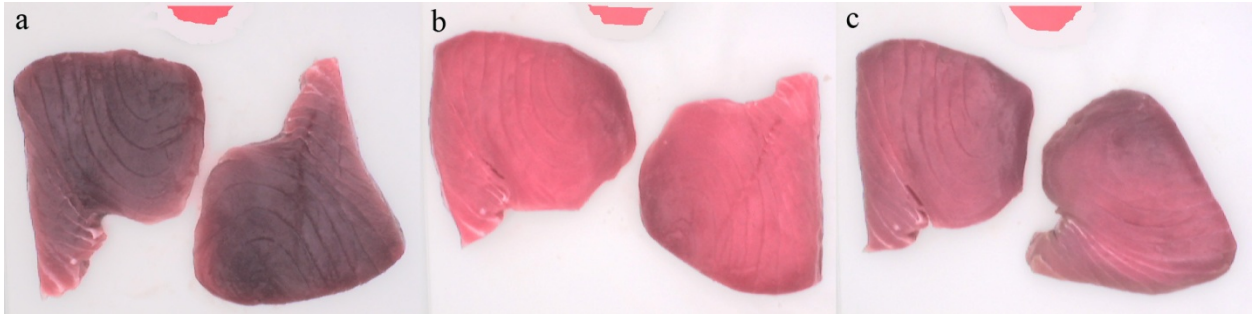

Figure B-4. Artificial smoke (24h-treatment) group images for the frozen storage study captured by the CMVS. a) Image of samples before treatment was applied. b) Image of samples immediately after treatment was applied and ceased. c) Image of samples after 30 days of frozen storage (-20ºC) followed by 14 days of refrigerated storage (4ºC).

Figure B-5. Artificial smoke (48h-treatment) group images for the frozen storage study captured by the CMVS. a) Image of samples before treatment was applied. b) Image of samples immediately after treatment was applied and ceased. c) Image of samples after 30 days of frozen storage (-20ºC) followed by 14 days of refrigerated storage (4ºC).

Figure B-6. Control group images for the fresh storage study captured by the CMVS. a) Image of samples before treatment was applied. b) Image of samples immediately after treatment was applied and ceased (here no treatment – control group). c) Image of samples after 14 days of refrigerated storage (4ºC).

Figure B-7. Filtered smoke (24h-treatment) group images for the fresh storage study captured by the CMVS. a) Image of samples before treatment was applied. b) Image of samples immediately after treatment was applied and ceased. c) Image of samples after 14 days of refrigerated storage (4ºC).

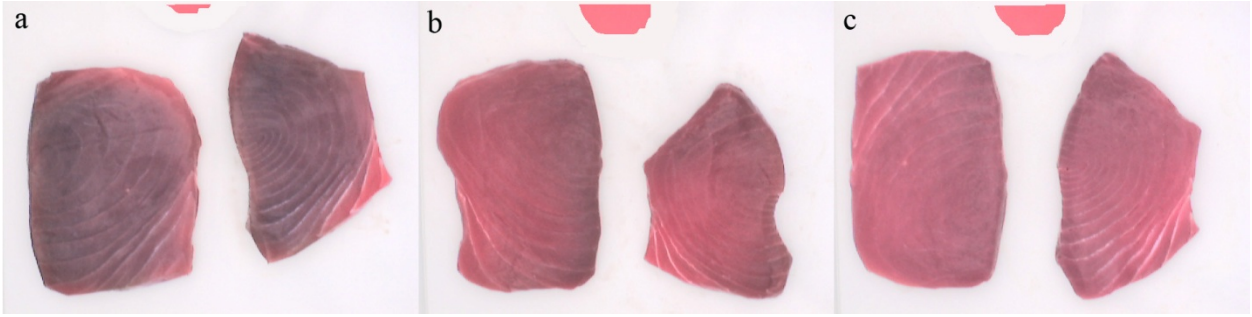

Figure B-8. Filtered smoke (48h-treatment) group images for the fresh storage study captured by the CMVS. a) Image of samples before treatment was applied. b) Image of samples immediately after treatment was applied and ceased. c) Image of samples after 14 days of refrigerated storage (4ºC).

Figure B-9. Artificial smoke (24h-treatment) group images for the fresh storage study captured by the CMVS. a) Image of samples before treatment was applied. b) Image of samples immediately after treatment was applied and ceased. c) Image of samples after 14 days of refrigerated storage (4ºC).

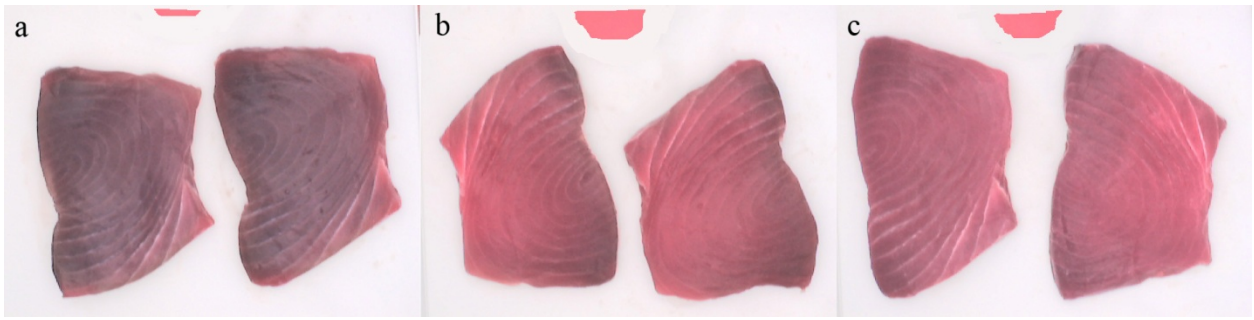

Figure B-10. Artificial smoke (48h-treatment) group images for the fresh storage study captured by the CMVS. a) Image of samples before treatment was applied. b) Image of samples immediately after treatment was applied and ceased. c) Image of samples after 14 days of refrigerated storage (4ºC).

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BIOGRAPHICAL SKETCH

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