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ABSTRACT

THE EFFECT OF PROCESSING VARIABLES ON THE SAFETY AND ACCEPTABILITY OF SMOKED GREAT LAKES WHITEFISH

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The effect of various levels of salt concentrations (water-phase) on the lipid stability and the botulinal safety of smoked whitefish was investigated. A 4 percent (water-phase) salt concentration prevented the outgrowth of Clostridium botulinum type E spores in smoked whitefish held for 42 days at 27°C. The addition of nitrite to smoked salted (4%) whitefish prevented spore outgrowth for 63 days at 27°C.

Unsalted smoked whitefish prepared with or without nitrite was significantly ($P<0.01$) less rancid after 22 days of refrigerated storage than smoked whitefish containing salt concentrations (water-phase) of 2, 4 or 6 percent. However, sensory evaluations of these treatment samples throughout the refrigerated storage period indicated a significant ($P<0.05$) panelist preference for smoked whitefish containing 4 percent salt (water-phase).

The effect of smoke type (woodsmoke or liquid) and the level of liquid smoke on lipid stability and organoleptic acceptability of smoked whitefish was investigated. Woodsmoke-treated whitefish prepared with or without nitrite

had significantly ($P<0.01$) lower levels of rancidity than fish treated with increasing levels of liquid smoke during 22 days of refrigerated storage. Sensory evaluation of the whitefish samples treated with woodsmoke or liquid smoke indicated a significant ($P<0.05$) panelist preference for the woodsmoke-treated samples on day 0. However, by the 14th day of refrigerated storage, panelists were unable to distinguish differences between sample treatments.

Nitrite was shown to be an effective antioxidant and antibotulinal agent in smoked whitefish. In addition, nitrited whitefish did not contain any detectable levels of volatile N-nitrosamines or N-nitrosothiazolidine carboxylic acid. Therefore, the addition of nitrite in combination with four percent salt (water-phase) can be recommended for use in the production of smoked whitefish.

To my mother for her continued support
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to Dr. J.F. Price for his friendship

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INTRODUCTION

Considerable amounts of processed smoked foods including meats, cheese and fish are consumed in the United States. These products are smoked by traditional wood smoking methods or by the use of liquid smokes (Daun, 1979). Smoking of foods was originally a means of food preservation. However, smoking of foods today is done primarily to add smoke flavor and color to the product (Sink, 1979).

The production of smoked fish in the state of Michigan requires that the finished product contain 5% salt in the water-phase portion of the finished product (Stachiw et al., 1984). This stipulation was established to prevent botulism. Botulism is a food borne disease caused by the ingestion of the potent neurotoxin produced by the bacterium Clostridium botulinum. Because of the widespread distribution of C. botulinum in marine and freshwater environments, contamination of fish products by this organism is difficult to avoid (National Academy of Sciences, 1981). The most prevalent type of C. botulinum in the marine environment is type E (Schmidt et al., 1962; Pelroy et al., 1982). Type E is non-proteolytic and can grow at refrigerator temperatures. Therefore, it can be present in refrigerated

foods without any obvious signs of putrefaction and its associated off-odors (Eklund, 1982).

The safety of smoked fish relies on the ability of the salt concentration in the water-phase of the finished product to inhibit the outgrowth of C. botulinum type E spores. Since the consumer is becoming more aware of the relationship between dietary salt intake, hypertension and its associated health problems, long term acceptability of this salty product could be limited in today's society.

Warmed-over flavor (WOF) is a term used to describe the rapid development of oxidative rancidity occurring in meats that are cooked, held at refrigerator or freezer temperatures, and then reheated before serving. WOF is important in red meats and poultry. Research involving red meats and poultry indicates that the phospholipid fraction of the animal fat is primarily responsible for the development of WOF (Igene et al., 1979). In these products, the phospholipids contain a greater percentage of the unsaturated fatty acids than the triglyceride fraction. It has been shown that the rate of lipid oxidation is affected by the degree of unsaturation occurring in the lipid system (Dawson and Gartner, 1983). WOF is anticipated to occur in smoked fish products since fish lipids contain a significant amount of long chain polyunsaturated fatty acids (Khayat and Schwall, 1983). In addition, smoked fish can be held at refrigerator temperatures for as long as 14 days prior to

consumption.

A primary catalyst of WOF has been shown to be the heme compounds of the muscle tissue (Pearson et al., 1977) or free iron released from the meat pigments during cooking (Igene et al., 1979). It has been suggested that during cooking, the heme-containing proteins denature and unfold, exposing the iron molecule. The free iron molecule is a strong catalyst of lipid oxidation. Nitrite has been shown to be an effective inhibitor of WOF development in both red meats and poultry, but it has not been tested in fish products. Nitrite is believed to bind the iron in the heme-containing pigments of meats forming a non-reactive species, thus preventing the accelerated rate of lipid oxidation normally seen in the development of WOF.

The addition of nitrite to smoked fish has been clearly demonstrated to allow a decrease in the salt concentration while maintaining safety against botulinal toxin production in temperature abused products (Pelroy et al., 1982). However, the addition of nitrite to fish products could result in the formation of N-nitrosamines from the reaction of the added nitrite and the free amines of fish (Shewan, 1951; Castell et al., 1971; Golovyna, 1976). Research has shown that N-nitrosamines can be formed in fish products, although the data are extremely variable (Malins et al., 1970; Sen et al., 1970; Fazio et al., 1971; Gadbois et al., 1975). The formation of N-nitrosamines in fish products

occurs primarily in saltwater species. This is understandable, since the saltwater species contain high levels of trimethylamine and trimethylamine oxide, which readily break down to produce formaldehyde and dimethylamine. Dimethylamine is readily nitrosated to produce N-nitrosodimethylamine (NDMA) (Dyer and Mounsey, 1949; Spinelli and Koury, 1979; Sikorski and Kostuch, 1982).

The purpose of this project was to investigate the effect of brining and brine salometer levels on the salt uptake in whitefish and to study the feasibility of lowering the level of salt in the finished smoked fish product, while maintaining organoleptic acceptability and safety against botulinal toxin production. This required investigating the antibotulinal and the antioxidant activity of nitrite in the smoked fish product. Another objective of this study was to investigate the effect of smoke (wood and liquid), with or without the addition of nitrite, on the organoleptic acceptability of smoked fish. Before advocating the use of nitrite in smoked fish, it was necessary to assess whether the addition of nitrite would result in the formation of N-nitrosamines in the fish products. Research was conducted to correlate the TBA values of baked whitefish, stored in the refrigerator, with the sensory evaluation of rancidity of the product. Finally, this study involved profiling and identifying the major flavor volatiles in baked whitefish, particularly as they pertain to lipid oxidation.

LITERATURE REVIEW

Michigan Fishing Industry

The state of Michigan is surrounded by four of the five Great Lakes; Superior, Michigan, Huron and Erie (Stachiw et al., 1984). Therefore, it is understandable that Michigan has a strong fishing industry. Michigan's first commercial fishing industry was established on Lake Erie about 1820. By 1840, fisheries were established on Lakes Michigan and Huron (Bulletin E-1000, 1977). Whitefish was the mainstay of these early fisheries (Bulletin E-1000, 1977), and whitefish has remained the major utilized species in the Great Lakes, with an average annual catch of 3.6 million pounds (Stachiw et al., 1984). Approximately two-thirds of the whitefish caught in Michigan are from Lake Michigan. Lake Superior and Lake Huron each produce about one half of the remaining catch. These figures do not account for the catch taken by the Indian tribes along the Lakes by Canadian commercial catches. Their catch is estimated to account for approximately 1.9 million pounds (Stachiw et al., 1984). Approximately half of all whitefish caught commercially are eventually processed and sold as a smoked product (Booren, 1984).

Regulation of Smoked Fish Production in Michigan

Fish processors are being confronted with an increasing demand for higher quality product by the consumer. Because the fish processing industry is a food industry, it is subject to food regulations at both federal and state levels (Stachiw et al., 1984). In Michigan, the production of smoked fish is controlled by Michigan Regulation 541 (Michigan Department of Agriculture, 1965).

This regulation requires that the fish processor must (1) cook all product to an internal temperature of 180°F in the coldest part of the fish, and maintain that temperature for not less than 30 minutes; (2) insure that all smoked fish product contains in its water phase portion, a salt content of not less than 5%; (3) maintain the product under refrigeration (36°F) at all times, excluding the time necessary for smoking operations; and (4) must provide a label on the finished product which carries a warning statement indicating smoked fish should not be sold or consumed after 14 days of refrigerated storage (Michigan Department of Agriculture, 1965).

The Salting/Brining of Fish

Salting is one of the oldest methods used for the preservation of meats and, in particular, fish. Traditionally, the high levels of salt used in smoked fish acted to preserve the product by lowering its water activity (Deng,

1977). Today, because of the availability of refrigeration, there is less need for heavy salting and the levels of salt and smoke used are primarily for flavor acceptability (Deng, 1977).

There is little information on the rate of salt penetration into muscle. Kormendy and Gartner (1958) reported that the diffusion of salt into meat depended on the ratio of the amount of brine to the amount of meat within certain limits, and on the duration of the curing time. They also noted that during prolonged curing, the amount of salt penetration into the muscle would cease, although equilibrium had not been established. They theorized that the swelling that occurred during salting causes the external layers of the muscle to become closed to further salt penetration.

Crean (1961) studied the problem of salt penetration in fish muscle. He reported that during dry-salting, the average water and salt contents of fish muscle were inversely and linearly related, indicating that the rate of salt uptake was in constant ratio with the rate of water loss. This led to the postulation that the salt and water exchange was primarily confined to a region of "denatured" muscle which acted to create a "front" at which denaturation occurred. This front could move into the muscle and it was behind this front that the bulk of the salt and water exchange occurred. A final suggestion by Crean (1961) was that the driving force for the salt penetration into fish muscle might be

the difference in the concentration between the average internal fish brine and the ambient brine.

Hamm (1960) studied the factors which affected the amount of swelling occurring in fish muscle during brining. He reported that the amount of tissue swelling depended upon the manner in which the brine immersion was carried out. If the strength of the brine was increased gradually, the muscle's weight gain was much larger than if the brining occurred in full strength brine. During the brining equilibration, one of two events could occur. These were: a) at low or intermediate salt concentrations, the water is transferred from the brine into the muscle and the muscle swells, or b) at salt concentrations beyond a certain point, water is transferred from the muscle to the brine. If the salt content of the brine is high enough, the muscle protein will coagulate or salt-out. Final equilibrium between the brine of a given concentration and the muscle which is immersed in the brine has been taken as an equality of the salt concentration of the brine with the total water inside the muscle (Del Valle and Nickerson, 1967a).

The factors which affect the rate of salt penetration in fish muscle have been studied. However, most of the research has been directed towards producing a dried salted fish such as found in countries where fish is a staple in the diet, i.e., developing countries (Del Valle and Nickerson, 1967; Deng, 1977; Doe et al., 1982; Poulter

et al., 1982).

Del Valle and Nickerson (1967) in a series of studies investigated the equilibrium considerations that apply to the salting and drying of fish. They found that the coefficient for the penetration of salt into fish muscle (swordfish) was not constant but depended upon the salt concentration of the brine and the temperature.

In a second set of studies, Del Valle and Nickerson (1967) determined that the primary variables affecting salt uptake by the fish muscle were: 1) salt concentration of the brine, 2) volume of the muscle, 3) distribution coefficient of the salt between the muscle volume and the brine and 4) distribution coefficient of the salt between the muscle tissue water and the brine. Secondary variables affecting the salting equilibria were functions of the salt concentration in the brine.

Deng (1977) found that salt penetration into whitefish muscle can be affected by the storage conditions prior to the actual salting. Freezing acted to increase the rate of salt penetration. Fish that had been processed fresh had a salt uptake rate of 0.006 g salt/g sample/min and a rate constant of 0.018 min^{-1}. Fish frozen for 1 week had a salt uptake rate of 0.014 and a rate constant of 0.029. However, fish frozen for 3 and 5-9 weeks had salt uptake rates of 0.011 and 0.009 and rate constants of 0.025 and 0.018, respectively. These changes in salt penetration closely

followed changes in the amount of extractable actinomyosin in the muscle, indicating that the rate of salt uptake depends on the degree of denaturation of the fish proteins that occurred during freezing. Deng (1977) reported that when the brine concentration used with fresh fish exceeded 20% salt, the water migrated from the muscle into the brine, and the reverse occurred in brine less than 15% salt. In fish frozen for 2 months, a brine of 25% or more salt was required before the water was transferred from the muscle to the brine. Fish could be brined in salt concentrations up to 20% and still have salt penetration.

Incidence of *Clostridium botulinum* Type E in Smoked Whitefish

Botulism is a foodborne disease caused by ingesting the potent neurotoxin produced by the bacterial genus *Clostridium botulinum*. *C. botulinum* is a rod shaped, anaerobic, spore-forming bacterium of which there are 7 types (Kautter and Lynt, 1978). The toxins of all seven types of *C. botulinum* are probably toxic to man, but in the United States only types A, B and E are epidemiologically important. This is a result of the natural distribution of the *C. botulinum* types in the environment, the food habits of various ethnic groups and the method(s) of food processing used, especially prior to consumption (Pivnick and Bird, 1965).

Type E is the most prevalent type of C. botulinum in the marine environment, except in Southern California where type A predominates (Schmidt et al., 1962; Pelroy et al., 1982). C. botulinum type E was first isolated and identified from Russian sturgeon in 1936 (Kautter, 1964). C. botulinum type E has never been isolated in the southern hemisphere (Insalata et al., 1967). Epidemiological evidence indicates that C. botulinum type E occurs in significant numbers only in latitudes higher than 40^{o} North. Type E C. botulinum occurs most frequently in Northern Japan, British Columbia, Alaska, the Soviet Union and Western Europe. It occurs less frequently in other areas such as the Pacific Northwest, along the sub-Arctic and Arctic Perimeters, and it may occur in such areas as the Mediterranean, the Gulf of Mexico and the Great Lakes (Johannsen, 1965).

In 1957, it was established that C. botulinum type E was distributed in the marine sediments of the Canadian Pacific Coast (Johannsen, 1965; Emodi and Lechowich, 1969). The organism was not identified in the Great Lakes until 1960, when a botulinal outbreak was traced to smoked ciscoes (Christiansen et al., 1968; Pace et al., 1972; Pace and Krumbiegel, 1973). Further evidence of the presence of C. botulinum type E in the Great Lakes was provided in 1963 when two additional outbreaks of type E botulism were traced to smoked fish that had been processed in the Great Lakes area (Bott et al., 1966; Christiansen, 1968; Pace

et al., 1972). Bott et al. (1966) reported that fish caught in Lakes Erie, Superior, Huron and Michigan had C. botulinum spores and vegetative cells in their intestinal tract, at levels of contamination of 1%, 1%, 4% and 9%, respectively. They also noted that 56% of the 835 fish samples taken from the Green Bay area of Lake Michigan harbored C. botulinum type E.

Pace et al. (1968) examined 1071 whitefish chub samples throughout the eight stages of processing in order to demonstrate the frequency or source of C. botulinum contamination in smoked fish products. They tested samples from different stages aboard ship, the routine in the smoking plant, and on display in retail cases. They reported that 13-14% of freshly caught, eviscerated chubs were contaminated. The highest level of contamination (20%) was found among chubs sampled at the brining stage of processing. Levels of contamination in the chubs at processing stages before smoking ranged from 6-14%. After smoking at 180^{o}F for 30 minutes, 10 out of the 858 samples tested were found to be contaminated. Nine of these samples contained C. botulinum type E, while one had type B spores. Pace et al. (1972) attributed post smoking contamination to the processor's practice of taking the cooked product back into the raw processing area before packaging. Regardless of process treatment, this problem will not change and must be corrected by improved handling practices.

The differences in the growth requirements of Clostridium botulinum types A and E are summarized in Table 1. The types of C. botulinum can be characterized as proteolytic or nonproteolytic. The proteolytic types (A, B and F) are capable of producing putrefactive growth and its associated off-odors. They have a minimum required growth temperature of 10°C (50°F) and growth is inhibited by pH levels of less than 4.6. These types of C. botulinum are the most heat resistant. High water-phase salt concentrations (8-9%) and water activities of 0.93 are necessary for their inhibition (Eklund, 1982; Sperber, 1982).

The non-proteolytic types of C. botulinum (B, E and G) are more sensitive to heat. They are inhibited by pH levels of less than 4.6, water-phase salt concentrations of 5-6% and water activities of 0.96. However, they can grow at temperatures as low as 3.3°C (38°F), and because they are non-proteolytic, their growth is not accompanied by detectable putrefaction and off-odors (Eklund, 1982).

Fresh or frozen fish products have never been implicated in any recorded human botulism outbreaks in the United States. There are several reasons for this: (1) most fish products are cooked prior to consumption; (2) the endogenous microflora causes rapid quality deterioration, preventing consumption of the fish product; and (3) the spoilage organisms usually outnumber C. botulinum and, in some cases, their proteolytic enzymes inhibit and/or inactivate the

Table 1. Summary of the growth characteristics of *Clostridium botulinum* types A and E.[a]

Characteristic	Type A	Type E
Proteolytic activity	+	-
Heat-resentant spores	+	-
Minimum growth temperature (^{o}F)	50	38
(^{o}C)	10	3.3
Minimum water activity	0.94	0.97
Salt, water-phase	(8-9%)	(5-6%)
Minimum pH	4.6	4.6
Maximum Eh (mV)	-250	-250

[a]From Sperber (1982).

botulinal toxin (Eklund, 1982).

Preservation of fish by salting and/or smoking dates back into antiquity. The development of refrigeration has resulted in modifications of the traditional salt-smoking procedures. There is less need for the heavy salt and smoke levels, and the product does not need to be so severely dehydrated (Eklund, 1982). The hot-process smoking procedures used today inactivate the endogenous spoilage microflora and/or inhibit their growth. However, the hot-process smoking procedures do not destroy even the most heat sensitive of the C. botulinum spores. This can possibly lead to botulinal outbreaks (Eklund, 1982). Most of the reported botulinal outbreaks have been traced to a lack of understanding by both the consumer and the retailer of the proper handling procedures for this product. Salted smoked fish must be handled in the same manner as any perishable food product (Morbidity and Mortality, 1963).

Factors Affecting the Outgrowth of Clostridium botulinum Type E Spores in Smoked Whitefish

The safety of salted-smoked fish products against the outgrowth of C. botulimum spores is dependent on the inhibitory effects of the salt and the cooking parameters used during processing. Other factors which affect the rate of spore outgrowth are storage temperature, water activity, pH of the product and whether any antibotulinal

agents such as nitrite or sorbate are used (Sperber, 1982).

Salt

Abrahamsson et al. (1966) reported that the lowest level of salt necessary to inhibit toxin production by C. botulinum type E spores varied with incubation temperature and length of storage. The inhibitory effect of salt on toxin production was greatest at lower temperatures. Using Robertson's meat medium inoculated with 10^5 spores/g, they reported that a salt concentration of 4.5% was completely inhibitory to C. botulinum type E toxin formation even at optimal temperature (30^oC.

Cann and Taylor (1979) reported that in naturally contaminated hot-smoked trout and mackerel, a minimum of 2.5% water-phase salt concentration was needed to prevent toxin production for 30 days by C. botulimum type E spores. However, when processed fish were inoculated with 10^3 spores/g, vacuum packaged and stored at 10^oC and 20^oC, a minimum of 3.0% water-phase salt concentration was necessary to prevent toxin production for 30 days and 1 day, respectively. Roberts and Ingram (1973) studied the effects of various levels of pH, salt and sodium nitrite on the inhibition of C. botulinum types A, B, E and F vegetative cells. Type E vegetative cells were the most sensitive to each of the variables tested (pH, salt and nitrite) and to combinations of these variables. Using a medium containing trypticase, bacto-peptone, yeast extract and cysteine HCl,

C. botulimum type E spores were added at a level of 10^5 vegetative cells/20 ml of medium and incubated at 35°C for 3 months. The results indicate that a 4% salt concentration, a nitrite level of 156 mg/kg or a pH of 5.4 inhibited toxin production by the type E vegetative cells.

Cooking/Liquid Smoke

The survival of C. botulimum type E spores during the cooking and smoking of chub loin muscles was studied by Christiansen et al. (1968). Raw chub loin muscles, which had been brined in a 30° salometer brine (7.8% salt), were inoculated with 10^6 spores of C. botulinum type E per gram of muscle tissue. The fish were cooked to an internal temperature of 180°F and held for 30 minutes. After processing, the chubs were sealed under vacuum in triple laminated plastic bags and incubated at room temperature (20-25°C) for 7 days. Due to the variability of brine uptake, the tested samples had salt contents that ranged from 1.17-5.06%. Toxin was found in all samples containing less than 2.75% salt, but spores survived in all the samples tested. Christiansen et al. (1968) also tested the effect of a moist atmosphere during cooking on the survival of C. botulinum type E spores. Using steam, hot air or a combination of the two, unbrined chubs that had been inoculated with 10^6 spores/g were heated to 180°F and held for 30 minutes. The samples were sealed in plastic and stored at room temperature. They reported that moisture in the heated

atmosphere did not reduce the incidence of C. botulinum type E spore survival.

Pace et al. (1972) reported that the destruction of C. botulimum spores on whitefish chubs depended on the relative humidity (RH) in the smoke chamber during the cook. Using spore types B and E and conditions designed to simulate those used in commercial fish smoking plants, low numbers of the type E spores were destroyed within 30 minutes in fish held at an internal temperature of 77°C (170.6°F) in an atmosphere of at least 70% RH. However, when several hundred thousand type E spores were present, an internal temperature of 82°C (179.6°F) and a minimum RH of 70% were required for spore destruction. Pace et al. (1972) made quantitative estimates of spore destruction as a function of cooking conditions. Type E spore populations heated in an atmosphere of 70% RH were reduced by 2 to 4 logarithms when heated at 77°C (170.6°F), by 5 to 6 logarithms when heated at 82°C (179.6°F) and by more than 6 logarithms when heated at 88°C (190.4°F).

Kosak and Toledo (1981) developed a heating/smoking process for fish that was equivalent to a 12 logarithmic reduction of C. botulinum type E spores. The process, although severe, resulted in a product that did not appear excessively dehydrated. However, no attempt was made to evaluate the effect of their process on the overall organoleptic acceptability of the fish, initially or over time.

Eklund et al. (1982) studied the ability of liquid smoke, in combination with salt, to inhibit the outgrowth of C. botulinum type E spores in hot-processed whitefish. A mixture of C. botulinum type E spores was added to whitefish at a level of 10^3 spores/100 g just before processing. They reported that the combination of liquid smoke (2.0%) and a 3.7% water-phase salt concentration effectively inhibited spore outgrowth in hot-processed whitefish during 14 days of temperature abuse (25°C). However, liquid smoke (2.0%) in combination with 2.0% water-phase salt concentrations did not inhibit the outgrowth of the C. botulinum type E spores in temperature abused (25°C) hot-processed smoked whitefish.

Temperature

Schmidt et al. (1961) evaluated growth and toxin production of four strains of C. botulinum type E (VH, Beluga, Iwanai and BE) at temperatures less than 40°F (i.e., 34, 36 and 38°F). They inoculated a sterilized beef stew with mildly heat shocked spores at a level of 4-12 million spores/30 g. They reported that the strains of C. botulinum differed in their rate of toxin production. However, the results of this study clearly showed that at 38°F (3.3°C), toxin production was detected by day 31-45 in all strains tested, while no toxin was detected at day 104 at either 36°F (1.1°C) or 34°F (2.2°C). These results indicate that type E C. botulinum is a risk in products stored at normal

refrigerator temperatures ($4^{o}C$).

Abrahamsson et al. (1966) studied the effect of temperature on the formation of toxin by C. botulinum type E spores (10^{5} spores/g) in both Robertson's chopped meat medium and a fish dialysate medium. They found that the C. botulinum type E spores/vegetative cells were able to grow and produce toxin at temperatures between 3^{o} and $30^{o}C$. No toxin was found in the samples incubated at $1^{o}C$ after 1 year.

Nitrite

Considerable research has been conducted to elucidate the role of nitrite in controlling the growth of C. botulinum in cured meat systems (Christiansen, 1980). Most cured meat products do not have the levels of acid and/or salt required to completely inhibit the growth of C. botulinum spores (Riemann et al., 1972).

The use of nitrite in smoked fish products has been limited. This is unfortunate because the control of C. botulinum type E in this product requires a salt level which could be considered excessive. The addition of nitrite to smoked fish could allow a reduction of the salt level in the product, while still maintaining safety against botulinal outgrowth.

Pelroy et al. (1982) evaluated sodium nitrite and sodium chloride as inhibitors of C. botulinum types A and E spore outgrowth and toxin production in temperature-abused ($25^{o}C$), hot-processed salmon steaks. The fish samples were

inoculated intramuscularly before processing. After processing they were vacuum packaged in oxygen-impermeable plastic packages and incubated at 25°C. Pelroy et al. (1982) reported that the addition of nitrite permitted a decrease in the salt concentration required to maintain safety against botulinum toxin production. Salmon steaks processed without nitrite required a 3.8% water-phase salt concentration in order to inhibit C. botulinum type E spore outgrowth. A 6.1% water-phase salt concentration was required to inhibit C. botulinum type A toxin production. If nitrite was added at a level of 100 mg/kg or greater, a 2.5% water-phase salt concentration was required to inhibit the type E spore outgrowth. In order to inhibit the outgrowth of the type A spores, a minimum of 150 mg/kg nitrite and a 3.5% water-phase salt concentration was required. These values were valid for samples inoculated with 10^2 spores/gram of tissue and held for 7 days at 25°C. As the level of inoculation and/or the storage time increased, more nitrite and salt were required to inhibit toxin production.

Holley (1981) stated that initial concentrations of 100-150 mg/kg of sodium nitrite are inhibitory to C. botulinum in processed meats and that the maximum inhibition of C. botulinum occurred under anaerobic conditions at pH values of 4.5-5.5.

Nitrite inhibition of C. botulinum occurs by inhibiting or delaying the emergence of the vegetative cell from the spore and during cellular division (Sofos et al., 1979c). Pivnick et al. (1970) showed that nitrite acts to inhibit C. botulinum between the stages of germination and outgrowth. Research by Duncan and Foster (1968) revealed that nitrite allows germination and swelling but prevents emergence from the spore coat or elongation. The inhibitory effect of nitrite can be increased by the addition of reducing agents such as ascorbic acid, cysteine and thioglycolate (Johnston and Loynes, 1971).

The exact mechanism(s) of nitrite inhibition of C. botulinum has not been fully elucidated, since the nitrite ion is capable of a variety of reactions in a given system and because meat products are also complex and lack uniformity (National Academy of Sciences, 1981). Some of the mechanisms proposed for the antibotulinal effects of nitrite are that nitrite (1) could react with other components during heating forming a substance capable of inhibiting spore outgrowth; (2) acts as either an oxidant or reductant on cellular components such as enzymes, enzyme cofactors, nucleic acids and cellular membranes; (3) reacts with thiols, which can react with components of the spore membrane and interfere with spore metabolism or (4) reacts with cellular iron, interfering with energy metabolism and repair mechanisms (Benedict, 1980).

In meat systems, the inhibitory effect of nitrite on the outgrowth of C. botulinum has been related to the ability of nitrite to bind iron. Tompkin et al. (1978b) investigated the role of iron in controlling the inhibition of C. botulinum in perishable canned meats cured with 156 mg/kg of sodium nitrite. They inoculated cured pork ham, beef round, and beef and pork organ meats (liver and heart) with a mixture of C. botulinum spores (types A and B) at a level of 10^2 spores/g sample. The inoculated samples were sealed in retort cans and stored at 27^{o}C for up to 110 days. When supplemental iron was added to these systems, there was an increase in the rate of toxin production by the C. botulinum spores, indicating that nitrite acts to inhibit C. botulinum by binding the iron necessary for sporulation and/or cell outgrowth.

Freeze et al. (1973) studied the role of nitrite in C. botulinum inhibition and reported that nitrite, as undissociated nitrous acid, appeared to inhibit the energy dependent transport systems within the cell. Nitric oxide is theorized to react with essential iron containing compound(s) within the botulinal cell and therefore prevents cell outgrowth (Tompkin et al., 1978b). Tompkin et al. (1978b) reported that there appeared to be an inverse relationship between the amount of muscle pigment and the degree of inhibition by nitrite on the occurrence of C. botulinum outgrowth.

Added iron has been found to reduce the action of the nitrite by replacing the cation that had been bound (scavenged) by the nitrous oxide. The effect of added iron can be countered by the addition of metal chelators, such as polyphosphate or ethylenediaminetetraacetic acid (Tompkin et al., 1978, 1979). Reddy et al. (1984) showed that nitric oxide forms an iron-nitrite complex which results in the destruction of iron-sulfur enzymes. They asserted that nitrite, therefore, inhibits the outgrowth of C. botulinum cells by interferring with their iron-sulfur enzymes.

The duration of nitrite inhibition is temperature and/or spore load dependent (Geingeorgis and Riemann, 1979). The level of residual nitrite has been considered to be important in the ability of sodium nitrite to maintain its inhibitory effect, and the level of residual nitrite is time and temperature dependent (Christiansen et al., 1982). The inhibition of C. botulinum by nitrite essentially is a balance between nitrite depletion and the death of the germinated botulinal spores present in the system. This means that the safety of a meat product depends on having a high enough level of ingoing nitrite so that there is sufficient residual nitrite present until the number of viable botulinal cells has decreased to a point at which growth can no longer occur (Christiansen et al., 1978; Christiansen, 1980; Cook and Pierson, 1983; Reddy et al.,

1984).

Sorbate

During the last two decades, the role of nitrite as a precursor in the formation of N-nitrosamines in foods has received much attention, and research has been directed towards finding a substitute for nitrite in meat systems. Sorbic acid and its potassium salt have been investigated as possible replacements for nitrite. The use of sorbates in conjunction with small amounts of nitrite (40 mg/kg) for color and flavor purposes has also been suggested (National Academy of Sciences, 1981). Research has shown that sorbate when used at a level of a 200 mg/kg is as effective as 100-120 mg/kg nitrite in delaying toxin production by C. botulinum in a variety of temperature-abused cured meats (Sofos and Busta, 1980).

Tompkin et al. (1974) added 1000 mg/kg of potassium sorbate to skinless, precooked, uncured sausage links inoculated with C. botulinum. They reported that potassium sorbate delayed the growth of the normal spoilage organisms. Sorbate also reduced the growth of C. botulinum, delaying toxin production for 6 days. The results of this study were in contrast to the findings of previous investigators and acted to stimulate further studies (Sofos and Busta, 1980). The significant ($P<0.01$) effect of potassium sorbate on the occurrence of gas production, package swelling (bloating) and toxin production has been shown in bacon

(Ivey et al., 1978; Sofos et al., 1979a), in chicken frankfurters (Robach et al., 1978a; Sofos et al., 1979c; Huhtanen and Feinberg, 1980), and in turkey frankfurters (Huhtanen and Feinberg, 1980).

Tanaka et al. (1977) demonstrated that when potassium sorbate (2600 mg/kg) was added to frankfurters, it had an antibotulinal effect as effective as 1000 mg/kg of nitrite. Robach (1980) reported that the addition of 1 to 5% salt increased the ability of potassium sorbate to inhibit clostridia. It has been shown by many workers that the combination of nitrite (40 mg/kg) and sorbate (2600 mg/kg) is more effective in retarding C. botulinum than either nitrite or sorbate used individually (Sofos and Busta, 1980; Widdus and Busta, 1982).

The inhibition of C. botulinum by sorbate is pH dependent, with an upper limit of pH 6.5 necessary for inhibitory action (Smoot and Pierson, 1981; Cook and Pierson, 1983). Smoot and Pierson (1981) reported that potassium sorbate is a strong inhibitor of C. botulinum at a pH of 5.7. Because the undissociated acid form is responsible for the inhibition of C. botulinum, the hydrogen ion concentration (pH) is a major factor in the antibotulinal efficacy of sorbate thus the inhibitory effect of sorbate increases as the pH decreases (Samson et al., 1955). This change in inhibitory effect occurs because the cell is only permeable to the acid in the undissociated

form. Raevori (1976) stated that sorbic acid as the free acid enters the bacterial cell and is then capable of inhibiting several of the cells enzyme systems. Researchers have linked sorbic acid to inhibiting fumarase activity (York and Vaughn, 1955b), sulfhydryl-containing enzymes, ficin and alcohol dehydrogenases (Whitaker, 1959), aspartase and succinic dehydrogenase (York and Vaughn, 1964), and malate and α-ketoglutarate dehydrogenases (Rhem, 1967). Harada et al. (1968) suggested that sorbate competitively combines with Coenzyme A and acetate, inhibiting all reactions involving these compounds.

Water Activity and pH

Two main factors controlling the growth of spore forming organisms are water activity (Aw) and pH. The Aw of foods influences the growth of microorganisms by affecting their metabolic activity, reproduction, as well as their resistance to environmental conditions (Leistner et al., 1981). The Aw affects the lag and stationary growth phases as well as the death rate of the organism (Troller and Christian, 1978). Since most of the organisms associated with foods require a relatively high Aw level to grow, lowering the Aw in foods would reduce the organisms ability to grow or multiply (Leistner et al., 1981).

Pace et al. (1972) noted fish processed in low moisture environments would undergo desiccation causing a gradient in water activity to occur at the surface of the

fish. This gradient would move into the fish until an equilibrium is established with the relative humidity of the environment. The loss of water from the fish could result in a reduction of the Aw to levels as low as 0.2 to 0.4. Murrell and Scott (1966) have shown that C. botulinum type E spores, while relatively intolerant to heat, are the most resistant to heat at Aw of 0.2 to 0.4.

Baird-Parker and Freame (1967) investigated the interrelationships of Aw, pH and temperature in controlling the outgrowth of C. botulinum type E spores. Type E spores were incubated in Reinforced Clostridial Medium (RCM) that had pH values between 5 and 7, and Aw's between 0.997 and 0.890. The spores were incubated at either 20^{o} or 30^{o}C. They reported that C. botulinum type E spores grew at Aw's between 0.99 and 0.997 at pH 5.3, 0.98 and 0.997 at pH 6.0 and 5.5 and at 0.97 and 0.997 at pH 7. These data indicate that as the pH increased, a lower water activity was required to inhibit the outgrowth of the C. botulinum type E spores. Ohye and Christian (1967) reported that the minimal Aw levels for C. botulinum types A, B and E were 0.95, 0.94 and 0.97, respectively. Riemann (1967) reported that toxin production by C. botulinum type E in brain heart infusion, ceased at a higher Aw than did growth.

Emodi and Lechowich (1969) studied the interaction of water activity and temperature on the outgrowth of C. botulinum type E spores in a TPSY (trypticase-peptone-sucrose-yeast

extract) medium. They reported that at 30°C, C. botulinum could grow at a water activity of 0.97, but growth only occurred at 3.3°C if all other factors were optimal. They also reported that at the same Aw, the time for spore outgrowth increased as the temperature decreased.

The use of salt to lower the Aw of a system is an effective means of inhibiting Clostridia. It has been reported that salt concentrations which have little inhibitory effect at optimal temperatures (30°C) have a strong inhibitory effect at lower temperatures (Roberts and Smart, 1976). This is important in the control of C. botulinum type E since type E is more sensitive to salt than types A and B. This means that at lower temperatures, the limiting Aw for C. botulinum type E would increase (Roberts and Smart, 1976).

Mechanism of Lipid Oxidation

Lipid oxidation is one of the most deteriorative processes occurring in food systems. It can, if allowed, result in reduction of quality, nutritional value and safety of foods (Dugan, 1968). Lipid oxidation is responsible for the formation of short chain aldehydes, ketones and fatty acids in fat-containing foods. These products of lipid oxidation are believed to be responsible for the development of the oxidized flavors in red meats, poultry and fish (Watts, 1962; Pearson et al., 1983).

The classical mechanism of lipid oxidation involves three steps: 1) initiation, 2) propagation and 3) termination (Farmer and Sutton, 1943; Uri, 1961; Lundberg, 1962; Labuza, 1971). This mechanism is a free radical process that utilizes unsaturated fatty acids as the initial substrate. It has been accepted as the primary event in autoxidation. The three steps of the lipid oxidation mechanism can be illustrated as follows:

Initiation: $RH \text{ ---------- } R\cdot + H\cdot$

$RH + O_2 \text{ -------- } ROO\cdot + H\cdot$

Propagation: $R\cdot + O_2 \text{ ------ } ROO\cdot$

$ROO\cdot + RH \text{ --------- } ROOH + R\cdot$

Termination: $ROO\cdot + R\cdot \text{ -------- } ROOR$

$R\cdot + R\cdot \text{ ---------- } RR$

$ROO\cdot + ROO\cdot \text{ ------ } ROOR + O_2$

where ROO· is a lipid peroxy radical, R· is an alkyl radical and RH is an unsaturated fatty acid. Once initiated, the reaction is propagated by the level of hydroperoxides produced due to their ability to decompose to free radicals. Termination begins at the point where the level of free radicals is such that they begin interacting and forming non-reactive species. The rate of lipid oxidation is affected by such factors as lipid composition, temperature, presence or absence of light, metal catalysts, inhibitory compounds and oxygen (Lea, 1962; Labuza, 1971). Under

extreme conditions, the hydroperoxides formed during the autoxidation of unsaturated fatty acids can undergo further free radical chain reactions that can result in the formation of polymers, other oxygenated compounds, cleavage products and/or reaction products with proteins (Gardner, 1979; Pearson et al., 1983).

Measurement of Lipid Oxidation

The major effect of lipid oxidation on food acceptability is the development of off-odors and off-flavors. These time-related organoleptic changes occur in food products as a result of oxidative rancidity. They have been associated with the accumulation of malonaldehyde and other oxidative reaction products some of which may be potentially harmful to human health (Mukai and Goldstein, 1976; Shamberger et al., 1974, 1977; Caldironi and Bazan, 1982; Pearson et al., 1983).

Malonaldehyde has been shown to be a secondary oxidation product of polyunsaturated fatty acids containing two or more double bonds (Dahle et al., 1962). To date the most widely used test for measuring the malonaldehyde content of muscle foods has been the 2-thiobarbituric acid (TBA) test (Gray, 1978; Rhee, 1978a; Melton, 1983). This test expresses the malonaldehyde content of foods in mg/kg values and this value is commonly referred to as the TBA number (Melton, 1983). The TBA test has been used by many researchers to follow lipid oxidation in cooked beef, pork and poultry

(Huang and Greene, 1978; Igene et al., 1979), in refrigerated and frozen stored beef, pork and poultry (Igene et al., 1979; Judge and Aberle, 1980; Younathan et al., 1980; Drerup et al., 1981) in freeze-dried beef and pork (Chipault and Hawkins, 1971) and in fish (Lee and Toledo, 1977).

Despite abundant literature on its use as a measure of rancidity, the validity of the test has been questioned, particularly in food systems containing nitrite. Nitrite has been reported to decrease the TBA values in oxidized meat systems. Nitrite decreases the TBA value by nitrosating the malonaldehyde during the distillation step in the analysis (Hougham and Watts, 1958; Swain, 1972). The reduction of TBA values by nitrite can be overcome by the addition of sulfanilamide before the distillation step (Zipser and Watts, 1962).

It has been shown that the TBA test is subject to interference by the reaction of the TBA reagent with other substances. Aldehydes have been reported to react with the TBA reagent to form red complexes that absorb at the same wavelength (532) as the malonaldehyde-TBA complex (Melton, 1983). It has also been reported that the TBA reagent can react with alkanals, 2-alkenes and 2,4-alkadienals to form a yellow complex that absorbs at 452 nm (Marcuse and Johansson, 1973; Patton, 1974).

The significance of these other TBA reactive substances (TBARS) to the quantitation of malonaldehyde has been

studied. Igene et al. (1985a) reported that in cooked chicken meat (white and dark), the TBA values had a correlation of -0.87 with the sensory evaluation of rancidity in the cooked product (warmed-over flavor). They noted that the malonaldehyde-TBA complex accounted for 93.3% and 83.0% of the total TBARS in the distillates from the cooked white meat after 0 and 3 days, respectively. Corresponding values for the dark meat were 95.5 and 94%, respectively, indicating that in cooked chicken, malonaldehyde is the primary TBARS. Yamauchi (1972a) reported that malonaldehyde is responsible for 99.2% of the TBARS in cooked rancid pork.

Lipid Oxidation in Muscle Foods

Table 2 lists the unsaturated fatty acid composition of the major muscle foods. From the data shown, it is understood why fish and poultry lipids are so vulnerable to lipid oxidation. The fish lipid system contains significant quantities of the long chain polyunsaturated fatty acids (Khayat and Schwall, 1983).

Lipid Oxidation in Red Meat and Poultry

Igene et al. (1980) using a beef and a poultry model system, reported that during frozen storage both the triglycerides and the phospholipids contribute to the development of rancidity in meat products. The influence of the triglycerides on the development of rancidity during frozen storage depended on the degree of unsaturation of the

Table 2. Unsaturated fatty acid content of lipids in some muscle foods (Melton, 1983).

Fatty Acid	Content (%)				
	Lamb	Beef	Pork	Chicken	Fish
C18:1	9.51	33.44	12.78	20.25	19.59
C18:2	18.49	10.52	35.08	14.20	5.88
C18:3	0.43	1.66	0.33	0.90	8.07
C20:2	0.34	0.69	--	--	0.20
C20:3	0.62	2.77	1.31	1.30	0.36
C20:4	13.20	8.51	9.51	11.60	3.75
C20:5	--	0.76	1.31	1.55	7.16
C22:4	--	0.88	0.98	2.10	0.65
C22:5	--	0.92	2.30	5.75	2.39
C22:6	--	--	2.30	5.75	2.39

component fatty acids and the length of storage of the meat and poultry products. They also found that during frozen storage of intact raw meat, the major changes in total lipids occurred in the triglyceride content, while the phospholipid content remained relatively unchanged over time.

Lipid Oxidation in Fish

The development of rancidity in fish muscle from fatty species such as salmon and whitefish can be rapid. It is governed by complex factors common to many biological systems such as inherent metal ions, presence of natural antioxidants, kind and amount of fatty acids, age, season of harvest and storage conditions before and after processing (Braddock and Dugan, 1972).

The effects of frozen storage on the overall quality of fish have been investigated and several reviews are available (Mills, 1975; Kelly, 1969; Olley et al., 1969; Shewfelt, 1981; Khayat and Schwall, 1983). Most of the studies concerning the oxidation of fish lipids have been conducted with lean fish whose primary lipid constituents are phospholipids. Therefore little research has been concerned with the triglyceride fraction of fish lipids. Fish phospholipids can oxidize during frozen storage. This has been noted by many researchers and in a variety of species including cod (Lovern et al., 1969), lemon sole, halibut (Olley et al., 1962), trout (Jonas and Bilinski, 1976b),

herring (Bosund and Ganrot, 1969b), freshwater whitefish (Awad et al., 1969), salmon (Botta et al., 1973), silver hake (Hiltz et al., 1976), carp, red sea beam (Toyomizu et al., 1977) and capelin (Botta and Shaw, 1978).

Mechanism of Warmed-over Flavor Development in Muscle Foods

A primary catalyst to warmed-over flavor in meat systems has been shown to be the heme compounds of the muscle tissue. This is the topic of an in-depth review by Love (1983). The ability of heme to accelerate the oxidation of lipids was shown by Tappel (1962). Sato and Hegarty (1971) and Igene et al. (1979) have shown that beef muscle thoroughly extracted with water does not develop warmed-over flavor. This indicates that the factor(s) responsible for initiating warmed-over flavor are water soluble, as would be the heme and nonheme irons.

Younathan and Watts (1959) reported that cooked meats develop rancid off-flavors and TBARS more rapidly than raw meat. They proposed that the Fe^{3+} hemes were active catalysts of lipid oxidation in cooked meat systems. Brown et al. (1963) reported that ferric and ferrous hemes were both catalysts, but that the ferric heme was the most active form of heme in lipid oxidation. Fox (1966) stated that during cooking, heme pigments are converted to the ferric hemochromogen.

It has been speculated that during cooking, the protein portion of the hemoprotein is denatured and unfolds. This unfolding of the protein would expose/release the iron molecule and allow contact with the lipids of the system, increasing the rate of lipid oxidation (Labuza, 1971; Pearson et al., 1977). Igene et al. (1979) demonstrated that the myoglobin is not responsible for the increased rate of lipid oxidation in cooked meats. Rather, it is the Fe^{2+} released from the pigment that causes the accelerated rate of lipid oxidation.

The role of nonheme iron as a prooxidant in meats has been investigated (Sato and Hegarty, 1971; Love and Pearson, 1974). Torrance and Bothwell (1968) and Schricker (1982) have reported that the heme iron represents 62% of the total iron in beef muscle, while nonheme iron accounts for only 5.4-5.5%.

Igene et al. (1979) reported that heating of meat results in the release of iron from the heme molecules. They noted that when extracts of beef pigments were heated to 70°C, the amount of nonheme iron increased from 8.7 to 27% of the total iron in the system. Schricker et al. (1982) reported that the nonheme iron content in beef heated to 100°C increased from 9.9 to 20.9 mg/kg.

Chen et al. (1984) showed that slow cooking of meat pigment extracts increased the amount of nonheme iron released more rapidly than did fast cooking. They theorized

that since meats generally are cooked slowly, the increased rates of lipid oxidation could be the result of the increased nonheme levels that would be produced.

Warmed-over Flavor in Red Meats and Poultry

Warmed-over flavor in cooked meats has been well documented (Timms and Watts, 1958; Younathan and Watts, 1960; Ruenger et al., 1978) and has been attributed to lipid oxidation. Oxidation of the tissue lipids appears to occur in two stages, i.e., the phospholipids are oxidized first followed by the triglycerides (Igene, 1979). Younathan and Watts (1960) demonstrated that flavor deterioration in cooked meats involved the unsaturated fatty acids of the lean tissue or cellular lipids, which exist primarily in the form of phospholipids. Wilson et al. (1976), however, reported that while the phospholipids are major contributors to rancidity in beef and lamb, the total lipids are more important in pork. Igene and Pearson (1979) have also shown that the total phospholipids are primarily responsible for the development of warmed-over flavor in cooked beef and poultry. Being less susceptible to oxidation, the triglycerides appear to exert only a minor influence on the development of warmed-over flavor.

The level of phospholipids in muscle foods remains relatively constant when they are expressed as a function of the total lipid (Dugan, 1971). However, between species

there is considerable variation in the actual phospholipid content (Pearson et al., 1977). Pearson et al. (1977) found that poultry meat and fish are higher in phospholipids than are red meats. Within the same species, it has been found that phospholipids generally have a higher level of unsaturated fatty acids than the triglycerides. Also within the same species, the red muscles are more prone to the development of WOF than are the white muscles. Between species there is a great difference in the susceptibility to the development of WOF, with turkey being the most susceptible followed by chicken, pork, beef and mutton (Pearson et al., 1977).

Warmed-over Flavor in Fish

Based on the high correlation between the TBA number and the oxidation of polyunsaturated fatty acids, fish would suffer extensively from WOF (Pearson et al., 1977). Theoretically, fish would behave more like turkey than red meats in this aspect. Wide differences would be expected among the fish species in their susceptibility to the development of WOF. These differences would be due to the variability in the lipid content and composition among the species of fish (Pearson et al., 1977).

The role of the phospholipids and the triglycerides in the development of WOF in fish is unclear. Since there is little difference in the fatty acid composition between the

phospholipid and the triglyceride fractions in fish (Braddock and Dugan, 1972), both lipid fractions contribute to the rapid development of oxidative rancidity in fish (Zipser and Watts, 1951; Lovern, 1959; Braddock and Dugan, 1972; Pearson et al., 1977). In addition, the occurrence of warmed-over flavor in fish has not received much attention since few fish products are cooked and then rewarmed prior to serving. However, a study by Sen and Bhandary (1978) showed that when sardine fish were cooked and then stored at refrigerated temperatures there was a significant decrease in the rate of lipid oxidation when compared to raw fish stored under the same conditions. The raw sardines became rancid in 2-3 days while the cooked product became rancid in 6 days as evaluated by TBA numbers and peroxide values. They concluded that this decrease in lipid oxidation was due to: 1) the destruction of a "lipoxygenase" inherent to the fish, 2) the formation of water-soluble antioxidants as a result of the cooking process and/or 3) the destruction of heme compounds.

The role of metal ions, Fe^{2+}, Fe^{3+}, Cu^{2+}, and hemin in the oxidation of lipids in frozen fish and fish oils have been investigated. Ke and Ackman (1976) found that when copper, iron and zinc were added to mackerel skin and meat there was acceleration of the lipid oxidation process in this nonaqueous system. Mizushima et al. (1977) added Fe^{2+}, Fe^{3+} and Cu^{2+} to fish homogenates and found that the relative effectiveness of these metals to oxygen uptake, i.e.,

oxidation, was in decreasing order: $Fe^{3+} > Fe^{2+} > Cu^{2+}$. They went on to investigate the effect of iron, copper and hemin on lipid oxidation in fish homogenates and found all three were capable of accelerating the process. The relative activity of these ions were: Fe^{2+} > hemin > Cu^{2+} > Fe^{3+}. The addition of up to 50 mg/kg copper to fish muscle prior to freezing acts to reduce the induction period for lipid oxidation to a few days (MacLean and Castell, 1964). Cations have also been shown to be capable of accelerating the formation of secondary reaction products (Seblacek, 1974).

Various biochemical compounds such as heme proteins, organic acids, amino acids, pigments and various fish tissues have been shown to catalyze lipid oxidation reactions, alone or in combination with trace metals (Castell and Bishop, 1969; Jurewicz and Salmonowicz, 1973; El-Zeany et al., 1974; Yu et al., 1974).

Antioxidant Role of Nitrite in Meat Systems

Warmed-over flavor can be inhibited in meat systems by the addition of 50 mg/kg nitrite and eliminated by the addition of 220 mg/kg of nitrite (Sato and Hegarty, 1971). Fooladi et al. (1979) reported that nitrite-free cooked pork had TBA values 5 times higher than cooked pork containing nitrite. The addition of nitrite to cooked beef and chicken reduced their TBA values by half.

The mechanism by which nitrite inhibits the development of WOF has never been fully explained. Igene et al. (1985b) suggested that nitrite acts as an antioxidant by (1) strongly binding the heme pigments, thus preventing the release of the nonheme iron during the cooking process; (2) serving as a chelator or metal sequestor and/or (3) to a lesser degree, nitrite could act to stabilize the lipids in muscle membranes.

The primary process occurring in meat systems appears to be the stabilization of the heme porphyrin ring. Chen et al. (1984) heated meat pigments extracts, with or without added nitrite. They reported that the nonheme content of the extract heated to 62^{o}C without added nitrite, increased from 1.31 to 1.78 mg/kg. When the same system was heated to 88^{o}C, the nonheme content increased to 2.34 mg/kg, an overall increase of 78.6%. However, when a similar extract of pigments were heated to 88^{o}C in the presence of nitrite, the nonheme content went from 1.29 to 1.14 mg/kg, an 11.6% decrease. They attributed this finding to the ability of nitrite to stabilize the heme proteins, thus preventing the release of nonheme iron. Chen et al. (1984) asserted that the increase in nonheme iron in the heated pigment extract, without added nitrite, indicated that the iron molecule was being released from the porphyrin ring. Schricker and Miller (1983) have also suggested that the nonheme iron originates from the porphyrin ring of the heme molecule.

The role of nitrite as a membrane stabilizer has been discussed in the literature. Liu and Watts (1970) noted that in meat muscle, myoglobin is in solution in the cytoplasm and is separated from the phospholipids in the membranes. During cooking the membranes are destroyed and contact between the heme components and the phospholipids is established. Liu and Watts (1970) asserted that the addition of nitrite to this system would have the same effects as stabilization of the membrane. Love and Pearson (1976) suggested that it seemed more probable that nitrite complexes and stabilizes the membranal lipids, thus preventing the development of WOF in cooked meats.

Evidence indicates that nitrite can react with heme proteins, nonheme proteins, low molecular weight peptides, amino acids and trace metals in meats (MacDonald et al., 1980). Nitrite has also been found in adipose tissue and may react with unsaturated fatty acids (Frouin et al., 1975; Walters et al., 1979). Woolford and Cassens (1977) reported finding levels as high as 20-25% of the nitrite added to bacon in the adipose tissue. Goutefongea et al. (1977) recovered 35% of the added nitrite in whole adipose tissue. They also reported that 80-90% of the added nitrite found was in the free state.

Mechanism of N-nitrosamine Formation

Nitrite contributes to the color, flavor, lipid stability and botulinal safety of cured meats (Gray, 1981). However, its continued use has been debated as it has been shown that nitrite reacts with secondary amines found in foods to form N-nitrosamines (Gray, 1976). It has been shown that the majority of the N-nitrosamines tested in animal experiments are carcinogenic (Crosby and Sawyer, 1976; Preussman et al., 1976; Gray and Randall, 1979; Sen, 1980).

N-Nitrosamines are formed primarily from the reaction between secondary amines and nitrous acid. In this reaction, R^1 is an alkyl group, while R may be an alkyl, aryl or a wide variety of functional groups.

The extent of N-nitrosamine formation is governed by a variety of factors such as the basicity of the amine, concentration of the reactants, pH, temperature and the presence or absence of catalysts and inhibitors. N-Nitroso compounds have been identified in a variety of food systems, including cured meat products, non-fat dried milk, dried malt and beer (Gray and Randall, 1979).

N-Nitrosamines in Fish Products

In the state of Michigan, nitrite has clearance for use in smoked chub, and it may be added to levels up to 200 mg/kg (Michigan Department of Agriculture, 1965). This often results in initial nitrite levels of 75 to 150 mg/kg and a residual nitrite level of 10-20 mg/kg (Holley, 1981; Cook and Pierson, 1983). Because of these levels of nitrite it is possible that the formation of carcinogenic N-nitrosamines could occur from the reaction of the added nitrite, and the free amines of the fish. This would be especially true in some marine species of fish which have been shown to have high levels of trimethylamine which readily breaks down to dimethylamine and formaldehyde (Dyer and Mounsey, 1949; Shewan, 1951; Castell et al., 1971; Spinelli and Koury, 1979; Sikorski and Kostuch, 1982). Malins et al. (1970) investigated the possibility of nitrosamine formation in smoked chub and found that in all probability N-nitrosodimethylamine (NDMA) does not form in concentrations greater than 10 µg/kg during the processing of the fish. Malins et al. (1970) were unable to find any evidence of amine nitrosation in smoked chub.

Gadbois et al. (1975) treated sablefish with 0-1300 mg/kg nitrite before cold-smoking. The product was analyzed for N-nitrosamine levels at day 0 and after 2 weeks storage at 40°F. They reported that in fish containing 0-500 mg/kg nitrite only trace amounts (<10 µg/kg) of NDMA were present in the samples. Increasing levels of nitrite did not increase

the levels of NDMA detected, and there was a slight decrease in the amount of NDMA during storage at 40°C.

Fazio et al. (1971) investigated the effect of processing, with or without nitrite and/or nitrate, on N-nitrosamine levels in commercially processed shad, sable and salmon. Raw and processed samples of these fish were collected from 2 different commercial processing plants. Analysis of these species of fish showed that the raw sable contained 4 ug/g NDMA while no NDMA was found in the other species of fish in the raw state. The processing of these three species of fish with nitrite or nitrate, increased the NDMA level in sable to 9-26 ug/g. Salmon and shad, processed with nitrite, were found to contain 0-17 ug/kg and 0-12 ug/g NDMA, respectively.

Sen et al. (1970) analyzed 23 samples of fish products, i.e., 18 smoked and 5 canned. All of the 23 samples had been cooked with or without added nitrite (200 mg/kg). The 18 smoked fish samples included cod, haddock, hake, mackerel and salmon, while the canned samples included salmon and mackerel. They reported that when the fish were cooked without added nitrite no N-nitrosamines were detected. When the fish were cooked in the presence of the added nitrite it was found that the cod contained 0.5 ug/kg N-nitrosodipropylamine (NDPA), the haddock contained 1 ug/kg N-diethylnitrosamine (NDEA), the mackerel contained 1 ug/kg NDMA, the hake contained 4 ug/kg NDMA and the salmon contained <0.5 ug/kg NDMA. The canned fish (salmon and mackerel) when cooked

without added nitrite had no detectable levels of N-nitrosamines. When cooked with the nitrite, salmon was found to contain <0.5 ug/kg NDMA and mackerel contained 4-8 ug/kg NDMA.

Pelroy et al. (1982) reported that when nitrite was added to hot-processed salmon steaks, no detectable N-nitrosamines were found.

N-Nitrosothiazolidine in Smoked Meats

Recently, interest has focused on the presence of a specific N-nitrosamine in smoked foods. Pensabene and Fiddler (1983) analyzed smoke processed bacon samples and detected N-nitrosothiazolidine (NTHZ) at levels between 5-25 ug/kg. The bacon was processed using three smoking procedures. These were (1) a commercial liquid smoke in a gas-fired smokehouse that used sawmill trimmings; (2) a commercial liquid smoke in an electric smokehouse, and (3) a gas heated smokehouse with medium to heavy smoke introduced after the drying period under constant air exhaust. The results of this study indicated that the NTHZ found in the bacon appeared to be associated with the smoking step. However, it is still uncertain whether the NTHZ is formed during actual wood smoking then deposited onto the meat, or whether one or more smoke components react with the meat constituents to form the NTHZ.

The role of woodsmoke in the formation of NTHZ in smoked meats has been further investigated by Mandagere et al.

(1984). They proposed that formaldehyde could react with cysteamine to produce thiazolidine which can nitrosate to form NTHZ. A second route proposed to explain the formation of NTHZ was that formaldehyde could react with cysteine to form thiazolidine carboxylic acid. This compound could then react with nitrite to form N-nitrosothiazolidine carboxylic acid (NTCA) which could undergo decarboxylation to form NTHZ during cooking. Average NTHZ levels for smoked meats, including bacon, pepperoni, Canadian bacon and lebanon bologna, have been determined to be 2.5-10, 2.7, 4.1 and 3.1 ug/kg, respectively (Gray et al., 1982; Pensabene and Fiddler, 1983b; Mandagere et al., 1984). Sen et al. (1985) reported no detectable levels of NTHZ in smoked kipper and cod or in salted cod and herring.

Smoking of Meat Systems

Smoking is one of the oldest food preservation techniques developed by man. Considerable amounts of smoke processed foods are consumed in the United States using both woodhouse generated and liquid smokes to impart the characteristic flavoring and coloring (Daun, 1979).

Woodsmoking Process

Smoke consists of a dispersion of solid and liquid particles in addition to the gaseous phase (Foster and Simpson, 1961). The composition of these dispersions/phases is dependent on combustion temperature, combustion conditions,

oxidative changes smoke undergoes, moisture content, equipment and design, smoke density, amount of smoke treatment and wood type used for smoke generation (Porter et al., 1964; Pearson and Tauber, 1984; Toth and Potthast, 1984).

Depending on the level of humidity used, smoke can be characterized as being either wet or dry. In traditional woodhouse generated smoking of foods, smoke development and smoking occur in the same system. Smoke entering the smokehouse is equilibrated by the smokehouse temperature, which depends on the product being produced. During the smoking process, smoke constituents are absorbed or condensed on the surface of the product. The amount of absorption that occurs depends on the surface properties of the product. The relative fatness and leaness, humidity, temperature, the presence of casings, smoke conditions and smoke composition can affect the deposition of smoke on a food (Toth and Potthast, 1984).

Hardwoods produce different flavors in foods than softwoods due to basic compositional differences of the woods. Hardwoods contain more pentosans than softwoods, therefore pyrolysis of the hardwood produces smoke which contains higher levels of acids (Gilbert and Knowles, 1975). Tilgner and Wierzbricka (1959) noted that, although hardwoods are preferred for smoking meats, softwoods, when used to smoke fish, gave flavors almost as desirable as those produced by the traditionally-used hardwoods. Lantz and Vaisey (1970)

reported that in the production of canned smoked fish, softwoods gave a more desirable salty, sweet, smokey taste than did the conventional maple or hickory.

Smoke Absorption of Fish

Foster and Simpson (1961) found that the deposition of smoke on fish occurred as vapor absorption, in which the surface and interstitial water of the fish acts as the principal absorbent. Chan et al. (1975) found that the smoke absorption by fish fillets follows first order kinetics, where there is a very rapid initial rate of absorption followed by a gradual reduction in the rate of absorption until saturation is reached. Smoke saturation was reached faster on the skin side of the fillet than on the flesh side. They reported that the optimum conditions for smoking fish consisted of a two-stage process. An initial cook using a dry bulb (DB) temperature of 160^{o}F and a wet bulb (WB) of 140^{o}F with 60% relative humidity in the chamber followed by at least 33 minutes at 200^{o}F DB and 190^{o}F WB.

Deng et al. (1974) investigated the effect of smoking temperature on the organoleptic acceptability of smoked Spanish mackerel. Using a cold smoking process, a hot smoking process and a combination of both processes, they reported that the temperature of the cooking process did not affect the acceptability of the final product's saltiness, smoke flavor or color. However, panelists found significant differences in texture and appearance of the products.

Composition of Woodsmoke

The components of smoke can be classified as water soluble or water insoluble. The water insoluble fraction contains the tar, solid particles and polycyclic aromatic hydrocarbons (PAH), some of which have been shown to be carcinogenic (Howard and Fazio, 1969; Barnett, 1976; Engst and Fritz, 1977; Walker, 1977; Toth and Potthast, 1984). The water soluble fraction contains both high and low molecular weight compounds. These compounds are important in the production of liquid smoke, since the water soluble fraction is responsible for the desirable effects of the smoke treatment of foods (Toth and Potthast, 1984).

The chemical composition of smoke has been investigated by many researchers (Porter et al., 1964; Hamid and Saffle, 1965; Doerr et al., 1966; Fiddler et al., 1966; Love and Bratzler, 1966; Lustre and Issenberg, 1969, 1970). The number of compounds that occur in smoke have been estimated to range from about one thousand to several thousand, but only approximately 500 are responsible for the characteristic smoke flavor (Tilgner, 1977). A number of compounds that have been identified in smoke are shown in Table 3 (Toth and Potthast, 1984).

Although a wide variety and number of compounds are present in woodsmoke, not all of these compounds are found in smoked foods. The occurrence of the woodsmoke components in smoked foods has been shown. Bratzler et al. (1969)

Table 3. Compounds identified in curing smoke classified by chemical group (Toth and Potthast, 1984).

Chemical Group	Number Identified	Chemical Group	Number Identified
Hydrocarbons	58	Ketols	1
Alcohols	10	Phenols	85
Ketones	24	Acids	33
Cetoalcohols	5	Esters	7
Aldehydes	15	Ethers	4
Aldols	1	Alicyclics	66

extracted and analyzed smoked and unsmoked bologna for total carbonyl, acid and phenol content. They found that the phenols were the only class of compounds found in the smoked product that was entirely absent from the unsmoked bologna. Lustre and Issenberg (1969) analyzed smoked belly strips and commercially produced summer sausages for their phenol contents. Both samples contained 4-methylguaiacol, phenol, 4-ethylguaiacol, m- and p-cresol, cis- and trans- isoeugenol, syringol, 2,6-methyl-4-allhyphenol, vanillin, acetovanillin and cyclotene. In addition the pork strips were reported to contain eugenol, 4-vinylguaiacol, syringaldehyde, aceto-syringone and maltol, indicating that the meat systems varied in the smoke constituents they absorbed.

Sensory Attributes of Smoked Foods

While the smoking process was originally a means of preserving foods, its use today is primarily as a flavoring/coloring agent (Sink, 1979). The treatment of meat products, fish and some other foods with smoke produces changes in color and flavor. Because the addition of smoke to meats has occurred in an arbitrary manner since its initial use, the final flavor and color existing in the finished product is subject to regional smoking habits and traditions (Toth and Potthast, 1984). The flavor of smoked foods ranges from a very light to a very heavy smokey taste, while the color of the products may vary from a golden yellow to a very dark mahogany brown (Potthast, 1975, 1981).

It is well established that smoking contributes strongly to the sensory attributes of foods. The desired flavor of smoke is the result of a blending of smoke constituents with certain components in the foods (Draudt, 1963). Smokiness is affected by the type of processing system used and the extent of smoke penetration into the product (Sink, 1979). The perception of smokiness in a food is a function of both color and the perceived palatability of the product. The perception of smokiness has been found to occur primarily in the outer layers of the food (Bratzler et al., 1969).

Bratzler et al. (1969) reported that there was a high correlation (r=0.81) between the sensory perception of smoke in foods and their phenol content. Phenols associated with smoke odor and flavor have been identified as guaiacol, 4-methylguaiacol and 2,6-dimethoxyphenol (syringol). The guaiacols give a smokey taste and the syringol gives a smokey odor (Gilbert and Knowles, 1975; Daun, 1979). These compounds are the major components of both the vapor and dispersed phases of smoke (Wasserman, 1966; Kornreich and Issenberg, 1972). Wasserman (1966) mixed guaiacol, 4-methylguaiacol and syringol in the proportions found in curing smoke, but the solution had a taste only slightly reminiscent of the original condensate. Phenols, in general, were found to give an incomplete smoke cured aroma, indicating that whole smoke aroma and taste are the result of a complex mixture of compounds (Wasserman, 1966).

Antimicrobial Activity of Woodsmoke

The bacteriostatic effect of woodsmoke is related to several events that accompany smoking. Smoking occurs along with the cooking of the product and the dehydration that results acts to stabilize the product. Another factor that affects the ability of smoke to function as a preservative is that generally salting is a preliminary treatment to smoking and salt has been long known for its antimicrobial effects (Deng et al., 1974; Chan et al., 1975).

The bacteriostatic effect of smoke has been found to occur primarily at the product surface (Toth and Potthast, 1984). This has been related to the fact that during smoking, the smoke is deposited on the surface, and a secondary skin is formed. A decrease in surface pH occurs, which can act to deter microbial growth (Toth and Potthast, 1984). The presence of formaldehyde in the smoke that is deposited on the surface of the product is also suspected to act as a bactericide (Hess, 1928; Kochanowski, 1962; Incze, 1965; Kersken, 1973). However, the impact of formaldehyde as a bactericide in meat systems has been disputed. Kurko and Perova (1961) in their studies on the bacterostaic properties of smoke reported that only the organic acid and the phenol fraction of smoke had any ability to prevent bacterial growth.

Gibbons et al. (1953) reported that the bactericidal effect of smoke in bacon was dependent on smoke density and

smoke duration during cooking. Smokehouse temperature also had an effect on the amount of bacterial destruction that occurred during cooking. They reported that when high smoke densities were used with high temperatures (132-135^{o}F), there was a greater reduction in bacterial load than when high smoke densities were used in combination with lower temperatures (68-76^{o}F). The combination of low smoke density with low temperature had little bactericidal effect. The level of humidity in the smokehouse had little effect on the ability of smoke and/or temperature to reduce the bacterial load on this product.

Piggott (1979) reported on a study designed to establish the relationship between smoke, smoking temperature and microbial population. Brined haddock were prepared with and without smoking. The unsmoked fish had a bacterial count of greater than $200x10^{6}$ organisms/g, while the smoked fish had a count of $62x10^{3}$ organisms/g. The study was repeated using a higher temperature during the preparation. Again, the unsmoked fish had higher bacterial counts ($75x10^{3}$ organisms/g) than did the smoked fish (104 organisms/g).

Antioxidant Activity of Woodsmoke

The antioxidant effect of smoke in foods is influenced primarily by the phenol fraction. This is not surprising since the phenol structure is basic to most commercial antioxidants (butylated hydroxyanisole, butylated hydroxytoluene, propyl gallate, tertiarybutyl hydroguinone) used in the food

industry today (Fretheim et al., 1980; Toth and Potthast, 1984). Fretheim et al. (1980) noted that the antioxidant effect of smoke is not a linear function of the amount of smoke employed. They also found that as the generation temperature used for the smoke production increased from 350 to 500°C, there was a decrease in the concentration of the active antioxidant and antimicrobial compounds. They explained this decrease in activity as being more of a dilution effect, since at higher generation temperatures more water, alcohols and carbonyls are produced in the same volume of smoke than at the lower generation temperatures.

Pearson and Tauber (1984) noted that the maximum level of phenols are produced at smoke generation temperatures of 590°F or greater. This is where the maximum lignin decomposition occurs. During normal smoking, the smoke generation temperatures range from 212-750°F or higher. The best quality smoke is produced using a combustion temperature of 650-750°F and an oxidation temperature of 390-480°F (Pearson and Tauber, 1984).

Kurko (1959) studied the antioxidant effectiveness of the acidic, basic and neutral fractions of smoke in pork fat. It was shown that the acid fraction contained only minor antioxidant properties; the basic fraction was actually prooxidant in effect, while the neutral fraction contained most of the antioxidant activity. It was further shown that the lower boiling point phenols (phenol, cresol and guaiacol

as the main constituents) were less effective antioxidant than the fraction containing the higher boiling point phenols (mainly syringol and its derivatives) (Draudt, 1963).

Other higher boiling point phenols that have been shown to be effective antioxidants include 2,6-dimethoxyphenol, 2,6-dimethoxy-4-methylphenol and 2,6-dimethoxy-4-ethylphenol (Daun, 1979; Pearson and Tauber, 1984). Shewan (1949) showed that woodsmoke could act as a preservative on fish. However, salting and the dehydration of the fish during cooking did not contribute significantly to this preservative effect.

Liquid Smoke in Foods

Woodsmoke is composed of three phases, i.e., solids, gases and condensibles. The condensibles are responsible for the primary effects of smoke, preservation, coloring and flavoring (Wistreich, 1979). The collection of these condensibles results in production of liquid smoke. Methods of producing liquid smoke consist of trapping the condensible phase in water or in the water vapor of the smoke (Wistreich, 1979).

Use of refined liquid smoke flavorings is expanding in both the United States and other countries. The major reason for this is that liquid smokes can perform all the desired functions of woodsmoke and they have several advantages over conventional woodsmoke (Hollenbeck, 1979). Draught (1963) reviewed the advantages of using liquid smokes in food

systems. These included: (1) increased control of the levels of smoke flavor in the product; (2) the ability to incorporate smoke flavor into the body of the product; (3) increased variety of smoked foods that can be produced; (4) lower cost of producing smoked foods due to the reduced amount of equipment required and (5) the methodology for producing liquid smokes removes compounds that are carcinogenic in nature. These carcinogenic compounds would include polcyclic aromatic hydrocarbons (PAH) that are associated with the crude tar fraction of the woodsmoke (Daun, 1979; Hollenbeck, 1979; Wistreich, 1979).

Functionality of Liquid Smoke

The production of liquid smoke, while removing the PAH, does not appear to remove the phenols that have been shown to give curing smoke its antioxidant and bacteriostatic properties (Gorbatov et al., 1971; Knowles et al., 1975). Potthast (1976) reported that liquid smoke contained between 4 and 219 g phenol/liter. The majority of the liquid smoke preparations that have been analyzed contain syringol and guaiacol, 4-methylguaiacol or phenol and cresol as their primary ingredients (Toth and Potthast, 1984). Fiddler et al. (1970) fractionated an ether extract of a commercial liquid smoke and determined which fraction was considered to be the most "smokey" by sensory evaluation. Gas chromatographic analysis of this "smokey" fraction revealed that it was composed primarily of phenols and carbonyls. The role

of wood type on the flavor of liquid smokes was investigated by Fujimaki et al. (1974). They reported that the differences in the flavor of the liquid smokes was related to the amount of carbonyl, non-carbonyl and phenol fractions produced when each wood type was used to generate smoke. From these observations, it is obvious that the flavoring properties of liquid smokes can have wide variation, since the phenols are primarily responsible for the smoke flavor (Toth and Potthast, 1984).

Flavor Characteristics of Whitefish

The flavors that are associated with fish and seafoods have been investigated, but a definitive flavor chemistry of these products has not been developed (Josephson et al., 1983). In salt-water fish, ammonia, dimethylamine and trimethylamine have been implicated in fishy aromas and flavors. However, these compounds have been shown not to characterize the aroma of seafood and are considered to have only a modifying role (Josephson et al., 1983). Trimethylamine is thought to contribute to the stale odor in fish since its concentration increases as the fish loses its freshness (Reay and Shewan, 1949).

Other compounds contributing to the fishy flavor and odor of fish have been identified. However, there is little agreement on which ones are the primary contributors. All fresh uncooked fish are characterized by a common green,

seaweed-like aroma which is modified to create the aromas that are characteristic of species (Josephson et al., 1983). Geiselman (1972) studied the aroma of fresh rainbow trout and observed odors that could be described as cucumbers, violets and rushes. Positive identification of the compounds responsible for these aromas was not made. Berra et al. (1982) identified the fresh cucumber-like aroma of Australian grayling as being due to (E,-Z)-2,-6-nonadienal. A metallic off-flavor found with deep-sea prawn and sand lobster has been identified as (Z)-1,-5-octadien-3-ol by Whitfield et al. (1981, 1982). Josephson et al. (1983) using headspace analysis identified two distinct families of compounds that are characteristic of the cucumber, melon-like and mushroom aromas of fresh uncooked whitefish. They reported that the compounds responsible for the cucumber-like odor were identified as (E)-2-nonenal, (E,Z)-2,-6-nonadienal and 6-nonen-1-ol. The principal contributors to the heavy, plant-like aroma were identified as 1-octen-3-ol, 1-octen-3-one, 1,5-octadien-3-ol, 1,5-octadien-3-one and 2,5-octadien-1-ol. Josephson et al. (1983) also reported that the development of the aromas in fresh whitefish involved enzymatic induced changes.

Flavors of cooked fish in all probability contain some of the already mentioned compounds, but the distinctive cooked flavor of fish will be characterized by those compounds that are formed during the cooking (Pokorny, 1980). Obata

(1950) found that a fishy aroma could be attributed to piperdine, ε-aminovaleric acid, and ε-aminovaleraldehyde. He also reported that the reaction products of piperidine and piperidinealdehyde exhibited a freshwater fish aroma. When trimethylamine was added to the mixture, a saltwater fish aroma was created.

In a study on the volatile compounds occurring in cooked whitefish, Josephson et al. (1984) identified several compounds present in cooked whitefish that were absent in the raw fish. These included: (E,Z)- and (E,E)-2,4-heptadienal, (E,Z)- and (E,E)-2,4-decdienal, pentanal, (Z,E)- and (E,E)-3,5-octadien-2-one, (Z,E) and (E,E)-3-5-nonadien-2-one,3-octen-2-one, 1-penten-3-one, 2,3-pentaneione and 1-pentadecene. They also reported that hexanal accounted for approximately 40% of the volatile compounds making it the most abundant volatile. However, they failed to find any (Z)-4-heptenal which has been reported to be responsible for the cold-storage flavor in cod.

MATERIALS AND METHODS

Materials

Clostridium botulinum Spores

Five strains of *C. botulinum* type E spores (8E,304E, Saratoga E, Birmingham E and Minneapolis E) were obtained from the Disease Control Center, Atlanta, Georgia. The spores were blended and a spore count was determined using the Most Probable Number (MPN) technique (FDA Bacteriological Analytical Manual, 1978).

Vacuum Packaging

Polyethylene-laminated nylon pouches (3 mil) were obtained from Koch, Kansas City, MO. These bags have an oxygen transmission rate of 9 $ml/m^2/24$ hours at 4^oC (32^oF).

Salt

Alberger^R fine flake salt was obtained from Diamond Crystal Salt Company, St. Clair, MI.

Sodium Nitrite

Sodium nitrite was obtained from Fisher Scientific, Detroit, MI.

Sorbate

Potassium sorbate was obtained from Monsanto Co., St. Louis, MO.

Liquid Smoke

Royal Smoke^{R}, H Special a neutralized, solubilized liquid smoke generated from hickory sawdust, was obtained from Griffith Labs., Alsip, IL.

Sawdust

The sawdust used to smoke fish in this study was obtained from O.P. Link Co., Salem, IN.

Experimental

Salt Uptake by Whitefish

Two studies were conducted to evaluate salt uptake in whitefish. The first study was designed to investigate the salt uptake over time in whole, eviscerated whitefish and whitefish fillets. Using a 30^{o} salometer (7.89% salt) brine, eight lots of fish in brine were prepared for both the whole fish and the fillets. The fish were added to the brine in a ratio of 1 part fish to two parts brine. Every two hours, beginning with time 0 and continuing for 14 hours, a set of fillets and whole fish were debrined, rinsed, placed in a clean plastic bag and held at 4^{o}C until all samples were ready to be cooked. The samples were cooked using the schedule of Bratzler and Robinson (1967). This cook cycle

is shown in Table 4. After cooking, the samples were cooled, ground, then analyzed for salt and moisture.

The second study was designed to investigate the effect of the salt concentration in the brine on salt uptake of whitefish fillets only. Fillets were brined for 14 hours in one of ten brines (Table 5). These brines were calculated, based on a 26.3% salt saturation, to have salt concentrations that would represent 10, 20, 30, 40 and 50^{o} salometer brines. Nitrite was added at a level designed to give an ingoing concentration of 156 mg/kg (220 mg/kg in the brine), and ascorbate was added at a level to give an ingoing concentration of 550 mg/kg (820 mg/kg in the brine). These levels are representative of those used in the meat industry for the curing of meats, except bacon.

Taste panel evaluations of the samples were made using a hedonic rating procedure (Appendix 1). During the taste panel, the ten samples were divided into two sets of five and placed on a plate. All five salt levels and nitrited samples were represented on each plate. The ten untrained panelists were given unsalted crackers and water containing lemon juice to clear their palates between samples. These panelists were asked to evaluate the samples in degree of perception of the traits asked.

Table 4. Cook cycle used in the smoking of whitefish[a].

Time Period	Dry Bulb °F	Wet Bulb °F	Relative Humidity (%)
1st 60 min	120	110	72
3rd 30 min	150	115	34
4th 30 min	200	165	45
5th 30 min	200	165	45
6th 30 min	195	165	50

[a]Bratzler and Robinson, 1967.

Table 5. Brine compositions for determining the effect of salt concentration on the rate of salt uptake by whitefish fillets during a 14 hour brining period at 4°C.

Treatment	Salt (%)	Water (%)	Sodium nitrite (mg/kg)	Sodium ascorbate (mg/kg)
10° Brine	2.63	97.37	--	--
10° Brine + nitrite	2.63	97.29	220	820
20° Brine	5.26	94.74	--	--
20° Brine + nitrite	5.26	94.66	220	820
30° Brine	7.89	92.11	--	--
30° Brine + nitrite	7.89	92.01	220	820
40° Brine	10.52	89.48	--	--
40° Brine + nitrite	10.52	89.38	220	820
50° Brine	13.15	86.85	--	--
50° Brine + nitrite	13.15	86.75	220	820

The Role of Nitrite, Salt and Sorbate on the Incidence of C. botulinum in Smoked Whitefish

This study was designed to study the effect(s) of salt, nitrite and sorbate on the incidence of C. botulinum in smoked whitefish. Nine brines (Table 6) of varying levels of salt, alone or with nitrite or a combination of nitrite and sorbate, were used. The concentrations of these brine additives were calculated to produce specific ingoing levels that were to be achieved once an absorption equilibrium was reached. The nitrite was added to the brine at an ingoing level of 156 mg/kg (220 mg/kg in the brine), sodium ascorbate was added (as a cure accelerator) at an ingoing level of 550 mg/kg (820 mg/kg in the brine) and sorbate was added at a level of 2600 mg/kg in the brine. These levels are representative of the levels used in the meat industry for the curing of meats, except bacon.

Fish were added to the brine in a ratio of one part fish to two parts brine. After 14 hours at 4°C, the fish were removed from the brine, rinsed for 10 seconds in cold running water and placed on a cooking rack that had been coated with lecithin to prevent sticking. The fish were smoked and cooked according to the procedure of Bratzler and Robinson (1967) described previously.

After cooking, the fish were chilled to 3°C, then transferred to plastic bins, covered with plastic film and refrigerated (4°C) until the following morning. At this

Table 6. Brine compositions for determining the effect of salt, nitrite and sorbate on the outgrowth of Clostridium botulinum type E spores in smoked whitefish.

Treatment	Salt (%)	Water (%)	Sodium nitrite (mg/kg)	Sodium ascorbate (mg/kg)	Potassium sorbate (mg/kg)
10^{o} Brine	2.63	97.37	--	--	--
10^{o} Brine + nitrite	2.63	97.27	220	820	--
10^{o} Brine + nitrite + sorbate	2.63	97.01	220	820	2600
20^{o} Brine	5.26	94.74	--	--	--
20^{o} Brine + nitrite	5.26	94.64	220	820	--
20^{o} Brine + nitrite + sorbate	5.26	94.36	220	820	2600
30^{o} Brine	7.89	02.11	--	--	--
30^{o} Brine + nitrite	7.89	92.01	220	820	--
30^{o} Brine + nitrite + sorbate	7.89	91.75	220	820	2600

time the fish were cut into 75 g portions and placed into plastic pouches. The uninoculated samples, to be used for parallel chemical analyses, were vacuum sealed and removed from the area. The remaining samples were inoculated with 0.25 ml (10^6 spores/ml) of the mixed C. botulinum type E spore suspension and vacuum sealed. A vacuum of 21-24 inches of mercury was established in each bag. Both sets of samples were placed in the same 27°C incubator.

Samples to be assayed for toxin production were removed from storage, starting on day 0 and every 7 days until day 63, then every 10 days until day 83. Samples were taken in triplicate for the treatments brined in the 20 and 30° salometer brines. However, due to the limited amount of product, only duplicate samples were taken for the samples prepared in the 10 degree brines.

The samples to be chemically analyzed were taken in triplicate every 7 days until day 56 or until no more samples were available. Analyses were carried out for salt, nitrite, moisture, pH, water activity and sorbate.

The Role of Salt in the Stability of Smoked Whitefish

This study was conducted to determine the effect of salt, alone or in combination with nitrite, on the lipid stability and the organoleptic acceptability of smoked whitefish during refrigerated storage (4°C). The salt concentrations of the brines, based on the results of the previous brining studies, were designed to produce 0, 2, 4 and 6% salt in the water-phase

of the finished product. A total of eight brines were used. These included a control brine which contained no salt, a control with nitrite and ascorbate added. The other six were composed of 10, 20 and 30° salometer brines prepared with and without the added nitrite and ascorbate (Table 7). The fish containing no salt were held in water during the soaking period. Brining, cooking and smoking were as described previously.

After smoking, the fish were cooled, cut into quarters, randomly assigned to treatment days, placed in unsealed plastic bags and held in a refrigerator (4°C) until needed for analysis. Samples were taken on days 0, 7, 14 and 22 and analyzed for TBA numbers, salt, moisture and fat. In addition, the samples treated with nitrite were analyzed for their residual nitrite and N-nitrosamine contents.

Taste panel evaluations were made on all sets of samples except those sampled on day 22. This was because Michigan Regulation 541 prohibits the consumption of smoked fish after day 14. Taste panel evaluations of the samples were made using a ranking procedure (Appendix 2). During each taste panel, the eight samples were divided into 2 sets of four and placed on a sample plate. All four salt levels and nitrited samples were represented on each plate. The taste panelists were asked to evaluate the degree of desirability of each characteristic rather than absolute degree of perception. Each panelist was given unsalted crackers and water

Table 7. Brine compositions for determining the effect of salt and nitrite with ascorbate on the development of oxidative rancidity in smoked whitefish.

Treatment	Salt (%)	Water (%)	Sodium nitrite (mg/kg)	Sodium ascorbate (mg/kg)
Control	--	100.00	--	--
Control + nitrite	--	99.90	220	820
10^{o} Brine	2.63	97.37	--	--
10^{o} Brine + nitrite	2.63	97.29	220	820
20^{o} Brine	5.26	94.74	--	--
20^{o} Brine + nitrite	5.26	94.66	220	820
30^{o} Brine	7.89	92.11	--	--
30^{o} Brine + nitrite	7.89	92.01	220	820

containing lemon juice to clear their palates between samples.

The Role of Smoke Type and Level on the Stability of Smoked Whitefish

This study was conducted to determine the role of smoke type (liquid or woodhouse generated), the level of liquid smoke, and nitrite on the lipid stability and organoleptic acceptability of smoked whitefish during refrigerated storage. Four basic brines were used, alone or in combination with nitrite, creating eight treatments (Table 8). Liquid smoke was added to the brine at 0.7, 1.4 and 2.1% of the total brine. The amounts of liquid smoke used were based on values reported by Eklund et al. (1982). The whitefish fillets were brined and cooked as previously described. Samples were taken on days 0, 5, 14 and 22 for analysis. Taste panel evaluations, using the ranking procedure, were made on all sets except those taken on day 22. These samples were analyzed as described in the previous study. In addition these samples were also analyzed for their total phenol content and were characterized for the relative concentrations of each specific phenol present in the final product.

The Evaluation of Lipid Oxidation in Baked, Whitefish by TBA Number and Volatile Analysis

Whitefish fillets were skinned and blended to create a uniform product. The blended fish were packaged into 1 lb units and frozen (-20^{o}C) until needed. Samples were baked from the frozen state for 1 hour at 350^{o}F. Beginning 3 days

Table 8. Brine compositions for determining the effect of smoke type and level and nitrite with ascorbate on the development of oxidative rancidity in smoked whitefish.

Treatment	Salt (%)	Water (%)	Liquid smoke[a] (%)	Sodium nitrite (mg/kg)	Sodium ascorbate (mg/kg)
Woodsmoke	7.90	92.10	--	--	--
Woodsmoke + nitrite	7.90	92.00	--	220	820
0.7% Liquid smoke	7.90	91.40	0.7	--	--
0.7% Liquid smoke + nitrite	7.90	91.30	0.7	220	820
1.4% Liquid smoke	7.90	90.70	1.4	--	--
1.4% Liquid smoke + nitrite	7.90	90.60	1.4	220	820
2.1% Liquid smoke	7.90	90.00	2.1	--	--
2.1% Liquid smoke + nitrite	7.90	89.90	2.1	220	820

[a]Royal Smoke[R], H Special

prior to analyzing, samples were baked on days 3, 2, 1 and 0.

On day 0, the samples were analyzed for their TBA value and their flavor volatiles. At the same time, taste panelists were asked to evaluate the overall desirability of samples from days 0, 1, 2 and 3 using a ranking procedure (Appendix 3). Using the same panelists, triangle tests on samples baked on days 0, 1 and 2 were used to determine the threshold value for rancidity in this product based on the TBA value. In the triangle test, the panelists were asked to find the "odd" sample and then indicate if it was more or less desirable than the other two samples on the plate (Appendix 3).

Methods of Analyses

Assay for *Clostridium botulinum* Type E Toxin

The inoculated, temperature abused (27°C) whitefish samples were assayed for the presence of *C. botulinum* type E toxin using the FDA standard procedure (FDA Bacteriological Analytical Manual, 1978).

Proximate Analysis

Moisture, fat, salt, pH and nitrite determinations were performed according to standard AOAC procedures (1975). In the nitrite analysis, N-1-naphthylethylene diamine was used to replace the carcinogenic α-naphthylamine (Usher and Telling, 1975).

Sorbate Analysis

Residual potassium sorbate was determined using the method described by Robach (1980).

Water Activity

Water activity was determined using a thermocouple psychrometer (Decagon Devices, Inc., Seattle, WA). A confirmation of the data was made using the freezing point depression method of Lerici et al. (1983).

Thiobarbituric Acid Test (TBA)

The TBA distillation method of Tarladgis et al. (1960) was used to measure the development of rancidity in smoked fish. The modification of Zipser and Watts (1962) to prevent interference by nitrite was utilized for all samples containing nitrite. TBA numbers were expressed as mg malonaldehyde/kg fish.

Lipid Extraction and Fractionation

The total lipid was extracted from the whitefish using the procedure of Bligh and Dyer (1959). Separation of the total lipid into triglyceride and phospholipid fractions was accomplished using the method of Choudhury et al. (1960).

Fatty Acid Analysis

The methylation of the total, triglyceride and phospholipid fractions of the raw whitefish was done according to the boron-trifluoride-methanol procedure outlined by Morrison and

Smith (1964). The methylated samples were analyzed for their fatty acid composition using a Hewlett Packard gas chromatograph (Model 5840A) equipped with a flame ionization detector (FID) and a Hewlett Packard 18850A GC integrator. The glass column (2m x 2 mm i.d.) was packed with 10% SP 2330 on Chromosorb W. (Supelco, Bellefonte, PA). Operating conditions were as follows: Nitrogen was used as the GC carrier gas and the flow rate was set as 30 ml/min; the injection port temperature was 250^{o}C; the detector temperature was set at 350^{o}C; a temperature program that started at 140^{o}C for one minute then went to 190^{o}C at a rate of 10^{o}/min was used. Identification of the fatty acid peaks was made using the retention times of standard fatty acids assayed under identical conditions.

N-Nitrosamines

Smoked whitefish were analyzed for N-nitrosamines using the extraction procedure of White et al. (1974). The only modification was the addition of 1 gram of ammonium sulfamate prior to the distillation step to minimize possible artifactual formation of N-nitrosamines during sample work-up.

The quantitative determination of volatile N-nitrosamines was carried out using a gas chromatograph-thermal energy analyzer (GC-TEA) system comprised of a Varian 3700 GC coupled with a TEA model 502 LC (Thermo Electron Corp., Waltham, MA) via a 1/8" glass-lined stainless steel transfer line. The GC column was glass (3m x 2mm i.d.) packed with 10% Carbowax 20 MM TPA on Chromosorb WHP. The operating conditions were

as follows: nitrogen flow was 30 ml/min; initial temperature was 140°C held for 1 minute; the rate of temperature change was 15°/min to a final temperature of 180°C for 7 minutes. The injector temperature was 150°C and the TEA pyrolyzer furnace was set at 425°C. The TEA reaction chamber pressure was 1.5 Torr and the GC-TEA transfer line was heated to 200°C. Identification and quantitation of the N-nitrosamines were made by injecting known amounts of standards and comparing retention times and peak areas.

N-Nitrosothiazolidine and N-nitrosothiazolidine carboxylic acid were determined using the procedure of Sen et al. (1985).

Phenol Analysis

The total phenol content was determined spectrophotometrically using the method of Bratzler et al. (1969). The analysis of the samples for the individual phenols present involved the extraction method outlined by Lustre and Issenberg (1970). In this procedure, 100 g of smoked whitefish were blended with 200 ml of 5% sodium hydroxide until homogenous. Trichloracetic acid (40%, 200 ml) was slowly added during the blending to precipitate the proteins. The sample was transferred to 250 ml glass centrifuge bottles and centrifuged at 1800 rpm for 15 minutes. After centrifugation, the top liquid layer was decanted off and then filtered under suction through Whatman #1 filter paper. The samples were placed into a 1 l beaker and placed overnight at -20°C to solidify the lipid layer so that it could be physically removed from the sample.

The extract was taken to pH 12 with 40% sodium hydroxide (approximately 30 ml). The alkaline solution was then extracted with two 300 ml and one 150 ml volumes of ethyl ether and the ether was discarded. The remaining solution was taken to pH 6.8 by saturation with carbon dioxide. This step regenerated the phenols from their sodium salts. After regeneration, the sample was extracted with two 300 ml and one 150 ml volumes of ether. The ether was dried over anhydrous sodium sulfate and then concentrated to a volume of 20 ml using a rotary evaporator at room temperature. The concentrate was transferred to a centrifuge tube and dried to 2 ml under a stream of nitrogen. The extract of the samples were analyzed on a Hewlett Packard gas chromatograph (Model 5840A) equipped with a flame ionization detector (FID) and a Hewlett Packard 18850A GC integrator. A 50 meter 10% Carbowax 20 M column (Alltech Assoc. Inc., Deerfield, IL) was used under the following operating conditions. Helium flow was set at 28 psi; the injector temperature was 250^{o}C; detector temperature was 350^{o}C; initial oven temperature was 90^{o}C for 1 minute; rate was 5^{o}/minute to a final temperature of 190^{o}C for 60 minutes. Specific phenols present were tentatively identified by injecting standards and comparing retention times.

Gas Chromatography of the Volatiles from Baked Whitefish

Two hundred grams of the baked whitefish were placed in a 2000 ml boiling flask, 500 ml of water were added and the

mixture was placed in a Likens-Nickerson apparatus. Ethyl ether (25 ml) was used as the extracting solvent. The system was allowed to reflux for 6 hours followed by concentration of the ether to a final volume of 0.5 ml. Sodium sulfate was used to dry the ether extract of the fish volatiles. The extract was stored in a screw-top vial and held at -20^{o}C until analysis. The extracts were analyzed using a 3m x 2mm (i.d.) glass column packed with a 10% Carbowax 20M TPA on Chromosorb WHP (80/100 mesh) (Supelco, Bellefonte, PA). A Hewlett Packard 5840A Gas chromatograph was used as follows: the initial temperature was set at 50^{o}C and held for 2 minutes. The temperature changed at a rate of 5^{o}/minute until a final temperature of 190^{o}C was reached followed by a 20 minute hold at this temperature. Helium carrier gas was regulated at 30 ml/min, the injection port temperature was set at 275^{o}C and the flame ionization temperature was at 300^{o}C. Injection volume was 3 ul.

GC-MS Analyses of the Baked Whitefish Volatile Extract

GC-MS analyses of the extracts of the volatiles from baked whitefish cooked on day 0, 1 and 2 were performed. Three ul of the volatile extract were injected into a Hewlett Packard 5840A gas chromatograph equipped with a 3Mx 2mm (i.d.) glass column packed with 10% Carbowax 20 M TPA on Chromosorb WHP (80/100 mesh). The GC was operated using the conditions described previously. The injection effluent passed into a Hewlett Packard 5985A mass spectrophotometer having the

following parameters: electron impact voltage, 70 e V; electron multiplier voltage, 2410 e V; threshold 0.6; source temperature, 200^{o}C; analog/digital measurements, 3/sec; and ion detection in the positive mode.

Statistical Analysis

Statistical analyses of the data in this study were made using the ranking procedure of Kramer (1963), ANOVA analysis (Gill, 1978) and Bonferonni's t-statistic comparison (Gill, 1978).

RESULTS AND DISCUSSION

The Effect of Time, Filleting and Brine Salt Concentration on Salt Uptake in Whitefish

Whitefish fillets and whole, eviscerated whitefish were brined in 30° salometer brine at 4°C and sampled every two hours for 14 hours in order to establish the rate of salt uptake. The percent salt, moisture and salt (wp) concentrations of the fillets and whole, eviscerated whitefish over the brining period are listed in Table 9.

The relationship between salt uptake and time in both the fillets and the whole eviscerated whitefish is illustrated in Figure 1. The fillets had a significantly ($P<0.01$) faster rate of salt uptake than the whole, eviscerated fish. This is expected, since filleting of the fish increases the surface area, thus allowing the salt solution greater access to the fish tissue. The maximum level of salt uptake appeared to occur after 10 hours, indicating that an equilibrium between the salt in the brine and that in the fish tissue might exist. A similar phenomenon was observed with the whole, eviscerated fish, but at a much lower level of salt. The salt concentrations in the whole, eviscerated fish appeared to reach equilibrium with the salt in the brine after 10-12 hours.

Table 9. Salt and moisture levels in whole, eviscerated and filleted whitefish brined in 30° salometer brine for different times.

Fish Form	Time of brining (hours)	Salt[a] (%)	Moisture[b] (%)	Salt (water-phase) (%)
Fillets	2	3.0	57.2	5.0
	4	3.8	60.0	5.9
	5	3.8	59.5	6.0
	6	4.5	58.7	7.1
	10	4.9	58.5	7.7
	12	4.6	59.8	7.2
	14	4.7	58.7	7.4
Whole, eviscerated	2	0.4	69.6	0.6
	4	0.8	70.6	1.1
	5	0.9	68.9	1.3
	6	1.1	70.3	1.5
	10	1.1	67.9	1.6
	12	1.2	70.0	1.6
	14	1.0	69.3	1.4

[a]Each value represents the mean of six analyses.

[b]Each value represents the mean of triplicate analyses.

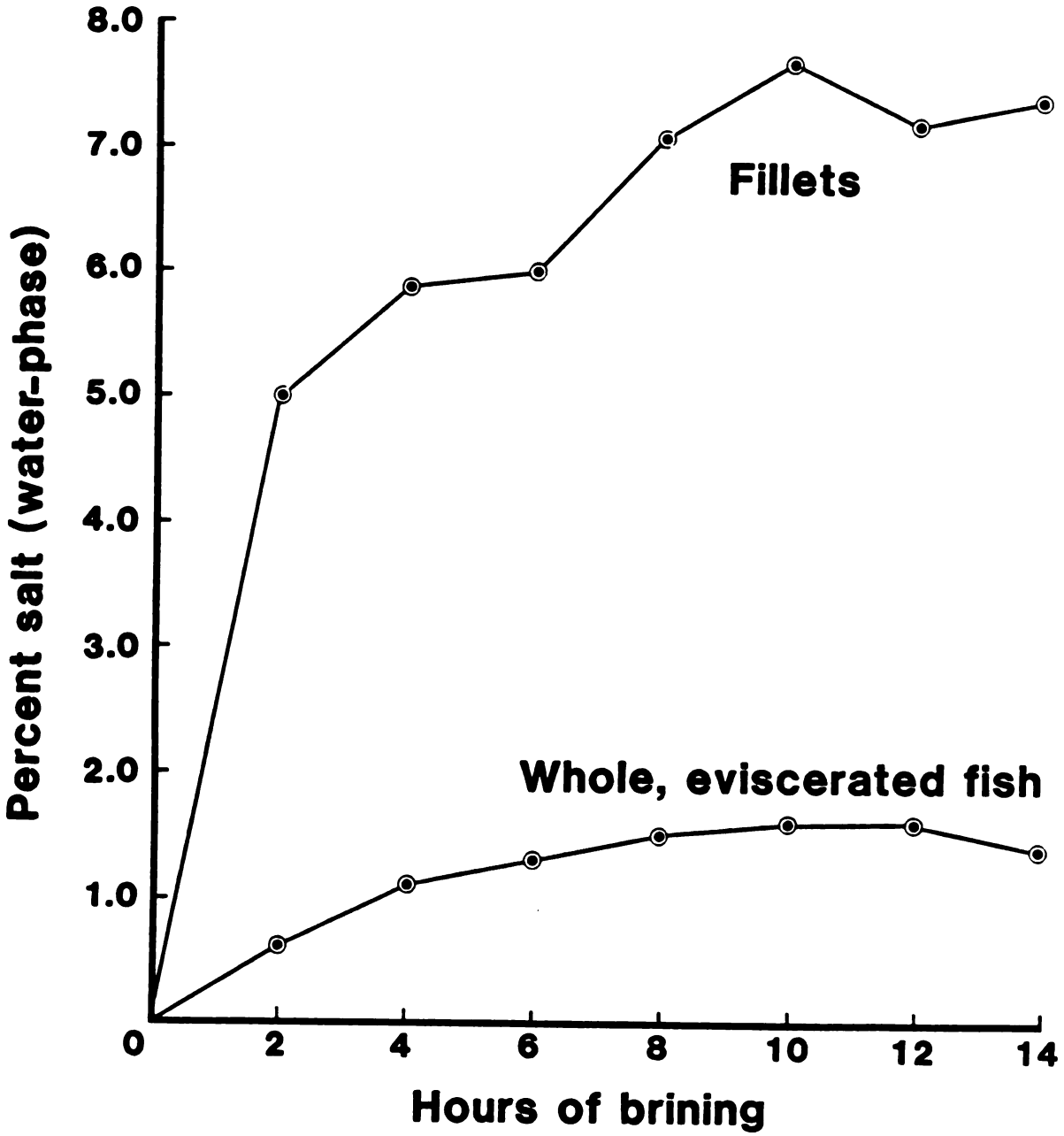

Figure 1. The effect of brining time on salt uptake in whole, eviscerated and whitefish fillets brined in 30° salometer brine.

In both brinings, there appeared to be a slight loss of salt from the fish tissue once equilibrium was established. However, a longer period of brining would be necessary to establish this loss as being real.

If the salting of fish follows the laws of simple diffusion as it has been suggested (Torry Research Station, 1960), then any loss of salt from the fish tissue could be the result of a higher salt concentration in the tissue relative to that in the brine. Crean (1961) reported that when muscle is immersed in a brine solution and allowed to equilibrate, two events can occur. At low or intermediate brine salt concentrations, water/salt is transferred from the brine to the muscle and the muscle swells. This swelling is the result of the adsorption of the chloride ions on the surface of the protein chains, which increases the net negative charge on the chains. The change in charge causes increased repulsion between and within the proteins so that protein structure expands. There is an increase in the water of hydration needed to hydrate the new negative charges and this results in muscle swelling. The second event that can occur during the brining of muscle is that at salt concentrations beyond a certain point, water/salt leaves the muscle and goes into the brine.

The percent salt, moisture and salt (wp) concentration of whitefish fillets brined for 14 hours at 4^{o}C in 10, 20, 30, 40 and 50^{o} salometer brines prepared with or without nitrite,

are summarized in Table 10. The relationship between the degree salometer of the brine and percent salt (wp) content of the finished product are shown in Figures 2 and 3. There was no significant difference ($P<0.01$) in the regression curve coefficients of the rate of salt uptake between the fillets brined with or without nitrite in the brine.

There was a high correlation ($r=0.981$) between the salt uptake by the fish and the salt content of the immersion brine. When nitrite was added to the brines there was a slight decrease in the correlation ($r=0.971$) between the salt uptake by the fish and the salt content of the brine. This difference was not found to be significant ($P>0.01$) as would be expected. These data agree with the findings of Del Valle and Nickerson (1967b) who showed that the salt content in swordfish muscle slices increased with increasing concentrations of salt in the brine. Their work showed a correlation of 0.99 between the rate of salt uptake in the fish tissue and the salt content of the brines.

Statistical analysis of the taste panel evaluations of the smoked whitefish prepared in the 10, 20, 30, 40 and 50^{o} salometer brines, with and without added nitrite, was carried out using an ANOVA analysis (Gill, 1978). There was no significant difference in the flavor, mouthfeel or overall acceptability of fish brined with and without nitrite. However, there was a significant ($P<0.01$) difference in the ability of the panelists to discern different salt levels in

Table 10. Salt, moisture and salt (water-phase) concentrations of whitefish fillets brined for 14 hours in different degree salometer brines.

Brine Composition	Salt[a] (%)	Moisture[b] (%)	Salt (water-phase) (%)
10° Brine	1.3	62.1	2.0
20° Brine	2.2	62.6	3.4
30° Brine	3.5	61.3	5.4
40° Brine	4.0	61.9	6.1
50° Brine	5.9	59.0	9.1
10° Brine + nitrite	1.0	62.3	1.5
20° Brine + nitrite	2.6	61.6	4.0
30° Brine + nitrite	3.7	61.0	5.7
40° Brine + nitrite	3.8	62.4	5.8
50° Brine + nitrite	6.3	63.1	9.0

[a]Each value represents the mean of six analyses.

[b]Each value represents triplicate analyses.

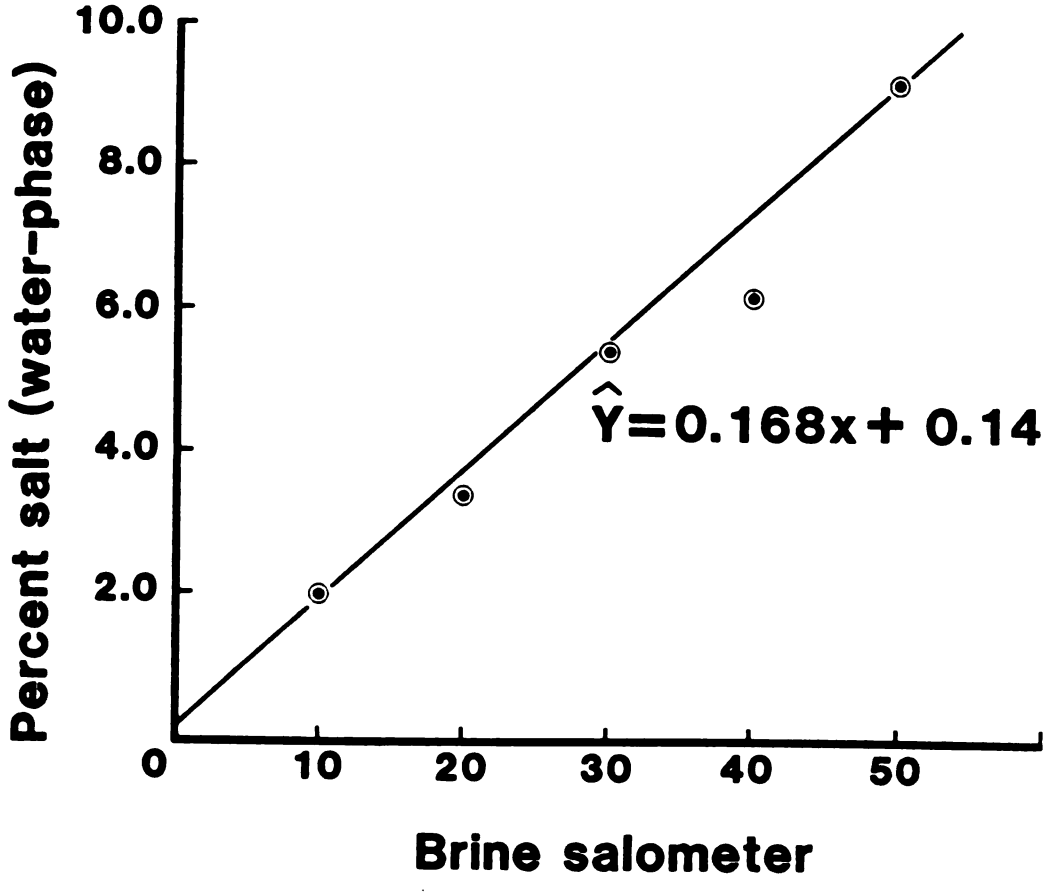

Figure 2. The effect of brine salometer concentration on salt uptake of whitefish fillets brined for 14 hours at $4^{o}C$.

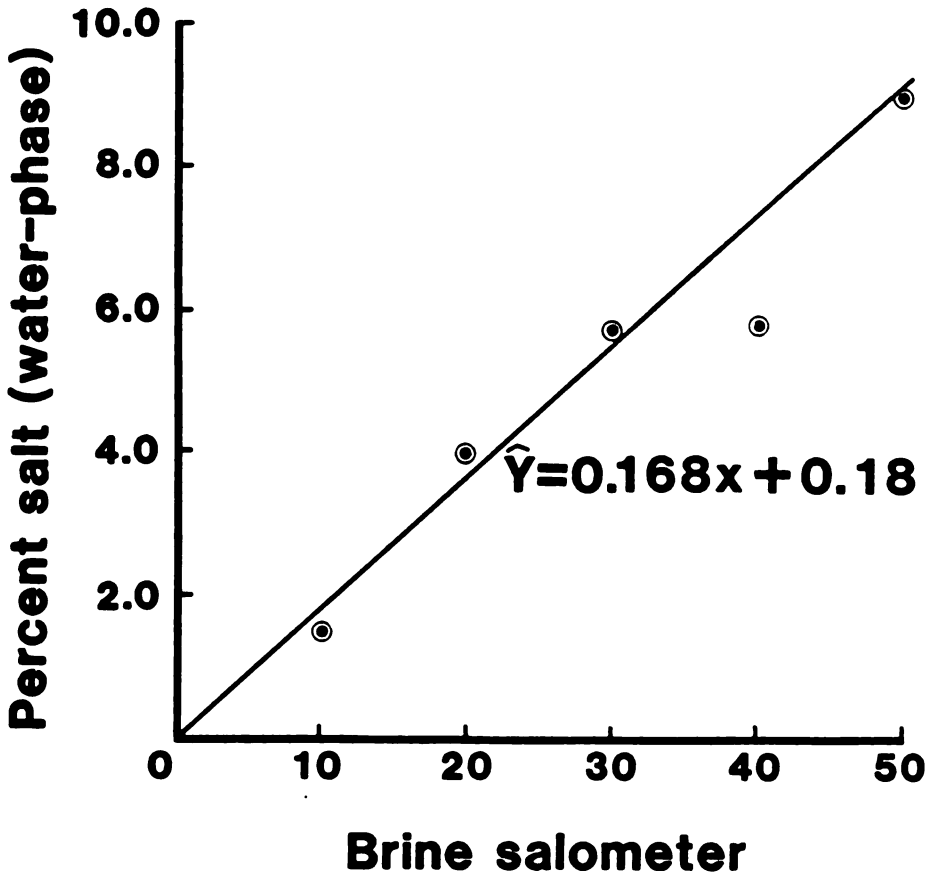

Figure 3. The effect of brine salometer and nitrite on salt uptake in whitefish fillets brined for 14 hours at 4°C.

the smoked whitefish. There was a correlation of 0.91 between the salt (wp) content of the sample and the panelists' response to the level of saltiness in the fish brined in the non-nitrited brines. When nitrite was added to the brines, the correlation between panelists response to the saltiness of the fish and the salt (wp) content of the fish was 0.95. These data reflected the ability of panelists to distinguish different salt levels, but it did not establish which level of salt was considered to be the most desirable. Nitrite appeared to heighten the perception of the panelists to salt in the smoked whitefish in this study.

The Effect of Salt, Nitrite and Sorbate on Toxin Production by *Clostridium botulinum* Type E Spores in Smoked Whitefish

The ability of salt, nitrite and sorbate to inhibit *C. botulinum* type E spore outgrowth and toxin production in smoked whitefish was evaluated. The presence of toxin in smoked whitefish samples inoculated with a 10^6 spores/ml mixture of *C. botulinum* type E spores and incubated at 27^oC is shown in Table 11. Due to a limited supply of fish, only 20 inoculated and 18 uninoculated samples were prepared for the treatments prepared in the 10^o salometer brines. However, for the treatments prepared in the 20 and 30^o salometer brines, a total of 48 inoculated and 36 uninoculated samples were prepared for each treatment. The uninoculated samples

Table 11. Presence of toxin in temperature abused (27°C) smoked whitefish samples inoculated with Clostridium botulinum type E spores.[a]

Treatment	Days of Storage											
	0	7	14	21	28	35	42	49	56	63	73	83
10° Brine	0/20[c]	12/20[b]	18/20[b]	--	--	--	--	--	--	--	--	--
10° Brine + nitrite	0/20	6/20[b]	10/20[b]	14/20[b]	--	--	--	--	--	--	--	--
10° Brine + nitrite + sorbate	0/20	3/20[b]	8/20[b]	10/20[b]	14/20[b]	--	--	--	--	--	--	--
20° Brine	0/48	0/48	0/48	0/48	0/48	0/48	2/48	2/48	5/48	8/48	11/48	14/48
20° Brine + nitrite	0/48	0/48	0/48	0/48	0/48	0/48	0/48	0/48	0/48	3/48	6/48	9/48
20° Brine + nitrite + sorbate	0/48	0/48	0/48	0/48	0/48	0/48	0/48	0/48	0/48	3/48	6/48	9/48
30° Brine	0/48	0/48	0/48	0/48	0/48	0/48	0/48	0/48	0/48	0/48	0/48	0/48
30° Brine + nitrite	0/48	0/48	0/48	0/48	0/48	0/48	0/48	0/48	0/48	0/48	0/48	0/48
30° Brine + nitrite + sorbate	0/48	0/48	0/48	0/48	0/48	0/48	0/48	0/48	0/48	0/48	0/48	0/48

[a]Inoculated with 0.25 ml of a 10^6 spores/ml blend of Clostridium botulinum type E spores.

[b]Bloated samples that proved to be toxic.

[c]Toxic samples/total number of samples prepared.

were used for chemical analysis.

Twelve of the 20 inoculated samples prepared in the 10^{o} salometer brine (without added nitrite or sorbate) were bloated and toxic by day 7. By day 14, the remaining samples (6) had bloated and were also found to be toxic. Only the 2 samples taken, just prior to placing the samples in the $27^{o}C$ incubator, on day 0 were non-toxic. The addition of nitrite to the 10^{o} salometer brine decreased the rate of toxin production, but by day 21, sixteen of the prepared 20 samples had bloated and were toxic. Only four samples, two taken on day 0 and two on day 7, had not bloated and were non-toxic.

The addition of sorbate in combination with nitrite, to the samples prepared in the 10^{o} salometer brine, decreased the rate of toxin production to a greater extent than did nitrite alone. Bloated toxic samples did occur by day 7, in the samples treated with nitrite and sorbate, but to a lesser extent than seen in the other samples prepared in the 10^{o} salometer brines. These results indicate that at this low brine salt concentration, the addition of nitrite or the combination of nitrite and sorbate decreased the rate of toxin production by C. botulinum type E spores in temperature abused smoked whitefish, but none of these treatments produce a safe product.

In the set of samples brined in the 20^{o} salometer brine, without nitrite or sorbate, a set of two and four bloated samples were found on days 14 and 21, respectively. However,

these bloated samples did not contain toxin and therefore were not recorded in the data presented in Table 11. In the triplicate samples taken for toxin analysis, toxicity was found in the samples taken on day 42. Toxicity did not occur again in this sample treatment until day 56 at which time all three samples taken were toxic. Thereafter, each set of three samples taken were toxic.

The addition of nitrite, or nitrite with sorbate, to the 20^{o} salometer brine delayed toxin production until day 63. At this time, the triplicate samples taken for both treatments were toxic. Toxicity was evident in all subsequent sets of samples taken until the end of the study on day 83.

The data indicate that in the samples brined in the 20^{o} brine, the addition of nitrite, and the combination of nitrite and sorbate, resulted in a reduced rate of toxin production by the C. botulinum type E spores (Table 11). These data also suggest that the use of a 20^{o} salometer brine, especially with the addition of nitrite, does produce a safer product than does the use of a 10^{o} salometer brine. Sorbate, at the level present in the fish, does not appear to greatly affect the rate of toxin production in smoked whitefish prepared in the 20^{o} salometer brine.

In the sets of samples prepared using the 30^{o} salometer brines, with or without added nitrite or sorbate, no toxic samples were found even by day 83. The addition of nitrite or nitrite in combination with sorbate in these higher salt

concentration treatments did not appear to influence the rate of toxin production by the spores of C. botulinum type E during the temperature abuse storage period of 83 days.

As a means of explaining the effect of salt, nitrite and sorbate on the rate of toxin production of C. botulinum type E spores in smoked whitefish, a set of uninoculated samples were prepared at the same time. These samples were analyzed for moisture, salt, pH and Aw values. The samples treated with nitrite and nitrite with sorbate were analyzed for residual nitrite and sorbate.

The Role of Salt Concentration (wp) on the Rate of Toxin Production by C. botulinum type E Spores in Smoked Whitefish

The percent salt in the water-phase (wp) of the smoked whitefish, prepared in 10, 20 or 30° salometer brines with or without added nitrite or nitrite and sorbate is shown in Table 12. This set of data was generated using the actual salt (Appendix 4) and moisture (Appendix 5) values in the following equation (Bratzler and Robinson, 1967).

$$\% \text{ salt, water-phase} = \frac{\% \text{ salt} \times 100}{\% \text{ salt} + \% \text{ moisture}}$$

The samples brined in the 10° salometer brines had an average salt concentration (wp) of 2.1 - 2.3%. Samples prepared in the 20° salometer brine contained an average of 3.4 - 4.5% salt, (wp), while the samples prepared in the 30°

Table 12. Percent salt (water-phase) of smoked whitefish brined in different degree salometer brines prepared with or without nitrite or nitrite and sorbate[a].

Treatment	Days of Storage									
	0	7	14	21	28	35	42	49	56	$\bar{X}$
	Percent Salt (water phase)									
10° Brine	2.9	1.6	2.8	2.3	2.1	2.0	--	--	--	2.3
10° Brine + nitrite	1.7	2.3	3.2	2.2	1.8	2.4	--	--	--	2.3
10° Brine + nitrite + sorbate	2.0	1.8	1.7	2.2	2.4	2.1	--	--	--	2.1
20° Brine	4.5	5.0	4.7	4.5	5.2	3.5	4.4	4.5	4.2	4.5
20° Brine + nitrite	3.2	4.2	4.7	4.5	5.2	3.5	4.4	4.5	4.5	4.3
20° Brine + nitrite + sorbate	2.5	3.0	2.5	5.2	4.0	3.1	4.1	3.8	3.1	3.4
30° Brine	6.2	6.6	6.7	6.5	6.3	7.0	6.5	5.8	5.7	6.2
30° Brine + nitrite	5.9	6.1	5.9	6.1	6.1	7.0	6.1	7.1	6.5	6.3
30° Brine + nitrite + sorbate	5.5	5.5	5.5	5.1	5.5	5.3	5.9	5.3	5.2	5.4

[a]Analyses were carried out in triplicate.

salometer brined averaged 5.4 - 6.3% salt, (wp). The ability of salt to inhibit the outgrowth and toxin production of C. botulinum type E spores has been shown in the literature to be concentration dependent. Christiansen et al. (1968) reported that as the salt concentration (wp) in brined chubs increased from <1 to >3.5%, there was a decrease in the rate of toxin production by C. botulinum type E spores. In the present study, it was found that toxin production occurred in 7 days in the whitefish samples incubated at 27°C when the salt concentrations (wp) were between 2.1 and 2.3% (Tables 11 and 12). These results are in agreement with the findings of Christiansen et al. (1968) who reported toxin production by C. botulinum type E spores at salt concentrations (wp) less than 2.75%.

The delayed toxin production by the C. botulinum type E spores in the samples prepared in the 20° salometer brine occurred in a system containing an average 4.5% salt concentration (wp). These results agree with those of Abrahamsson et al. (1966) who showed that in Robertson's chopped meat medium, a 4.5% salt (wp) inhibited toxin production by C. botulinum type E spores for 90 days at 30°C.

The addition of nitrite and sorbate to the 20° salometer brines decreased the rate of toxin production in the finished product, but the nitrite and/or sorbate at this salt level did not totally prevent toxin production by the C. botulinum type E spores.

In this study only the samples brined in the 30° salometer brines would be legal in the state of Michigan which requires a minimum 5% salt in the water-phase portion of the fish. The present study confirms the safety of a fish product containing >5% salt (wp), but it also indicates that a product containing 4% (wp) with added nitrite would be a safe product for up to 56 days at 27°C. The state of Michigan requires that smoked fish products must be processed and stored at refrigerator temperatures and must be sold or consumed within 14 days after processing. A lower salt containing smoked fish product, especially one containing nitrite, would be a safe product under these stipulations.

The Effect of Residual Nitrite on Toxin Production by *C. botulinum* type E Spores

The residual nitrite levels in the smoked whitefish samples are shown in Table 13. On day 0, i.e., immediately after the fish was packaged, there was an average nitrite concentration of 63 mg/kg fish. The nitrite depleted gradually until on day 42, nitrite was no longer detectable by the method used. These results are in agreement with those of Christiansen (1980) who reported that at two ingoing nitrite levels (156 and 50 mg/kg), the residual nitrite declines to approximately 5 mg/kg in meat systems after 21 days of storage at 0°C. He also found that the inhibition of *C. botulinum* was much greater with the higher concentration

Table 13. Residual nitrite levels[a] in smoked whitefish brined in different degree salometer brines containing nitrite and stored at 27°C.

Treatment	Days of Storage						
	0	7	14	21	28	35	42
10° Brine + nitrite	51.3	43.8	28.1	27.5	15.6	15.0	ND
10° Brine + nitrite + sorbate	66.3	41.3	28.1	22.3	16.3	15.6	ND
20° Brine + nitrite	62.5	40.7	33.1	26.3	21.9	15.0	ND
20° Brine + nitrite + sorbate	72.5	45.0	34.0	26.3	21.9	15.3	ND
30° Brine + nitrite	58.8	39.0	32.5	26.3	22.1	15.6	ND
30° Brine + nitrite + sorbate	65.0	43.8	3.90	29.4	27.5	15.3	ND

[a]Analyses carried out in triplicate.

ND = not detectable, limit of detection was 12.5 mg/kg.

of residual nitrite.

The decrease of detectable nitrite levels in cured meats during storage has been attributed to the reactivity of the nitrite with the meat components (Cassen et al., 1979). Cassens et al. (1974) reported that because nitrite reacts with various components of the muscle, less than 50% of the added nitrite can be detected immediately after processing. However, the measurement of residual nitrite does not always accurately measure all of the nitrite present. The sensitivity of the official AOAC method for residual nitrite analysis can be affected by the ascorbate in the meat system. The ascorbate can destroy some of the nitrite before the color reaction step. It has also been reported that breakdown products of other chemicals can be detected by this method (National Academy of Sciences, 1981).

The concentration of residual nitrite required to insure safety against the outgrowth of C. botulinum spores in cured meats has not been fully established. Bowen and Deibel (1974) and Hustad et al. (1973) reported that residual nitrite concentrations as low as 50 mg/kg were inhibitory to toxin production in frankfurters for 56 days at 27°C. These results generally agree with the results of the present study on fish.

It should also be recognized that as nitrite concentration decreases over time, the level of viable C. botulinum cells also decrease (Christiansen, 1980). This means that the

botulinal safety of the meat system depends on the presence of a residual nitrite concentration high enough to be maintained in the system until the viable cell level has decreased to a point where growth can no longer occur (Christiansen et al., 1978; Cook and Pierson, 1983).

The inhibition of C. botulinum type E spores by nitrite in combination with 4.3 percent salt (wp) as observed in this study is in agreement with the data reported by Pelroy et al. (1982). These authors reported that with the addition of nitrite (100 mg/kg, residual), the salt level could be lowered to 2.5% (wp) and still inhibit C. botulinum type E spores (10^2 spores/g) in smoked salmon steaks held for 7 days at 27°C. When the salmon was inoculated with 10^4 spores/g, a 3.1% salt (wp) was required with 100 mg/kg residual nitrite, to inhibit the type E botulinal outgrowth for 25 days at 27°C. Therefore, it is not unrealistic that with an inoculation level of 10^6 spores/g, the inhibition of outgrowth of C. botulinum type E spores for 56 days at 27°C would require a percent salt (wp) of 4.3 and a residual nitrite level of 63 mg/kg. The addition of sorbate with the nitrite provided the same inhibitory effect as the nitrite alone, but at a salt concentration (wp) of 3.4%.

The Effect of Residual Sorbate on Toxin Production by *C. botulinum* type E Spores

The residual sorbate concentrations of the smoked whitefish treated with nitrite and sorbate are shown in Table 14. Initially, the smoked whitefish had an average residual sorbate level of 195 mg/kg. Sorbate levels decreased and were no longer detectable by day 42. Since the sorbate was added to the brines at a level of 2600 mg/kg, the uptake of sorbate by the fish tissue appeared to be approximately 10% of the amount added to the brine. Although the level of residual sorbate in the smoked whitefish was low, the addition of sorbate in combination with nitrite appeared to enhance the inhibition of toxin production by the *C. botulinum* type E spores. As discussed earlier, the nitrite/sorbate combination prevented toxin production for 63 days at 27°C at a salt concentration (wp) of 3.4%, thus indicating that this treatment was equally as effective as the nitrite treatment. The ability of this low level of sorbate in combination with nitrite to inhibit toxin production at a lower salt level than the salt and nitrite combination indicates the possibility that a stronger, synergistic effect would be possible if the level of sorbate in the fish had been greater, i.e., closer to the 2600 mg/kg level recommended for inhibition of toxin production in cured meats (Robach, 1980).

The synergistic interaction between nitrite and sorbate noted in the literature is thought to be the result of the

Table 14. Residual sorbate levels[a] in smoked whitefish brined in different degree salometer brines with nitrite and sorbate and stored at 27°C.

Treatment	Days of Storage						
	0	7	14	21	28	35	42
	Sorbate concentration (mg/kg)						
10° Brine + nitrite + sorbate	188	117	71	47	47	33	ND
20° Brine + nitrite + sorbate	202	80	65	40	31	14	ND
30° Brine + nitrite + sorbate	196	109	64	43	40	24	ND

[a]Analyses were carried out in duplicate.

ND = not detectable, sensitivity of method was 10 mg/kg.

combination of the individual effects of these compounds. Sorbate/sorbic acid affects spore germination and retards cell development. Nitrite inhibits the outgrowth of the germinated cell, therefore the combination of these two compounds results in an increased inhibition of botulinal toxin production (Sofos et al., 1979a). However, this effect has only been shown with sorbate/sorbic acid levels of 2000-2600 mg/kg (Sofos et al., 1979a, 1979c).

The Effect of pH on toxin production by C. botulinum type E Spores

The inhibitory effect of both nitrite and sorbate on C. botulinum type E spore outgrowth is pH dependent. With both compounds, the undissociated acid form is responsible for the inhibitory effect on C. botulinum outgrowth and toxin production (Samson et al., 1955; Freese et al., 1973).

Initially, the smoked whitefish had an average pH value of 6.4 (Table 15). However, during storage there was a gradual decrease to an average of 5.4 for the samples prepared in the 10^{o} salometer brines, and to 5.5 for the remaining samples. The pH was never acid enough to totally inhibit botulinal growth. It has been indicated that as the pH decreased from 7.0 to 5.5, the inhibitory capacity of nitrite on botulinal outgrowth increases (Pelroy et al., 1982; Roberts and Ingram, 1966).

Table 15. Effect of storage at 27° on the pH values[a] of smoked whitefish brined in different degree salometer brines.

Treatment	Days of Storage					
	0	14	28	42	49	56
	pH value					
10° Brine	6.2	5.9	5.3	--	--	--
10° Brine + nitrite	6.3	5.9	5.4	--	--	--
10° Brine + nitrite + sorbate	6.4	5.9	5.5	--	--	--
20° Brine	6.4	6.0	5.6	5.4	5.5	5.4
20° Brine + nitrite	6.4	5.8	5.8	5.6	5.2	5.4
20° Brine + nitrite + sorbate	6.5	5.7	5.6	5.7	5.5	5.5
30° Brine	6.4	5.9	5.7	5.8	5.6	5.8
30° Brine + nitrite	6.3	6.0	5.8	5.8	5.7	5.5
30° Brine + nitrite + sorbate	6.4	5.9	6.0	5.7	5.6	5.6

[a]Analyses were carried out in duplicate.

The maximum inhibitory effect of nitrite has been shown to occur at pH values between 4.5-5.5 (Holley, 1981). Potassium sorbate is a strong inhibitor of C. botulinum at a pH value of 5.7 (Smoot and Pierson, 1981). In the present study, the whitefish had an overall initial pH value of 6.4. The samples became more acidic during their storage at 27°C, reaching pH values of 5.4-5.5. These pH values are ideal for the maximization of the inhibitory effect of both the nitrite and the sorbate on the botulinal spores and cells present in the samples. The decrease in the pH during the storage of the samples is presumably due to the presence of lactic-acid producing bacteria.

The Effect of Water Activity (Aw) on Toxin Production by C. botulinum type E Spores

There are very few literature reports describing the effect of Aw on the outgrowth and toxin production by C. botulinum type E in smoked fish. However, it has been reported that lowering the Aw through the use of salt is an effective means of inhibiting clostridia (Roberts and Smart, 1976). In addition, C. botulinum type E has been shown to be more sensitive to salt than are types A and B (Roberts and Smart, 1976). Therefore, in the present study, the samples were analyzed for their Aw levels during the first 56 day of incubation at 27°C.

There was an apparent difference in the initial water activities of samples containing the three different levels

of salt (Table 16). The samples brined in the 10, 20 and 30° salometer brines had average Aw's of 0.988, 0.976 and 0.962, respectively. The decrease in the initial Aw's with increasing levels of salt observed in this study is not unexpected since salt has been shown to act as a preservative by lowering the Aw of food (Deng, 1977). During storage (56 days) at 27°C, there was a gradual decrease in the Aw levels in the samples. As noted earlier, there was also a decrease in the pH of these samples over time in these samples. Baird-Parker and Freame (1967) reported that at a pH value of 7.0, an Aw of 0.97 or greater was required for the outgrowth of C. botulinum type E spores. They also noted that at pH values between 5.5-6.0, an Aw of 0.98 or greater was necessary for botulinal type E outgrowth.

In the initial (day 0) sample which had an overall pH value of 6.4, C. botulinum spores should be able to grow and produce toxin in the samples prepared in the 10 and 20° salometer brines since the Aw's averaged 0.988 and 0.978, respectively. In this study, toxicity did occur in the samples prepared in the 10° salometer brines within the first seven days at 27°C (Table 11). However, no toxicity was observed in the samples prepared in the 20° brines until day 42 (Table 11). The absence of toxicity in the sample prepared in the 20° salometer brine alone requires an explanation. There are two events that could be occurring based on the results presented by Baird-Parker and Freame

Table 16. Water activity levels[a] in smoked whitefish brined in different degree salometer brines prepared with or without nitrite or nitrite and sorbate and stored at 27°C.

Treatment	Days of Storage								
	0	7	14	21	28	35	42	49	56
10° Brine	0.992	0.982	0.980	0.971	0.971	0.970	--	--	--
10° Brine + nitrite	0.988	0.978	0.986	0.973	0.973	0.974	--	--	--
10° Brine + nitrite + sorbate	0.985	0.981	0.981	0.976	0.976	0.972	--	--	--
20° Brine	0.974	0.967	0.968	0.965	0.964	0.964	0.960	0.962	0.956
20° Brine + nitrite	0.976	0.972	0.969	0.964	0.965	0.964	0.065	0.961	0.970
20° Brine + nitrite + sorbate	0.978	0.976	0.989	0.966	0.966	0.965	0.964	0.964	0.969
30° Brine	0.961	0.958	0.954	0.955	0.956	0.939	0.939	0.956	0.952
30° Brine + nitrite	0.963	0.960	0.960	0.951	0.950	0.953	0.953	0.949	0.951
30° Brine + nitrite + sorbate	0.963	0.964	0.962	0.954	0.955	0.054	0.054	0.959	0.948

[a]Analyses were carried out in duplicate.

(1967) that at pH values of 7 and 6, the level of Aw required for botulinal growth would be 0.97 and 0.98, respectively. Since the sample containing salt alone (20^{o} salometer brine) had an Aw value of 0.974, this level of Aw could be low enough to inhibit the growth of the botulinal spores present since the pH was closer to pH 6 than pH 7. By day 7, this sample had a pH of 6.0 (Table 15) and an Aw of 0.968 (Table 16). At this point, the Aw was well below the 0.98 required for botulinal growth at pH 6. The samples prepared in the 20^{o} brine alone became toxic on day 42. This indicates that the C. botulinum spores/cells were able to adapt to the lower Aw level and were able to grow and produce toxin.

This finding is in agreement with those of Troller (1983) who reported that bacteria in Aw values lower than minimum growth level suffer a prolonged lag or "resting" phase in their growth curve. This phase is therefore more an "adaptation" phase because the organism undergoes an adaptation in order to survive the lower Aw level. Once the adaptation occurs, growth can resume, although usually at a slower rate initially (Sperber, 1982). Sperber (1982) also noted that spores can usually germinate at Aw's substantially lower than would permit growth. Baird-Perker and Freame (1976) reported that the minimum germination Aw for C. botulinum type E spores was 0.93, while growth required a minimum of 0.97 Aw, when salt was used to establish the Aw level.

The production of toxin in the samples prepared in the 20^{o} salometer brines with added nitrite and nitrite/sorbate on day 63 was not unexpected. Both residual nitrite and sorbate were not detectable on day 42. Once these compounds were depleted, the surviving viable cells could begin growing and toxin production would occur (Christiansen et al., 1978).

The lack of toxin in the samples prepared in the 30^{o} brines indicates that the salt level initially reduced the Aw to a level where germination could possibly occur but growth could not. The combined effects of the decreasing pH and Aw's further inhibited the outgrowth and toxin production by the botulinal cells. The ability of salt at this level (6% wp) to inhibit botulinal outgrowth has been discussed previously. The addition of nitrite and nitrite/sorbate did not affect the rate of toxin production at this high level of salt. This indicates that the salt level/Aw effect in these treatments was the primary inhibitor of the C. botulinum spores. These results agree with the findings of Segner et al. (1966), who reported that in a trypticase-peptone-glucose medium, a salt concentration (wp) of 5% inhibited toxin production by C. botulinum type E spores for 1 year in samples incubated at 37^{o}C.

Effect of Salt Level and Nitrite on the Lipid Stability and Organoleptic Acceptability of Smoked Whitefish

Chemical Analyses

The state of Michigan (Regulation 541) requires that smoked fish must contain at least 5% salt (wp) in the finished product. However, the acceptance of this high salt product in today's society could be limited as consumers are becoming increasingly aware of the relationship between dietary salt and the incidence of hypertension and strokes or heart attacks. Therefore, the following study was conducted to determine the effect of salt and nitrite on the lipid stability and organoleptic acceptability of smoked whitefish.

Chemical analyses, including salt, moisture, fat, TBA number residual nitrite and N-nitrosamine determinations were carried out on samples taken on days 0, 7, 14 and 22. Day 0 represents the initial day of the storage study, approximately 20 hours post smoking (cooking). Taste panel evaluations were made on all sets of samples except day 22, because of the 14 day limitation dictated by Regulation 541.

The percent salt in the water-phase (wp) of the whitefish was calculated from the salt and moisture data (Appendices 6 and 7, respectively). These were obtained by chemical analysis. The percent salt levels (wp) of whitefish brined in different degree salometer brines prepared with

and without nitrite are presented in Table 17. The control samples, which were soaked in water during the brining period contained minimal salt levels (0.34 to 0.40%) in the water-phase portion of the fish. The average percent salt levels (wp) in samples brined in the 10, 20 and 30^{o} salometer brines were 1.8-2.1, 4.0-4.3 and 6.3-6.9, respectively. These values agree with the data presented earlier.

There was a wide variation in the levels of fat in the smoked whitefish samples that had been prepared with different levels of salt in the brine (Table 18). Even with sample randomization, the fat contents ranged from 7.95-12.30 percent, with overall average of 9.43 percent. These fat levels are higher than the 3.84% and 5.2% values reported by Awad et al. (1969) and Exler and Weichrauch (1976), respectively. This difference in fat levels could be due to seasonal variation in the fish since there is a seasonal cycle that occurs in the catching of the fish (Stachiw et al., 1984). However, the fat levels reported in this study agree with other fat values for whitefish that have been determined in this laboratory in other studies (Maruf, 1983, unpublished data).

Fish lipids have been reported to contain significant quantities of long chain polyunsaturated fatty acids (Khayat and Schwall, 1983). The complexity of the fatty acids in the total, triglyceride and phospholipid fraction of whitefish lipid are shown in Table 19. The data presented do not

Table 17. Percent salt (water-phase) of smoked whitefish brined in different degree salometer brines prepared with and without nitrite.

Treatment	Days of Storage				
	0	7	14	22	$\bar{X}$
	Percent salt (water-phase)				
Control[a]	0.3	0.5	0.3	0.5	0.4
Control[a] + nitrite	0.5	0.3	0.2	0.4	0.3
10° Brine	1.9	1.7	1.6	2.0	1.8
10° Brine + nitrite	2.1	2.1	2.1	2.1	2.1
20° Brine	3.3	3.9	4.6	4.2	4.0
20° Brine + nitrite	4.2	4.3	4.7	4.0	4.3
30° Brine	6.3	7.3	7.1	6.9	6.9
30° Brine + nitrite	6.0	6.5	6.4	6.4	6.3

[a]No salt used in the brine.

Table 18. Percent fat[a] in smoked whitefish brined in different degree salometer brines prepared with or without nitrite.

Treatment	Days of Storage[b]				
	0	7	14	22	$\bar{X}$
	Percent fat (%)				
Control[c]	9.22	14.66	8.26	10.01	10.54
Control[c] + nitrite	12.85	14.80	8.13	13.43	12.30
10° Brine	8.57	5.53	7.60	10.10	7.95
10° Brine + nitrite	7.77	7.87	8.86	9.70	8.55
20° Brine	8.23	8.87	8.51	8.56	8.54
20° Brine + nitrite	7.79	8.57	8.52	9.22	8.53
30° Brine	9.34	7.85	10.78	9.97	9.49
30° Brine + nitrite	9.36	11.20	7.44	10.25	9.56

[a]Analyses were carried out in duplicate.

[b]Stored at refrigerated temperature (4°C).

[c]No salt used in the brine.

Table 19. Fatty acid composition of the total, triglyceride and phospholipid fractions of whitefish lipid.

Fatty acid	Total	Triglyceride	Phospholipid
		Area percent	
12:0	0.19	0.10	1.05
12:1	0.03	--	0.71
13:0	0.04	0.21	0.56
14:0	4.31	1.94	3.38
14:1	0.43	0.19	0.71
15:0	0.34	0.23	0.66
16:0	13.59	19.55	8.51
16:1	10.57	7.93	13.60
18:0	2.45	4.78	2.13
18:1	24.30	11.75	16.29
18:2	2.23	0.82	2.32
18:3	2.36	1.56	2.93
20:0	1.39	0.50	1.60
20:1	1.05	0.24	2.00
20:2	0.19	0.31	0.85
22:0	0.61	0.35	1.06
22:1	8.63	10.68	6.33
22:5	0.52	0.52	1.47
24:0	2.40	14.49	2.43
24:1	5.79	1.38	4.19

represent all of the fatty acids present in the lipid fractions and only includes those that could be identified by fatty acid standards.

While similar fatty acids are found in the total, triglyceride and phospholipid fractions of the whitefish lipid, there were some differences in the quantities of specific fatty acids in each fraction. The predominant unsaturated fatty acids in the triglyceride fractions were, in order of prominence, C18:1 (11.75%), C22:1 (10.68%), and C16:1 (7.93%), while in the phospholipid fraction C18:1 (16.29%) was most predominant followed in sequence by C16:1 (13.6%), C22:1 (6.33%), C24:1 (4.19%) and C18:3 (2.93%).

Based on the fatty acid data generated, the phospholipid fraction contained 21.4% of saturated fatty acids and 54.0% of unsaturated fatty acids. This gives an unsaturated to saturated fatty acid ratio of 2.5. The triglyceride fraction contained 42.2% of saturated fatty acids and 35.4% of unsaturated fatty acids. Therefore the triglyceride fraction had an 0.8 ratio of unsaturated fatty acids to saturated fatty acids. The higher level of unsaturation in the phospholipid fraction is not unexpected. It has been reported that within the same species, the phospholipids generally contain a higher level of unsaturated fatty acids (Pearson et al., 1977).

Exler and Weichrauch (1976) analyzed whitefish for its fatty acid composition and reported values as follows for

total lipids: C14:0, 2.1%; C16:0, 12%; C16:1, 10.6%; C18:0, 1.05%; C18:1, 27.4%, C18:2, 5.48%; C18:3, 3.59%; C20:0, 2.11% and C22:1, 4.2%. In comparison, the total lipid fraction of the whitefish analyzed in the present study had a much higher level of C22:1 (8.63%); however, there were lower levels of C18:2 (2.23%) and C18:3 (2.36%).

Braddock and Dugan (1969) studied the fatty acid composition of Coho salmon and reported that fatty acids ranged from C14:0 to C22:6 in chain length and in degree of unsaturation. In the Coho salmon, the levels of C16:0 and C16:1 were 10.8 and 10.9%, respectively. These values were comparable to those reported for the total lipid fraction in the present study. Literature values for the levels of C16:0 and C16:1, in a variety of fish species, range from 10.8 to 24.8% and 4.0 to 20.8%, respectively (Braddock and Dugan, 1969; Ackman and Eaton, 1971; Ackman et al., 1975; Exler and Weichrauch, 1976; Mai and Kinsella, 1979; Leu et al., 1981; Gall et al., 1983). Therefore, the levels of C16:0 and C16:1 reported for the whitefish lipids in the present study are reasonable.

The whitefish used in this study had an average fat content of 9.43%. When this lipid was separated into its triglyceride and triglyceride fractions, it was found to contain 78% triglyceride and 22% phospholipid. These values agree with those of Awad et al. (1969), who reported that the whitefish lipid contained 80% triglyceride and 20% phospholipid.

In contrast, Igene et al. (1979) reported that beef lipids contain 8% phospholipids, while triglycerides account for the other 92%. In chicken, the phospholipid is 14% of the total lipid and the triglyceride is 86%. Pearson et al. (1977) indicated that poultry and fish contain more phospholipids than red meats. The results of this study support this statement.

Residual Nitrite Levels of Smoked Whitefish during Storage

Residual nitrite levels in these samples at the start of the refrigerated storage period ranged from 37.5 to 53.1 mg/kg with an average of 46 mg/kg (Table 20). There was a gradual depletion of nitrite during refrigerated storage as was expected. Residual nitrite is the amount of nitrite that is analytically detectable or recoverable by chemical analysis (Ito et al., 1983). The depletion of nitrite with time in cured meat is well established in the literature. The fate of the nitrite in the cured meat system has not been clearly demonstrated. Mirna and Hofmann (1969) reported that nitrite and thiol groups disappeared equimolarly in a minced meat system containing nitrite. They suggested that nitrosothiol formation was occurring and was responsible for nitrite depletion. Kubberod et al. (1974) showed that nitrosothiol formation did not play a major role in the nitrite loss from cured meats. Woolford et al. (1976) reported that one of the major pathways for nitrite loss in cured meats could be the

Table 20. Residual nitrite levels[a] of smoked whitefish brined in different degree salometer brines containing nitrite.

Treatment	Days of Storage[b]			
	0	7	14	22
	Nitrite (mg/kg)			
Control[c] + nitrite	46.9	36.3	28.8	10.0
10° Brine + nitrite	37.5	36.3	28.8	7.5
20° Brine + nitrite	53.1	37.5	31.3	16.3
30° Brine + nitrite	46.3	37.5	31.3	6.0

[a]Analyses were carried out in triplicate.

[b]Stored at refrigerator temperature (4°C).

[c]No salt used in the brine.

reaction between nitrite and heme proteins. Ito et al. (1983) noted that an assumption has been made that the protein-bound nitrite is unavailable for further reactions. However, they showed that the protein-bound nitrite complex is not stable and is potentially reversible. They suggested that the "bound" nitrite could function in transnitrosation reactions.

The use of nitrite in meat systems has been under scrutiny since it has been shown that nitrite reacts with secondary amines and amino acids to produce N-nitrosamines (Gray, 1976). N-Nitrosopyrollidine (NPYR) and NDMA, both commonly identified in cured meats, have been shown to be carcinogenic (Gray, 1976). Therefore, before advocating the use of nitrite in smoked whitefish, a product that could have high levels of amines (Castell et al., 1971), it was considered necessary to establish whether N-nitrosamines could be formed in nitrite-treated smoked whitefish.

Smoking has been shown to result in the formation of N-nitrosothiazolidine (NTHZ) in smoked cured meats (Pensabene and Fiddler, 1983a). Mandagere et al. (1984) has related the formation of NTHZ to the reaction of formaldehyde in smoke with cysteamine and/or cysteine in the meat. This reaction can subsequently result in the formation of NTHZ and/or N-nitrosothiazolidine carboxylic acid (NTCA). Since the production of smoked fish uses a heavy smoking process, it was necessary to evaluate the possible risk of NTHZ

formation in the finished product.

The nitrite-treated samples in this study were analyzed for volatile N-nitrosamines (NDMA and NTHZ) and NTCA. No detectable level of these compounds were found in any of the samples tested. The absence of NDMA in these samples is not unexpected since most of the N-nitrosamines reported in fish have been isolated in marine species fish. Marine species fish have been shown to contain high levels of trimethylamine which is degraded to formaldehyde and dimethylamine. The dimethylamine is readily nitrosated (Dyer and Mounsey, 1945; Shewan, 1951; Castell et al., 1971; Spinelli and Koury, 1979; Sikorski and Kostrich, 1982).

Stability of Smoked Whitefish Lipids during Storage

The stability of lipids in whitefish prepared in brines containing different salt concentrations was evaluated by the TBA procedure. The results of these analyses are presented in Table 21. The control samples prepared without nitrite had an average TBA number of 2.04 after 22 days of storage at $4^{o}C$. The addition of salt to the smoked whitefish resulted in increased levels of rancidity as measured by TBA number. The samples brined in the 10, 20 and 30^{o} salometer brines without nitrite had TBA numbers of 2.85, 2.54 and 2.68, respectively, after storage for 22 days. These data indicate that each level of salt had a significant ($P<0.01$) prooxidant effect in this system; however, no correlation was

Table 21. TBA values[a] of smoked whitefish brined in different degree salometer brines prepared with or without nitrite.

Treatment	Days of Storage[b]			
	0	7	14	22
	TBA value			
Control[c]	1.06	2.07	1.94	2.04[d]
Control[c] + nitrite	0.32	0.42	0.34	0.41[e]
10° Brine	1.06	2.30	2.16	2.85[f]
10° Brine + nitrite	0.45	0.67	0.52	0.87[g]
20° Brine	0.97	1.76	1.60	2.54[h]
20° Brine + nitrite	0.54	0.59	0.41	0.83[g]
30° Brine	0.85	1.77	2.27	2.68[h]
30° Brine + nitrite	0.22	0.48	0.44	1.86[i]

[a]Analyses were carried out in duplicate.

[b]Samples were stored at 4°C.

[c]No salt used in the brine.

Values with different letters are significantly different ($P<0.01$).

found between the increasing levels of salt and the increased rate of lipid oxidation (TBA number).

A prooxidant effect of salt has not been clearly shown in the literature. Originally, it was postulated that salt promoted the activity of lipoxidases in meats (Banks, 1937; Lea, 1939). However, Banks (1944) and Tappel (1952, 1953) reported that meat did not contain lipoxidases. They felt the prooxidant effect of salt was due to a catalytic effect of the heme pigments. These researchers failed to demonstrate any evidence of heme pigment's ability to catalyze the prooxidant effect of salt.

Gibbons et al. (1951) reported that when salted bacon sides were held at frozen temperatures, rancidity increased as the temperature decreased. Chang and Watts (1950) reported that the addition of salt, at levels of 15% or greater, to lard had a direct effect on the rate of rancidity development in the lard. Ellis et al. (1968) reported that in freezer stored salt cured pork, the rate of oxidation occurred at a rapid rate. Ellis et al. (1968) also reported that as the level of salt in the pork increased so did the rate of oxidation. They found that high proportions of lean increased the rate of oxidation and that the direct effect of the salt on oxidation did not appear to involve a reactive chloride ion. The prooxidant effect of salt has been suggested to be the result of heavy metal contamination of the salt. Denisov and Emanuel (1960) noted that heavy metals

(iron, copper and chromium) can catalyze oxidative rancidity. The flake salt used in curing has been found to contain iron and copper (Olson and Rust, 1973). More research is needed to clarify the effect of salt on the rate of lipid oxidation in food systems (Love, 1983).

The addition of nitrite to the unsalted whitefish samples resulted in a significant ($P<0.01$) decrease in the rate of lipid oxidation as measured by the TBA number (Table 21). This indicates the strong antioxidant effect of nitrite in this system. The efficacy of nitrite as an antioxidant was diminished as the level of salt in the sample increased. The TBA numbers of the samples brined in the 10, 20 and 30^{o} salometer brines with added nitrite were 0.87, 0.83 and 1.86, respectively. These data again suggest a prooxidant effect of salt in this system, although further studies are necessary to confirm this observed trend since only one experiment was carried out.

The antioxidant effect of nitrite on the development of WOF in cooked meats has been shown in red meats and poultry. Fooladi et al. (1978) reported that the addition of nitrite (156 mg/kg) to cooked beef, pork and chicken resulted in a two-fold reduction in their TBA numbers. MacDonald et al. (1980) reported that the addition of nitrite to cured hams significantly reduced TBA numbers. Chen et al. (1984) showed that the addition of nitrite to meat pigment extracts prevented the release of heme iron from the porphyrin ring. It

has been suggested that nitrite inhibits the development of WOF by reacting with iron porphyrins to form a stable non-reactive complex (Zipser et al., 1964; Igene et al., 1979). Kanner et al. (1981) proposed that the antioxidant effect of nitrite in cured meat could be attributed to the formation of nitric oxide. Nitric oxide is capable of interacting with metals, especially heme and nonheme compounds. Shahidi et al. (1985) reported that the addition of nitric oxide gas to a buffered solution containing hemin resulted in the formation of cooked cured-meat pigment, dinitrosyl ferro-hemochrome.

Igene et al. (1985) suggested that in addition to its ability to bind heme and nonheme pigments and to chelate metals, nitrite can inhibit the development of WOF by stabilizing the unsaturated lipids in the membranes of the meat tissue.

Overall the TBA numbers observed in this set of samples agree with literature values pertaining to the oxidation of fish, including whitefish. Biggar et al. (1975) reported that in canned whitefish that were opened and exposed to refrigerator temperatures for four days, the TBA values reached a maximum value of 1.97. These fish were assumed to contain 2% lipid. Awad et al. (1969) studied lipid oxidation in whitefish held frozen at -10^{o}C for up to 16 weeks. They found a maximum TBA value of 2.26 at the end of the 16 weeks of storage. These fish contained 3.8% lipid.

Mechanically deboned dogfish containing 8.7% lipid was stored at -20°C for 6 months had TBA values as high as 6-7 (Nakayama and Yamamoto, 1977). In contrast, it has been shown that cod containing 0.6% lipid and stored at -20°C for 6 months had TBA values between 0.5 and 1.0 (Nakayama and Yamamoto, 1977).

Sensory Analysis of Smoked Whitefish Samples

On each taste panel day, the treatment samples were divided into two sets of four in which all salt levels were represented as were nitrited samples. The panelists were asked to evaluate which treatment(s) produced the most desirable product relative to the perceived desirability of specific traits, which included odor, saltiness, texture, flavor and overall acceptability.

The procedure of Kramer (1963) was used to statistically analyze the sensory evaluation data. An ANOVA (Gill, 1978) was run using all eight samples on each day. This was done in order to determine if there were any significant differences between the eight samples on each test day. Bonferroni's t-statistic comparisons (Gill, 1978) were made on specific sets of treatment samples to determine if the panelists could detect significant differences between specific treatments. Bonferroni's comparisons were used because of the greater sensitivity to differences when limited comparisons are made between treatments (Gill, 1978).

On day 0, the first day of the study, no differences were recorded in the odor of any of the sample treatments (Table 22). The samples found to be significantly ($P<0.05$) more desirable in their saltiness, flavor and overall acceptability were those prepared in the 20^{o} salometer brine, with or without nitrite. There was no significant difference between textures of the samples.

On day 7, no significant ($P<0.05$) differences in odor of the sample treatments were perceived (Table 23). The most desirable ($P<0.05$) level of saltiness, texture, flavor and overall acceptability was found in the samples prepared in the 20^{o} salometer brine containing nitrite. The panelists found the unsalted sample containing no nitrite, significantly ($P<0.05$) less desirable in all of the traits except odor. On day 14, no differences in odor were detected among any of the eight treatment samples (Table 24). The samples found to be significantly ($P<0.05$) most desirable in all traits except odor were those prepared in the 20^{o} salometer brines, with or without nitrite.

The sensory data from all eight treatments were analyzed by ANOVA and Bonferroni's comparisons. On day 0, significant differences were found in the desirability of saltiness ($P<0.01$), texture ($P<0.01$), flavor ($P<0.01$) and overall acceptability ($P<0.001$) in all samples evaluated. Bonferroni's contrasts were made between the samples brined in the 20 and 30^{o} salometer brines only. The Bonferroni's contrasts

Table 22. Average sensory scores at day 0 for smoked whitefish samples brined in different degree salometer brines.

Treatment	Ranking[a]				
	Odor	Saltiness	Texture	Flavor	Overall Acceptability
Control[b]	2.2	3.2	3.5**	4.0**	3.8**
10° Brine	2.3	2.7	2.7	2.2	2.3
20° Brine	3.2	1.3*x	1.8	1.3*	1.3*x
30° Brine	2.8	2.3y	2.0	2.8	2.8y
Control[b]	2.7	3.8**	3.8**	3.7**	3.6**
10° Brine + nitrite	2.7	2.5	2.7	2.5	2.5
20° Brine + nitrite	2.2	1.2*x	1.5	1.3*	1.2*x
30° Brine + nitrite	2.0	3.0y	2.0	2.2	2.3y

[a]Scale: 1 = most desirable; 4 = least desirable

[b]No salt used in the brine

x,ySamples with different letters are significantly ($P<0.01$) different

*Most desirable (Kramer ranking analysis)

**Least desirable (Kramer ranking analysis)

Table 23. Average sensory scores at day 7 for smoked whitefish brined in different degree salometer brines.

Treatment	Ranking[a]				
	Odor	Saltiness	Texture	Flavor	Overall Acceptability
Control[b]	2.0	3.2**	3.4**	3.3**	3.6**
10° Brine	2.1	2.8	3.1	3.1	3.2
20° Brine	2.9	2.4	2.4	2.5[x]	2.3
30° Brine	2.9	2.4	2.3	2.2	2.1
Control[b] + nitrite	2.3	3.1	3.1	3.3**	3.2**
10° Brine + nitrite	2.6	2.1	2.4	2.0	1.9
20° Brine + nitrite	2.7	1.7*	1.4*	1.3*[y]	1.4*
30° Brine + nitrite	2.6	2.2	1.7	2.1	2.2**

[a]Scale: 1 = most desirable; 4 = least desirable

[b]No salt used in the brine

[x,y]Samples with different letters are significantly ($P<0.05$) different.

*Most desirable (Kramer ranking analysis)

**Least desirable (Kramer ranking analysis)

Table 24. Average sensory scores at day 14 for smoked whitefish brined in different degree salometer brines.

Treatment	Ranking[a]				
	Odor	Saltiness	Texture	Flavor	Overall Acceptability
Control[b]	2.3	3.1**	3.6**	3.3**	3.2**
10° Brine	2.9	2.1	3.0	2.9	2.9
20° Brine	2.1	2.0*	2.0*	2.0*	1.9*
30° Brine	2.5	3.0	2.0	2.1	2.3
Control[b] + nitrite	1.9	3.6**	3.8**	3.7**	3.8**
10° Brine + nitrite	2.4	2.1	1.9	2.6	2.6
20° Brine + nitrite	2.9	1.3*x	1.3*	1.3*	1.1*x
30° Brine + nitrite	3.1	2.7y	2.6	2.1	2.3y

[a]Scale: 1 = most desirable; 4 = least desirable

[b]No salt used in the brine

[x,y]Samples with different letters are significantly ($P<0.05$) different

*Most desirable (Kramer ranking analysis)

**Least desirable (Kramer ranking analysis)

showed that the samples brined in the 20° salometer brine with or without nitrite had a significantly more desirable ($P<0.01$) level of saltiness than the sample prepared in the 30° salometer brines prepared with or without added nitrite (Table 22). No differences in texture were found between the samples prepared in the 20 and 30° salometer brines. However, the 20° salometer brined samples were significantly ($P<0.01$) more desirable in flavor and overall acceptability than the 30° salometer brined sample.

On each sampling day, the eight treatments were tested for sensory differences that could result from the presence of nitrite in combination with the four levels of salt. However, no nitrite effect was found in any of the sensory data, indicating that the panelists were unable to distinguish between the nitrite and non-nitrited smoked whitefish.

On day 7, significant differences were found between the eight samples in texture ($P<0.05$), flavor ($P<0.05$) and overall acceptability ($P<0.001$) by ANOVA. However, the Bonferroni's comparison showed that the 20° salometer brined sample without nitrite was significantly ($P<0.05$) less desirable than the sample prepared in the 20° salometer brine with nitrite (Table 23).

On day 14, significant differences were found in saltiness ($P<0.01$), texture ($P<0.01$), flavor ($P<0.05$) and overall acceptability ($P<0.05$). Again it appeared that most of the significant differences occurred in the samples prepared in

the control and the 10^{o} salometer brines. The fish sample prepared in the 20^{o} salometer brine with nitrite was significantly ($P<0.05$) more desirable in saltiness and overall acceptability than the sample prepared in the 30^{o} salometer brine with nitrite (Table 24).

Over time, the majority of the differences seen in the ANOVA analysis was due to the unacceptability of those samples prepared in the control and the 10^{o} salometer brines. These samples were not tested using the Bonferroni's comparison because they would not be a commercially viable product.

These data indicate the preference by the panelists for a smoked whitefish containing 2.5 to 2.85% salt or 4.0 to 4.3% salt (wp). The 4% salt (wp) concentration is obviously lower than the 5% salt (wp) that is required by Michigan Regulation 541. The botulinal safety of the whitefish brined in a 20^{o} salometer brine and which contain 4 percent salt (wp) in the finished product has been demonstrated in the initial phase of this study. It was also demonstrated that the inclusion of nitrite increases the safety of this product by delaying the outgrowth of C. botulinum type E spores. In addition, the panel data indicate that the addition of nitrite to smoked whitefish does not affect the desirability of the product. Nitrite in the smoked whitefish did not result in the formation of detectable levels of N-nitrosamines. Therefore, the addition of nitrite in combination with a 4% salt (wp) concentration could be recommended for use in the production

of smoked whitefish.

Effect of Smoke Type and Level on the Lipid Stability and Organoleptic Acceptability of Smoked Whitefish

Chemical Analyses

The salt concentration in the water-phase of smoked whitefish prepared with different levels of liquid smoke or smoked with woodsmoke was calculated from the salt and moisture data (Appendices 8 and 9, respectively). The results are presented in Table 25. The fish samples had salt (wp) concentrations that ranged from 4.8 - 6.5% salt (WP) with an overall average of 5.9% salt (wp). These results are consistent with the values reported in previous phases of this study. Only one treatment had an average salt concentration (wp) that was less than the 5% required by Michigan law.

There was much variation in the level of fat found in these samples, even with randomization of samples (Table 26). The average fat content ranged from 9.11 to 12.72% with an overall average of 10.47%. The fat levels in these samples are comparable to the levels reported previously in this study.

The initial average residual nitrite levels in the samples, i.e. at day 0, brined with different levels of liquid smoke or smoked with woodsmoke was 53.8 mg/kg (Table 27). There was a gradual depletion of nitrite during the refrigerated storage period to a final average level of 27.6 mg/kg

Table 25. Percent salt concentration (water-phase) of brined whitefish[a] treated with various levels of liquid smoke or smoked with woodsmoke.

Treatment	Days of Storage[b]				
	0	7	14	22	$\bar{X}$
	Percent salt[c]				
Woodsmoke	6.6	7.7	6.3	5.8	6.2
Woodsmoke + nitrite	6.2	6.8	6.6	6.4	6.5
0.7% Liquid smoke	5.7	6.0	5.8	5.9	5.9
0.7% Liquid smoke + nitrite	6.1	6.0	6.4	6.8	6.3
1.4% Liquid smoke	5.3	5.9	6.1	6.6	6.0
1.4% Liquid smoke + nitrite	5.6	6.4	6.6	6.7	6.3
2.1% Liquid smoke	4.7	6.5	5.2	5.1	5.4
2.1% Liquid smoke + nitrite	5.3	4.9	4.5	4.5	4.8

[a]Brine contained 7.89% salt (30^{o} salometer)

[b]Stored at refrigerated temperature ($4^{o}C$)

[c]Analyses were carried out in triplicate

Table 26. Percent fat in brined whitefish[a] treated with various levels of liquid smoke or smoked with woodsmoke.

Treatment	Days of Storage[b]				
	0	7	14	22	$\bar{X}$
	Percent fat[c]				
Woodsmoke	9.89	10.65	11.99	10.51	10.76
Woodsmoke + nitrite	9.77	9.58	10.54	8.51	9.60
0.7% Liquid smoke	10.26	12.14	11.50	11.06	11.24
0.7% Liquid smoke + nitrite	9.52	9.18	9.42	9.33	9.11
1.4% Liquid smoke	7.35	9.25	10.62	10.11	9.33
1.4% Liquid smoke + nitrite	11.26	10.95	10.87	11.29	11.09
2.1% Liquid smoke	8.52	13.12	8.05	9.93	9.93
2.1% Liquid smoke + nitrite	11.77	13.54	12.08	13.48	12.72

[a]Brine contained 7.89% salt (30° salometer)

[b]Stored at refrigerated temperature (4°C)

[c]Analyses were carried out in duplicate

Table 27. Residual nitrite levels of brined whitefish[a] with or without nitrite and treated with varying levels of liquid smoke or smoked with woodsmoke.

Treatment	Days of Storage[b]			
	0	7	14	22
	Nitrite (mg/kg)[c]			
Woodsmoke + nitrite	63.8	41.3	36.3	28.7
0.7% Liquid smoke + nitrite	51.5	42.5	32.5	26.3
1.4% Liquid smoke + nitrite	46.3	45.0	37.5	28.8
2.1% Liquid smoke + nitrite	53.8	47.5	39.8	26.5

[a]Brine contained 7.89% salt (30^{o} salometer)

[b]Stored at refrigerator temperature ($4^{o}C$)

[c]Analyses were carried out in triplicate

after storage for 22 days.

The samples treated with nitrite were analyzed for their N-nitrosamine (NDMA and NTHZ) and NTCA content. No detectable levels of these compounds were found in any of the samples tested. These results indicate that the addition of nitrite to smoked whitefish would not result in the formation of any detectable levels of N-nitrosamines.

Stability of Whitefish Lipids During Storage

The TBA values for whitefish brined with different levels of liquid smoke or smoked with woodsmoke during refrigerated storage are shown in Table 28. The greater antioxidant activity of the woodsmoke was evident by the 5th day of the study. On day 14, it was found that as the level of liquid smoke increased, the rate of lipid oxidation significantly ($P<0.01$) decreased. However, the same level of effectiveness shown by woodsmoke as an antioxidant was never achieved by the liquid smoke. After 22 days of refrigerated storage, there was a decreased level of oxidation (as measured by TBA number) in the samples brined without nitrite. These lower levels of oxidation indicate a reduced level of malonaldehyde in the samples.

Malonaldehyde in the presence of water exists mainly as the enolate ion. The enolate ion can react with amino acids, proteins, glycogen and other food constituents to form products in which the malonaldehyde exists in a bound

Table 28. Effect of smoke type and level on the TBA values of whitefish brined[a] with or without nitrite.

Treatment	Days of Storage[b]			
	0	7	14	22
	TBA value (mg/kg)[c]			
Woodsmoke	0.75	0.99	1.17[d]	1.00[d]
Woodsmoke + nitrite	0.44	0.52	0.54[e]	0.47[e]
0.7% Liquid smoke	1.80	3.07	5.17[f]	3.25[f]
0.7% Liquid smoke + nitrite	0.80	0.72	1.45[d]	1.54[g]
1.4% Liquid smoke	0.99	1.77	3.09[g]	3.07[f]
1.4% Liquid smoke + nitrite	0.65	0.61	1.39[d]	1.45[g]
2.1% Liquid smoke	1.29	1.37	2.52[h]	3.22[f]
2.1% Liquid smoke + nitrite	0.66	0.51	0.59[e]	0.99[d]

[a]Brine contained 7.89% salt (30° salometer)

[b]Stored at refrigerated temperature (4°C)

[c]Analyses were carried out in duplicate

Values with different letters on a single day are significantly different ($P<0.01$)

form (Kwon et al., 1965). Buttkus (1967) showed that malonaldehyde reacted with the ε-amino groups in frozen trout myosin. Braddock and Dugan (1973) reported that in freeze-dried salmon steaks and salmon steaks held at -20°C for 1 year, there was an initial rapid increase in the level of oxidation as measured by the TBA number. However, over time the rate of oxidation as measured by increase in TBA number decreased. These researchers reported the presence of C=N functional groups, i.e, Schiff's base type compounds. When the freeze dried and frozen salmon samples were analyzed using UV, visible and fluorescence spectra and IR spectra before and after borohydride reduction, Kikugawa and Ido (1984) reported the formation of fluorescent products as a result of the reaction between malonaldehyde and primary amines. The reactivity of malonaldehyde with amino compounds has been related to the occurrence of browning (Maillard) in food systems (Porter et al., 1983). This reactivity of malonaldehyde with food components may account for the lower levels of detectable malonaldehyde seen in this study. Reduced levels of rancidity (TBA numbers) over storage time has been shown in fish. Botta et al. (1973) studied the rate of oxidation (TBA number) in Pacific halibut and Chinook salmon during frozen (-30°C) storage for 81 weeks. The maximum level of oxidation in the Pacific halibut occurred on week 62, thereafter the TBA number decreased to a level almost equal to the initial level. In the Chinook salmon, Botta et al.

(1973) found that the highest TBA number occurred during the 26th week of frozen storage and that this level of oxidation (TBA number) decreased until on week 77, it was almost equal to the initial level.

The addition of nitrite produced a significant ($P<0.01$) reduction in the rate of lipid oxidation in all systems tested (Table 28). The greatest effects were seen in the combinations of nitrite with woodsmoke and with 2.1% liquid smoke. These data confirm the antioxidant effect of nitrite in smoked whitefish during 22 days of refrigerated storage and confirm the results reported in the previous study. Day 14 data indicate that the 2.1% liquid smoke in combination with nitrite was equal to the woodsmoke with nitrite treatment in inhibiting oxidative rancidity in the smoked whitefish.

Bailey and Swain (1973) reported that woodsmoke in combination with nitrite inhibited oxidation in hams to a greater extent than woodsmoke alone. These results are in agreement with the findings of this study.

The greater ability of woodsmoke to inhibit oxidation in the smoked whitefish may be related to the method of application. The antioxidant effect of woodsmoke has been related to a surface effect. Draudt (1963) in a review of the smoking process in meats, reported that oxidation occurred most rapidly in the first half inch of the surface of unsmoked bacon. In smoked bacon, the surface oxidation rate was greatly reduced. Chen and Issenberg (1972) noted that

the preservative (antioxidant) effect of woodsmoke in foods was the result of partial surface dehydration and deposition of antioxidant compounds from the smoke onto the surface of the product. The antioxidant effect of smoke has been related to the presence of phenols in the smoke (Fretheim et al., 1980; Toth and Potthast, 1984). Bratzler et al. (1969) showed that the greatest quantity of phenols in smoked bacon occurred on the surface of the product.

In the present study, the outer layer of the whitefish treated with woodsmoke received a heavy smoking. This coating of the outer layer with smoke acted to prevent surface oxidation in these samples. In the samples treated with the liquid smoke, there was no surface protection, because the phenols were dispersed uniformly throughout the product. Therefore the samples treated with liquid smoke had no surface-barrier and oxidation could occur on the surface to a greater extent than in the woodsmoke-treated whitefish.

The whitefish samples prepared by the various smoking procedures were analyzed for their total phenol content by the method of Bratzler et al. (1969). The results of these analyses are presented in Table 29. The samples smoked with woodsmoke had a total phenol content of 79.2 mg/100 g of fish. The samples prepared in brines containing 0.7, 1.4 and 2.1% liquid smoke had total phenol contents of 46.1, 82.1 and 112.3 mg/100 g sample, respectively. As shown in Table 29, the levels of total phenols in the woodsmoke and

Table 29. Total phenol content of whitefish smoked with different levels of liquid smoke or smoked with woodsmoke.

Treatment	Total Phenols[a,b]
	(mg/100 g)
Woodsmoke	79.2
0.7% Liquid smoke	46.1
1.4% Liquid smoke	82.1
2.1% Liquid smoke	112.3

[a]Determined by the method of Bratzler et al. (1969).

[b]Analyses were carried out in duplicate on the sample taken on day 0.

the sample prepared with brine containing 1.4% liquid smoke were essentially the same. However, their effect on the rate of lipid oxidation were not equal (Table 28).

In an attempt to explain the significant difference ($P<0.01$) between the antioxidant effect of the woodsmoke and the liquid smoke (Table 28), the respective samples were analyzed for their phenol profiles (Figures 4 and 5). Some of the major differences in the relative percent concentrations of the individual phenols that could be tentatively identified through the use of standards and their retention times are shown in Table 30. The woodsmoke samples contained greater amounts of phenols which were tentatively identified as m-phenylphenol (1.1%), eugenol (0.5), isoeugenol (52.4%), 2,2-bisquinoline (9.2%) and o-hydroxyphenol (3.7%) than the samples prepared with the liquid smoke. The liquid smoke samples appeared to contain more guaiacol, 2-methoxy-4 methylphenol (10.4%), phenol (26.2%), and 4-methylsyringol (1.7%).

Knowles et al. (1975) studied the phenol uptake by bacon prepared by traditional kiln smoking and bacon smoked using electrostatic application of liquid smoke. They reported that bacon smoked using the traditional kiln smoking contained greater levels of eugenol, isoeugenol and 4-methylsyringol. The samples prepared with the liquid smoke had greater levels of guaiacol, phenol, o-cresol, 2-methoxy-4-methylphenol and m/p-cresols. These results agree with the findings of the

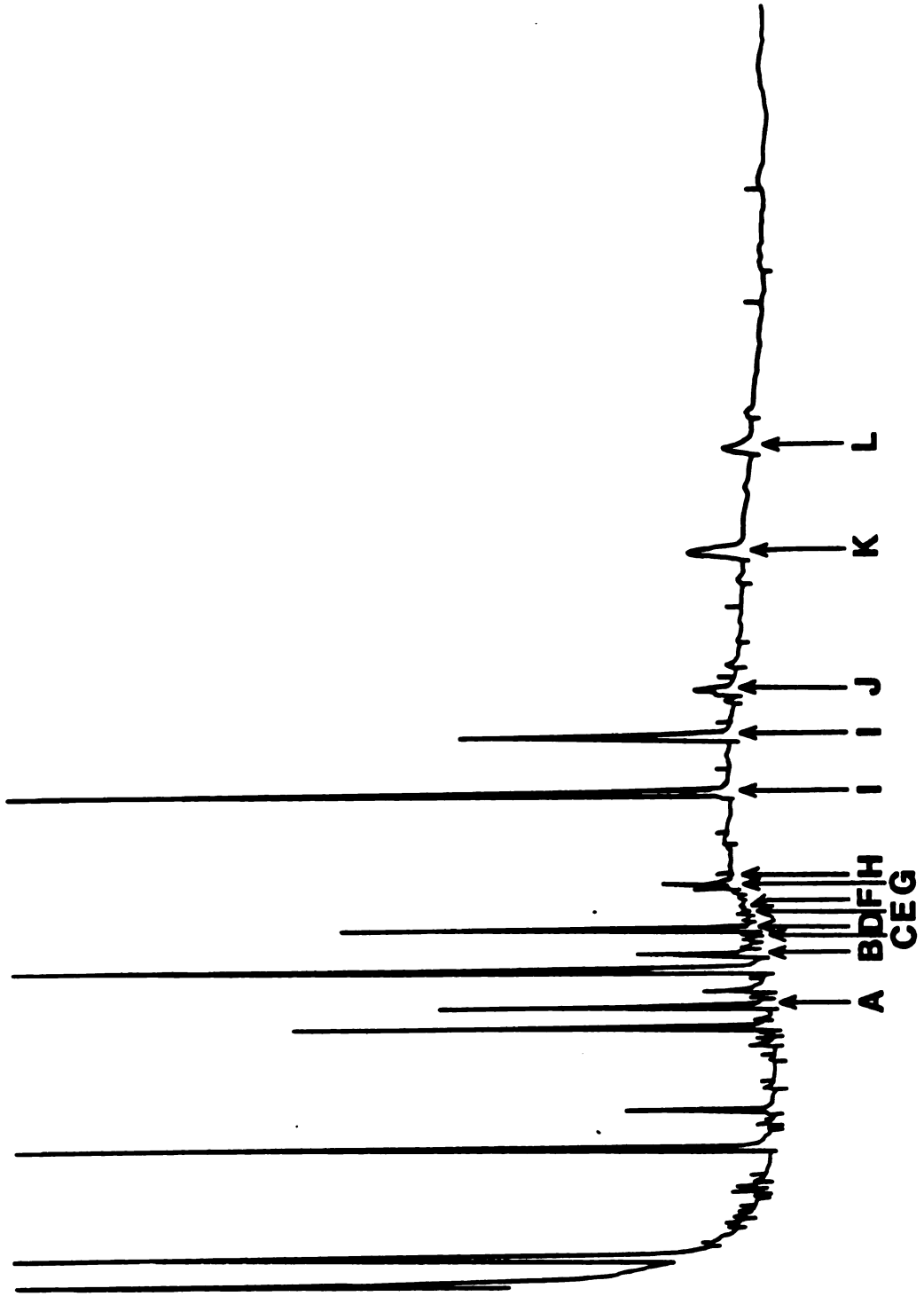

Figure 4. Gas chromatographic analysis of whitefish smoked with woodsmoke.

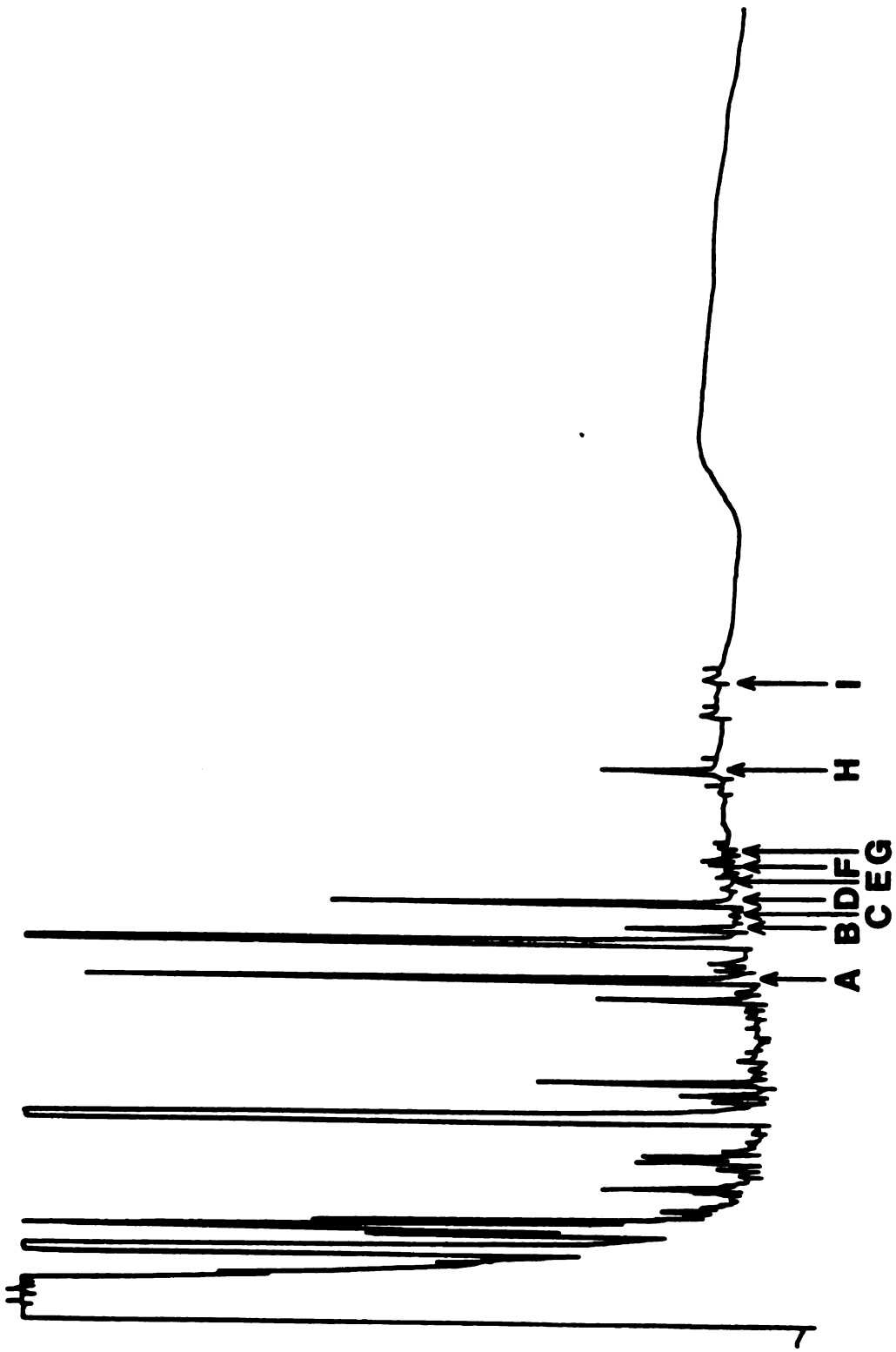

Figure 5. Gas chromatographic analysis of smoked whitefish prepared with 1.4% liquid smoke in the brine.

Table 30. Relative percent of phenols tentatively identified in whitefish prepared with 1.4% liquid smoke or smoked with woodsmoke.

Phenol	Woodsmoke (%)	1.4% Liquid Smoke (%)
Guaiacol	10.0	44.6
2-Methoxy-4-methylphenol	4.8	10.4
m-Phenylphenol	1.1	ND
Phenol	12.6	26.2
p-Cresol	0.7	0.7
m-Cresol	1.5	1.4
2,4-Dimethylphenol	2.6	2.5
Eugenol	0.5	ND
Isoeugenol (cis and trans)	52.4	12.2
4-methylsyringol	0.4	1.7
2,2-Biquiniline	9.2	ND
O-Hydroxyphenol	3.7	ND

ND = not detected, limit of detection was 1000 mg/kg

present study.

Daun (1979) noted that the antioxidant activity of smoke is due to the higher, rather than the lower, boiling point phenols. The lower boiling point phenols include phenol, cresols and guaiacol, while the higher boiling point phenols include syringol and its derivatives (Toth and Potthast, 1984). In the present study, fish samples prepared with the liquid smoke had greater levels of guaiacol, 2-methoxy-4-methylphenol (4-methylguaiacol), phenol and the cresols. All of these compounds are considered to be part of the lower boiling point phenols which have less antioxidant activity.

Toth and Potthast (1984), in a review on the chemical aspects of meat smoking, reported that pyrocatechol, guaiacol, eugenol and isoeugenol have been found to have antioxidant activity; however, no information of their relative effectiveness as antioxidants was reported. In the present study, the whitefish smoked with woodsmoke had higher levels of eugenol and isoeugenol which could help explain the greater antioxidant activity of the woodsmoke.

Sensory Analysis of Smoked Whitefish

The results of the statistical analysis of the taste panel evaluations of the samples prepared with different levels of liquid smoke in their brines or smoked with woodsmoke was carried out as described previously. On day 0,

the samples prepared with woodsmoke, with and without nitrite, were found to be significantly ($P<0.05$) more desirable in odor and in flavor (Table 31). The non-nitrited woodsmoked sample was also found to be significantly ($P<0.05$) more desirable in texture and overall acceptability relative to the other samples. In contrast, both samples prepared with 2.1% liquid smoke in their brine were found to be significantly ($P<0.05$) less desirable in their odor, flavor and overall acceptability (Table 31). These data indicate that there is an upper limit on the amount of liquid smoke that can be used in this product with respect to consumer acceptance.

By day 5, both woodsmoke treated samples were still significantly ($P<0.05$) more desirable in odor, but only the non-nitrited woodsmoke treated sample was significantly ($P<0.05$) more desirable in flavor and overall acceptability (Table 32). The samples prepared with the 2.1% liquid smoke in the brine containing nitrite were found to be significantly ($P<0.05$) less desirable in flavor and overall acceptability. By day 14, the panelists were unable to distinguish any specific sample treatment as being consistently most or least desirable (Table 33). Significant differences in the samples tested appeared to be occurring randomly indicating that the panelists were unable to identify any sample as being consistently more or less desirable.

Table 31. Average sensory scores at day 0 for brined[a] whitefish samples treated with varying levels of liquid smoke or smoked with woodsmoke.

Treatment	Ranking[b]				
	Odor	Saltiness	Texture	Flavor	Overall Acceptability
Woodsmoke	1.2*	2.7	1.6*	1.7*x	1.6*
0.7% Liquid smoke	3.1	2.5	2.3	3.0	2.8
1.4% Liquid smoke	2.5	2.9	2.6	2.5	2.7
2.1% Liquid smoke	2.8**	3.1	3.1**	3.1**	3.1**
Woodsmoke + nitrite	1.6*x	2.5	2.4	2.2*	2.1
0.7% Liquid smoke + nitrite	3.1	2.7	2.9	2.6	2.5
1.4% Liquid smoke + nitrite	2.5	1.9	2.2	1.7*	2.0
2.1% Liquid smoke + nitrite	3.2**	2.4	2.9**	3.2**y	3.2**

[a]All brines contained 7.89% (30° salometer) salt

[b]Scale: 1 = most desirable; 4 = least desirable

[x,y]Samples with different letters are significantly ($P<0.05$) different

*Most desirable (Kramer ranking analysis)

**Least desirable (Kramer ranking analysis)

Table 32. Average sensory scores at day 5 for brined[a] whitefish samples treated with varying levels of liquid smoke or smoked with woodsmoke.

Treatment	Ranking[b]				
	Odor	Saltiness	Texture	Flavor	Overall Acceptability
Woodsmoke	1.8*	2.3	2.4	1.9*x	1.8*
0.7% Liquid smoke	3.4	2.9	2.7	3.7	3.8
1.4% Liquid smoke	2.8	2.4	2.2	2.4	2.7
2.1% Liquid smoke	2.3	1.7	2.1	1.9	1.7
Woodsmoke + nitrite	1.4*	2.4	2.9	1.9	2.3
0.7% Liquid smoke + nitrite	2.4**	2.3	2.3	2.4	2.2
1.4% Liquid smoke + nitrite	2.8	3.0	2.3	2.4	2.2
2.1% Liquid smoke + nitrite	3.0	2.9	3.0	3.4**	3.3**

[a]All brines contained 7.89% (30° salometer) salt

[b]Scale: 1 = most desirable; 4 = least desirable

x,yWithin a column, means with different letters are significantly ($P<0.05$) different

*Most desirable (Kramer ranking analysis)

**Least desirable (Kramer ranking analysis)

Table 33. Average sensory scores at day 14 for brined[a] whitefish samples treated with varying levels of liquid smoke or smoked with woodsmoke.

Treatment	Ranking[b]				
	Odor	Saltiness	Texture	Flavor	Overall Acceptability
Woodsmoke	1.8*	2.4	2.8	2.1	2.3
0.7% Liquid smoke	3.4**	2.8	2.4	3.2	3.2
1.4% Liquid smoke	3.5	2.9	2.8	2.9**	3.0**
2.1% Liquid smoke	1.7	2.6	2.6	2.3	2.7
Woodsmoke + nitrite	1.7	2.8	2.1	2.6	2.4
0.7% Liquid smoke + nitrite	3.1	2.0	2.7	2.1	2.0
1.4% Liquid smoke + nitrite	2.4*	2.4	2.9	2.8	2.6
2.1% Liquid smoke + nitrite	2.3	2.1	1.8	2.0	1.8

[a]All brines contained 7.89% (30° salometer) salt

[b]Scale: 1 = most desirable; 4 = least desirable

*Most desirable (Kramer ranking analysis)

**Least desirable (Kramer ranking analysis)

ANOVA analysis, in combination with Bonferroni's contrasts, of the taste panel data revealed essentially the same trends as seen in the ranking analysis. On day 0, the woodsmoke treated samples were significantly ($P<0.05$) more desirable in odor, while the non-nitrited woodsmoke treated sample had the most desirable flavor ($P<0.05$) (Table 31). Also on day 0, the samples treated with the 2.1% liquid smoke were significantly ($P<0.05$) less desirable in flavor. On day 5, the woodsmoke treated samples were still more desirable ($P<0.05$) in odor and the non-nitrited woodsmoke treated sample was more desirable in flavor ($P<0.05$) (Table 32). By day 15, there was no significant distinction made between the samples (Table 33).

The results of this study indicate that woodsmoke treated whitefish undergo less oxidative rancidity than samples prepared with liquid smoke. The addition of nitrite significantly ($P<0.01$) reduced the level of oxidation occurring in the smoked whitefish. The addition of nitrite did not result in the formation of N-nitrosamines in the finished smoked whitefish product. Taste panel evaluation of the samples prepared with increasing levels of liquid smoke in the brine or smoked with woodsmoke indicate that there was an upper limit to the addition of liquid smoke in this product. The addition of 2.1% liquid smoke was found to be undesirable on the first day of the study. However, over time the panelists were unable to distinguish the samples

containing the 2.1% liquid smoke from the other treatments.

Evaluation of Lipid Oxidation in Baked Whitefish

The development of WOF in fish has not received much attention because fish, generally, is not cooked, refrigerated or frozen and then reheated prior to serving. However, smoked fish can be held at refrigerator temperatures for up to 14 days before consumption. That smoked fish undergoes oxidative rancidity has been shown in the present study. However, the relationship between the sensory evaluation of rancidity in the cooked, refrigerated whitefish and a chemical parameter of rancidity, such as TBA number and/or hexanal levels has not been established.

Therefore this study was designed to establish the level of TBA number in baked, refrigerated whitefish that is detectable by taste panelists and to investigate the changes in hexanal levels in the refrigerated baked whitefish.

Whitefish that was baked then held at refrigerated temperatures for 0, 1, 2 and 3 days had TBA values of 1.51, 4.77, 5.41 and 7.53, respectively (Table 34). The samples from day 0, 1 and 2 were analyzed for relative changes in their hexanal content. These values are shown in Table 34.

Statistical analysis of the taste panel evaluation of the baked whitefish held at refrigerated temperatures for 0, 1, 2 and 3 days, using the ranking procedure of Kramer (1963) showed that the day 0 sample was the most desirable in flavor

Table 34. The TBA number, hexanal changes and the sensory scores of baked whitefish refrigerated for 0, 1, 2 and 3 days.

Days Refrigerated	TBA Number[a] (mg/kg)	Hexanal[b] (area)	Sensory Score[c]
0	1.51	1854×10^5	0.54*
1	4.77	2075×10^5	2.14**
2	5.41	1423×10^5	1.00
3	7.53	--	2.14**

[a]Analyses were carried out in triplicate

[b]Hexanal area corresponds to computer printout of GLC analysis

[c]Scale: 1 = most desirable; 4 = least desirable

*Most desirable (Kramer ranking analysis)

**Least desirable (Kramer ranking analysis)

($P<0.05$) (Table 34). The sample that was 1 day old and the sample that was 3 days old were found to be the less desirable ($P<0.05$). The sample that was 2 days old was desirable but to a lesser degree than the day 0 sample.

When the samples that were 0, 1 and 2 days old were used in a triangle test, as a means of establishing a threshold level for the TBA value, it was found that the panelists were able to determine a significant ($P<0.05$) difference between the day 0 sample and the day 1 sample. However, they were unable to distinguish between the day 0 sample and the day 2 sample. The data indicates that the panelists were able to detect TBA values of 4.77 and 7.53. However, the panelists were unable to distintuish a TBA value of 5.41.

The sensory evaluation of off-flavor (rancidity) thresholds have been reported in the literature. Yu et al. (1969) reported that fish having a TBA value of 3.1 or greater were considered very rancid and unacceptable by panelists. However, TBA values of 2.4 in the same fish were judged as being acceptable.

The correlation between the TBA value and taste panel evaluation of rancidity in cooked meats has been shown. Igene (1979) reported correlation coefficients between TBA numbers and taste panel scores for beef, the dark meat of chicken and the white meat of chicken were -0.74, -0.91 and -0.87, respectively. Bussey (1981) reported that in turkey hams, the TBA number and the taste panel score for off-flavor

and acceptability had correlation coefficients of +0.81 and -0.87, respectively. Bussey (1981) also reported that in turkey bologna the correlation coefficient between TBA number and off-flavor and acceptability were +0.91 and -0.87, respectively. Zipser et al. (1964) reported a correlation coefficient of 0.92 between TBA number and rancid odor in cured and uncured pork.

The volatile extract from the baked whitefish (day 0) was subjected to gas chromatographic-mass spectrometric (GC-MS) analysis in order to identify the volatiles occurring in the baked whitefish (Figure 6). The volatiles that were identified in the baked whitefish volatiles were: decane, 1-penten-3-ol, hexanal, undecane, heptanal, 1-pentanol, 1,3,5-trimethylbenzene and benzaldehyde. These compounds were found by Josephson et al. (1984) in cooked whitefish; however, they identified 63 different volatiles in their sample(s). They reported that hexanal accounted for 40% of the volatiles in cooked whitefish. In the present study it was found that hexanal accounted for greater than 50% of the volatiles in baked whitefish; however, the sample analyzed by GC-MS only contained 14 compounds. Therefore the hexanal would be more predominant.

The concentration of hexanal in the samples of baked whitefish held for 0, 1 and 2 days (Table 34) increased from the initial level on day 0, then decreased on day 2. It appears that changes in the hexanal levels in the

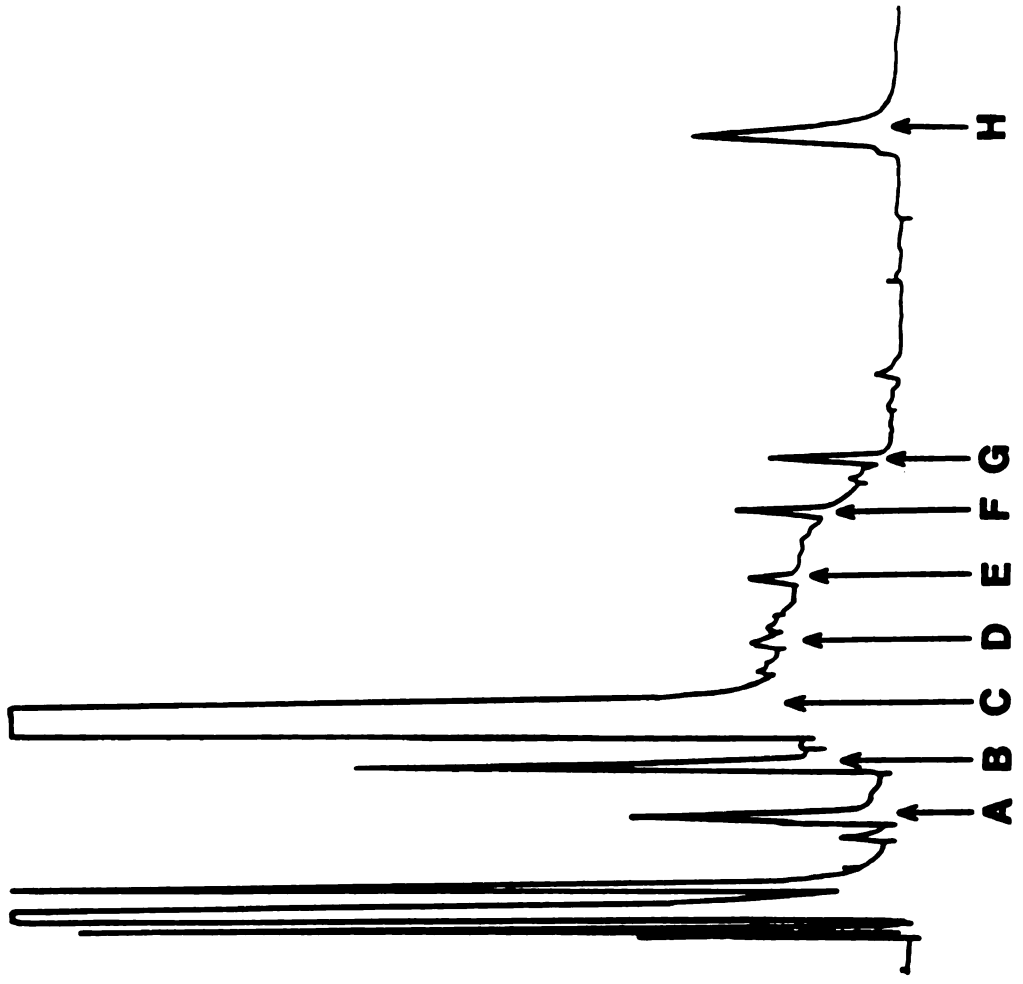

Figure 6. Gas chromatographic analysis of the volatiles from baked whitefish.

refrigerated baked whitefish should be investigated for their role in the evaluation of rancidity in this system.

The presence of hexanal as an indicator of oxidation in food systems has been investigated by a number of workers. MacLeod and Coppock (1976) reported that hexanal was produced during the cooking of beef, and that hexanal has been described to have a strong, unpleasant rancid odor. Gaddis and Ellis (1957) reported that hexanal has been identified as the major volatile in rancid turkey and pork fat. Cross and Ziegler (1965) noted that in uncured ham there were higher levels of hexanal and valeraldehyde than in cured ham. These hams had been cooked to an internal temperature of 70^{o}C, then held at 3^{o}C for several days before analysis. Based on the work by Fooladi et al. (1979), uncured cooked pork was shown to have a TBA number of 7.85 within 48 hours after cooking. Therefore it can be assumed that the uncured ham analyzed by Cross and Ziegler (1965) was rancid and the hexanal detected in the meat's volatiles was the result of this rancidity.

More research is needed to clarify the relationship between taste panel evaluation of rancidity with TBA and hexanal values in baked whitefish during refrigerated storage.

SUMMARY AND CONCLUSIONS

The salt uptake of whitefish during brining is affected by the salt concentration of the brine, the length of the brining period and whether the fish is brined whole or as a fillet. Fillets, due to their increased surface area, take up greater amounts of salt from the brine than the whole, eviscerated fish. Higher brine salt concentrations produce fish containing higher levels of salt.

The level of salt in smoked whitefish affected the rate of Clostridium botulinum type E spore outgrowth and toxin production. Fish containing 2 percent salt (wp) became toxic within 21 days at 27°C. Increasing the salt concentration to 4 percent (wp) inhibited toxin production for 42 days at 27°C. The addition of nitrite to the fish containing 4 percent salt (wp) prevented toxin production until day 63. Smoked whitefish containing 6 percent salt (wp) were not toxic even after 83 days at 27°C.

In another study concerned with the lipid stability and organoleptic acceptability of smoked whitefish containing various levels of salt (wp), it was found that unsalted smoked whitefish had a significantly ($P<0.01$) lower rate of oxidation (TBA number) during refrigerated storage. However, the unsalted product was found to be organoleptically

unacceptable initially (day 0) and over time (day 14). Taste panel evaluation showed that the smoked whitefish containing 4 percent salt (wp), with and without nitrite, was significantly ($P<0.05$) more desirable initially and over time. The addition of nitrite significantly ($P<0.01$) reduced the rate of oxidation in all salt treatment levels.

The role of smoke treatments on the lipid stability and organoleptic acceptability of smoked whitefish was investigated. The results showed that woodsmoke had a greater antioxidant effect than did the liquid smoke. Nitrite strongly inhibited the development of warmed-over flavor in this product. Taste panel evaluation indicated that initially (day 0) there was a significant ($P<0.05$) preference for the samples treated with woodsmoke but over time (day 14) the panelists were unable to distinguish between the woodsmoke and the liquid smoke treatments.

Smoked whitefish containing nitrite was shown to contain no detectable levels of N-nitrosamine or NTCA.

The evaluation of oxidative rancidity using taste panel evaluation in conjunction with TBA and hexanal analyses in whitefish baked then held at refrigerator temperatures for 3 days was studied. Sensory evaluation data indicated that the panelists could identify rancidity in fish with TBA numbers of 4.77 and 7.53. However, the panelists were unable to detect rancidity in fish having a TBA number of 5.41. Hexanal analysis indicated that in the samples with the 4.77

and 7.53 TBA number contained the highest level of hexanal. The sample with the TBA number of 5.41 had the lower level of hexanal. These data indicate that both chemical parameters, i.e, TBA and hexanal, appear to affect the panelists' ability to detect rancidity in whitefish that had been baked then held at refrigerated temperatures.

It can be concluded from these studies that it is feasible to produce smoked whitefish that contain a lower level of salt (4% water-phase) and still maintain safety against botulism. The addition of nitrite to smoked whitefish containing less salt increased the safety of the product against botulism. Taste panel data indicate that the addition of nitrite did not appear to affect the desirability of the smoked whitefish. Nitrite in the smoked whitefish did not result in the formation of detectable levels of N-nitrosamines. Therefore, the addition of nitrite in combination with a 4 percent salt (water-phase) concentration can be recommended for use in the production of smoked whitefish.

PROPOSALS FOR FUTURE RESEARCH

These studies on the safety and acceptability of smoked whitefish and the brining of whitefish have raised some questions which merit further study. These include:

(1) Determining the role of each lipid fraction (triglyceride and phospholipid) in the development of warmed-over flavor in smoked whitefish;

(2) Further clarification of the factors affecting salt uptake in whitefish tissue so that a more uniform product can be produced. This work should include an investigation of the cost effectiveness of using fillets as opposed to the whole, eviscerated fish in the production of smoked whitefish;

(3) To evaluate the effect of nitrite on the flavor of smoked whitefish;

(4) To evaluate the influence of higher levels of sorbate in combination with nitrite on the outgrowth of Clostridium botulinum type E spores.

APPENDIX

Appendix 1

Sensory Evaluation of Smoked Whitefish

Name _____ Sample # _____ Date _____

Flavor Acceptability	Mouthfeel	Saltiness	Overall Acceptability
Like Very Much	Very Juice	Very Salty	Very Desirable
Like Moderately	Moderately Juicy	Moderately Salty	Moderately Desirable
Like Slightly	Slightly Juicy	Slightly Salty	Slightly Desirable
Neither Like Or Dislike	Neutral	Neutral	Neutral
Dislike Slightly	Slightly Dry	Slightly Under Salty	Slightly Undesirable
Dislike Slightly	Moderately Dry	Moderately Under Salty	Moderately Undesirable
Dislike Very Much	Very Dry	Very Under Salty	Very Undesirable

Appendix 2

Taste panel form for the evaluation of the desirability of smoked whitefish using ranking analysis

SMOKED FISH EVALUATION

In this evaluation of smoked whitefish, you are asked to taste all of the samples, evaluate them and then decide how they relate to each of the traits listed. Using their assigned numbers, rank the samples from the most desirable to the least desirable in the spaces provided. There is no right or wrong answer, only your opinion.

Trait	Most	Desirability		Least
Odor				
Saltiness				
Texture				
Flavor				
Overall Acceptability				

Appendix 3

Cooked fish evaluation

COOKED FISH EVALUATION

The following taste panels are designed to evaluate the desirability of cooked fish. In order to complete this evaluation, you are asked to taste three (3) sets of samples. The first set is to be ranked as being most to least desirable. The next two sets will be used in triangle tests.

Ranking: Please rank the samples as to their desirability.

Most desirable			Least desirable

Triangle Tests: In these sets of samples, two of the samples are identical and one is different. Please taste and then decide which is the different sample; also indicate if the different sample is more or less desirable.

Test #1: Please list the number of the odd sample _____

Is this sample more or less desirable _____

Test #2: Please list the number of the odd sample _____

Is this sample more or less desirable _____

Name _____ Date _____

Appendix 4

Salt concentration of smoked whitefish brined in different degree salometer brines prepared with or without nitrite or nitrite and sorbate[a]

Treatment	Days of Storage[b]								
	0	7	14	21	28	35	42	48	56
	Percent salt (water-phase)								
10^{o} Brine	1.9	1.0	1.6	1.3	1.2	1.1	--	--	--
10^{o} Brine + nitrite	1.1	1.3	1.9	1.3	1.0	1.5	--	--	--
10^{o} Brine + nitrite + sorbate	1.3	1.1	1.0	1.4	1.3	1.3	--	--	--
20^{o} Brine	2.7	2.9	3.0	2.4	3.3	2.7	3.2	2.8	2.6
20^{o} Brine + nitrite	2.2	2.8	3.1	2.8	3.1	2.3	2.9	2.9	2.8
20^{o} Brine + nitrite + sorbate	1.7	2.0	1.7	2.7	2.5	2.0	2.5	2.3	1.9
30^{o} Brine	3.9	4.1	4.2	3.5	3.9	4.4	4.0	3.7	3.5
30^{o} Brine + nitrite	3.9	3.7	3.8	3.7	3.8	4.5	3.8	4.4	4.0
30^{o} Brine + nitrite + sorbate	3.5	3.7	3.5	3.2	3.6	3.4	3.9	3.3	3.3

[a]Analyses were carried out in triplicate.

[b]Stored at 27^{o}C.

Appendix 5

Moisture content of smoked whitefish brined in different degree salometer brines prepared with nitrite or nitrite and sorbate[a].

	Days of Storage[b]								
	0	7	14	21	28	35	42	49	56
	Percent moisture								
10° Brine	61.5	60.0	55.4	56.9	52.3	54.5	--	--	--
10° Brine + nitrite	60.9	57.3	58.1	57.5	54.4	61.7	--	--	--
10° Brine + nitrite + sorbate	60.7	57.0	55.9	60.8	53.5	58.9	--	--	--
20° Brine	61.3	63.8	62.8	62.9	62.6	60.6	61.4	59.0	60.3
20° Brine + nitrite	65.5	62.9	62.8	60.6	56.4	63.6	62.4	61.4	59.9
20° Brine + nitrite + sorbate	64.2	65.1	66.4	61.7	60.4	62.4	58.7	59.4	61.0
30° Brine	60.9	57.6	58.9	61.3	58.5	58.8	57.3	60.5	58.2
30° Brine + nitrite	63.4	57.8	60.6	57.8	59.4	60.3	58.4	57.6	57.6
30° Brine + nitrite + sorbate	58.9	62.6	60.6	58.1	61.3	61.6	61.6	59.3	58.2

[a]Analyses were carried out in triplicate.

[b]Stored at 27°C.

Appendix 6

Percent salt in smoked whitefish brined in different degree brines prepared with or without nitrite[a]

Treatment	Days of Storage[b]			
	0	7	14	21
	Percent salt			
Control[c]	0.2	0.3	0.2	0.3
Control[c] + nitrite	0.3	0.2	0.1	0.2
10° Brine	1.2	1.0	0.9	1.4
10° Brine + nitrite	1.3	1.3	1.3	1.3
20° Brine	2.2	2.5	3.0	2.6
20° Brine + nitrite	2.8	2.8	3.0	2.7
30° Brine	4.0	4.6	4.4	4.0
30° Brine + nitrite	4.0	4.2	4.1	4.0

[a]Analyses were carried out in triplicate.

[b]Stored at 4°C.

[c]No salt used in the brine.

Appendix 7

Moisture content of smoked whitefish brined in different degree salometer brines prepared with or without nitrite[a]

Treatment	Days of Storage[b]			
	0	7	14	21
	Percent moisture			
Control[c]	58.0	52.7	55.2	56.9
Control[c] + nitrite	55.7	52.5	54.7	52.8
10° Brine	62.4	59.4	58.9	57.8
10° Brine + nitrite	62.5	59.8	60.3	59.8
20° Brine	63.4	61.3	61.6	58.2
20° Brine + nitrite	63.8	62.6	62.2	61.9
30° Brine	59.3	58.5	58.0	57.7
30° Brine + nitrite	60.2	60.2	60.6	59.7

[a]Analyses were carried out in triplicate.

[b]Stored at 4°C.

[c]No salt used in the brine.

Appendix 8

Percent salt of brined whitefish treated with various levels of liquid smoke or smoked with woodsmoke[b]

Treatment	Days of Storage[b]			
	0	5	14	22
	Percent salt			
Woodsmoke	4.3	3.9	4.1	3.6
Woodsmoke + nitrite	3.9	4.2	4.1	4.1
0.7% Liquid smoke	3.6	3.7	3.6	3.5
0.7% Liquid smoke + nitrite	3.9	3.8	4.0	4.1
1.4% Liquid smoke	3.4	3.7	3.7	4.0
1.4% Liquid smoke + nitrite	3.5	3.9	4.0	4.0
2.1% Liquid smoke	3.0	4.0	3.3	3.1
2.1% Liquid smoke + nitrite	3.2	3.0	2.8	2.7

[a]Analyses were carried out in triplicate.

[b]Stored at 4°C.

Appendix 9

Moisture content of brined whitefish treated with various levels of liquid smoke or smoked with woodsmoke[a]

Treatment	Days of Storage[b]			
	0	5	14	22
	Percent moisture			
Woodsmoke	60.8	59.7	60.6	58.3
Woodsmoke + nitrite	58.8	56.9	58.1	59.5
0.7% Liquid smoke	59.9	57.0	57.9	56.0
0.7% Liquid smoke + nitrite	59.9	60.2	58.2	57.4
1.4% Liquid smoke	62.0	58.9	57.8	57.0
1.4% Liquid smoke + nitrite	57.7	57.3	56.1	55.6
2.1% Liquid smoke	61.4	56.8	59.6	58.6
2.1% Liquid smoke + nitrite	56.9	57.9	58.4	57.5

[a]Analyses were carried out in triplicate.

[b]Stored at 4°C.

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