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By

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ABSTRACT

THE INFLUENCE OF PROCESSING VARIABLES ON N-NITROSAMINE FORMATION IN BACON

By

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Various processing variables were investigated to determine their catalytic or inhibitory effect on N-nitrosamine formation in bacon. The use of curing adjuncts such as lactic acid-producing bacteria resulted in lesser concentrations of N-nitrosamines in the fried bacon, while the effects of α-tocopherol, rosemary oleoresin and acid phosphates were not conclusive. Oxidation of adipose tissue lipids of pork bellies did not influence N-nitrosamine formation in fried bacon. N-Nitrosamine concentrations in bacon processed from bellies from pigs fed oxidized feed were similar to those found in control bacon samples. The addition of malonaldehyde to a restructured bacon system did not promote N-nitrosamine formation. Bacon processed in Ireland had lesser concentrations of N-nitrosamines than bacon produced in the United States, due in part to its greater lean-to-adipose ratio. Components in liquid smoke were found to inhibit the catalytic effect of formaldehyde on the formation of N-nitrosopyrrolidine and N-nitrosothiazolidine in fried bacon.

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INTRODUCTION

Over the past two decades, N-nitroso compounds in foods have come under close scrutiny because of their possible carcinogenic activity in animals. Many N-nitroso compounds have been shown to elicit a carcinogenic response in a variety of test animals (Hotchkiss, 1987). N-Nitrosamines are detected in a number of food products including cured meats, dairy products, and alcoholic beverages (Hotchkiss, 1987).

N-Nitroso compounds present in bacon which are of primary concern include N-nitrosodimethylamine (NDMA), N-nitrosopyrrolidine (NPYR), and N-nitrosothiazolidine (NTHZ). Many factors influence the concentrations of these compounds in fried bacon, including slice thickness, frying time, cure ingredients, and pork belly composition, i.e., fat-to-lean ratio and degree of proteolysis in the adipose tissue (Skrypec *et al.*, 1985; Hotchkiss, 1987).

Due to the carcinogenic potential of volatile N-nitrosamines , extensive efforts have been undertaken to develop methods of reducing the formation of these compounds in bacon and to elucidate the factors influencing their formation. Ascorbic acid, a key ingredient in the curing brine, reacts with nitrite to form dehydroascorbic acid and nitric oxide, thus reducing the potential for N-nitrosamine formation in bacon. Nitric oxide does not readily react with

secondary amines to form N-nitroso compounds and is readily dissipated from the bacon system.

Three methods have been developed which can effectively inhibit N-nitrosamine formation in bacon. These are the addition to the brine of α-tocopherol (Gray *et al.*,1982), Bac-N-Phos (sodium polyphosphates/sodium hexametaphosphate/sodium bicarbonate system)(Stauffer Chemical Co., Westport, CT.), and lactic acid-producing bacteria (Tanaka *et al.*,1985) as curing adjuncts. In addition to these procedures, there is interest in the use of natural antioxidant systems such as the extracts from the spice, rosemary, as an anti-N-nitrosamine compound (Evans, personal communication). Like the active antioxidant α-tocopherol, certain compounds found in rosemary oleoresins such as rosemaridiphenol, possess antioxidant activity. Therefore, it is possible that these compounds can act in a manner similar to α-tocopherol by reducing nitrite to nitric oxide.

Liquid smokes are now used extensively in the smoking of cured meat products. Recent studies by Ikins *et al.* (1986) have shown that the use of liquid smokes instead of traditional woodsmoking can result in lesser concentrations of NPYR in fried bacon. It is thought that compounds within the liquid smoke, such as phenols, may act as inhibitors of N-nitrosamine formation (Hotchkiss, 1987). Certain phenols can be nitrosated up to 1000 times faster than dialkylamines to form N-nitrosophenols (Challis, 1973). Thus, phenols may

reduce the concentration of N-nitrosamines through a competitive reaction. N-Nitrosophenols can, however, under certain circumstances increase formation of N-nitroso compounds (Hotchkiss, 1987).

Formaldehyde is present in woodsmoke as a result of the combustion of wood (Toth and Potthast, 1984) and is also present in varying concentrations in commercial liquid smokes (Potthast and Eigner, 1985). Formaldehyde has been shown to react with cysteamine and nitrite to form NTHZ. Concentrations of NTHZ in bacon depend upon the liquid smoke used in processing (Ikins *et al.*, 1986). This can be attributed to the fact that different concentrations of formaldehyde and phenols are found in different liquid smokes (Toth and Potthast, 1984).

The major objective of this study was to evaluate some of the above factors which further influence the concentrations of N-nitrosamines found in fried bacon. Specific objectives of the study were:

1. To compare the efficacy of various processing procedures for minimizing N-nitrosamine formation in fried bacon.

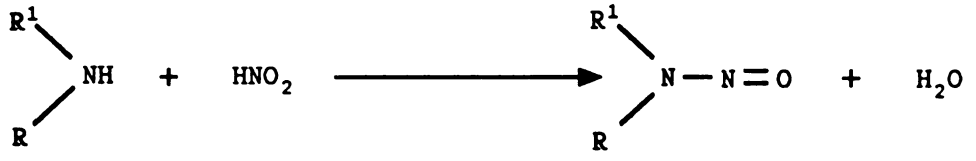
2. To compare the concentrations of N-nitrosamines in bacon processed in Ireland to bacon processed in the United States, and to establish the importance of the lean-to-adipose tissue ratio in the pork belly.

3. To evaluate the effect of lipid oxidation products in pork bellies on the formation of N-nitrosamines in fried bacon.

4. To determine the effect of formaldehyde in liquid smokes on the formation of NDMA, NPYR, and NTHZ in fried, restructured bacon.

REVIEW OF LITERATURE

N-Nitrosamines are formed primarily by the condensation of a nitrosating agent with secondary amines (Morrison and Boyd, 1976). An alkyl (R^1) group



is required, while R can be one of several different functional groups. N-Nitrosamines can also be formed from the reaction of nitrosating agents with primary amines, tertiary amines, and quaternary ammonium compounds (Fiddler *et al.*, 1972), although yields are much less than for secondary amines. The resulting N-nitroso compounds are generally stable under conditions commonly found in food (Hotchkiss, 1987).

N-Nitroso compounds were first recognized as potential toxicants in food during the 1950's (Ender *et al.*,1964). Animals, which were fed fish meal preserved with nitrite, became ill and died. Death was attributed to the presence of N-nitroso compounds in the animal feed. Over 90% of the 300 individual N-nitroso compounds tested in animals have been shown to produce carcinomas, although little direct evidence exists linking human carcinomas to N-nitrosamine exposure (Hotchkiss, 1987).

Concentrations of N-Nitrosamines in Foods

In early studies to determine the concentrations of N-nitrosamines in food systems, Fazio *et al.* (1971) surveyed 51 cured meat products and found 5 ug/kg NDMA in ham, while Crosby *et al.* (1972) detected NDMA in fried bacon, fish, and cheeses, at concentrations ranging from one to 40 ug/kg. More recently, extensive surveys of N-nitrosamines in food have been conducted by Gough *et al.* (1977) and Spiegelhalder et al. (1980). Gough *et al.* (1977) analyzed over 500 foods in the United Kingdom marketplace. They determined that the average person ingested approximately 1μg/kg N-nitrosamine per week based on the N-nitrosamine content of the foodstuffs analyzed. The only food product in which these researchers consistently found NDMA and NPYR was fried bacon.

Spiegelhalder *et al.* (1980) analyzed almost 3000 foods for their N-nitrosamine content. Included in the survey were cheeses, beer, meat and meat products, as well as a variety of other foods. Positive results were only occasionally obtained, and these were typically near the limit of detection for N-nitroso compounds. Out of the 395 meat and sausage products analyzed, 127 of these contained >0.5 μg/kg NDMA, while 51 contained >0.5 μg/kg NPYR. The highest concentrations found in the meat and sausage products were, 12 μg/kg NDMA and 45 μg/kg NPYR, respectively. Of the 215 different types of beer analyzed, 142 had concentrations of

NDMA > 0.5 μg/kg, with the highest concentration detected being 68 μg/kg.

Formation of N-Nitrosamines in Bacon

Although low concentrations of NDMA, N-nitrosodiethylamine (NDEA), and N-nitrosopiperidine (NPIP) are frequently detected in fried bacon, the major N-nitrosamine present is NPYR (Table 1). NPYR is produced during cooking with the final concentration of NPYR depending upon several factors. These factors include preprocessing procedures, lean-to-fat ratio of the bacon, nitrite concentration, presence of inhibitors, cooking method and temperature and time of cooking (Skrypec *et al.*, 1985). The majority of N-nitrosamines produced during cooking are volatile and escape in the vapor. Such losses have been quantitated by several workers including Gough *et al.*(1977), Bharucha *et al.*(1979), and Hotchkiss and Vecchio (1985).

Formation of N-Nitrosodimethylamine in Bacon

NDMA has been shown to be more carcinogenic than NPYR (Magee and Barnes, 1967) and is found consistently in bacon. The mechanism of NDMA formation in bacon has not been studied in great detail. However, model studies simulating food systems have indicated that several compounds including

Table 1. N-Nitrosamine concentrations (μg/kg) in fried bacon and cook-out fat.

Investigators	# Samples	NPYR		NDMA	
		Bacon	Cook-out Fat	Bacon	Cook-out Fat
Sen et al., 1973	8	13 (ND-25)[1]	NR[2]	6	NR
Fazio et al., 1973	8	63 (10-108)	100 (45-207)	NR	NR
Pensabene et al., 1979	8	20 (2-45)	30 (7-49)	5 (2-9)	15 (7-28)
Gray et al., 1982	5	20 (14-27)	18 (13-28)	5 (4-6)	5 (3-6)
Skrypec et al., 1985	5	6 (4-9)	13 (NR)	3 (1-4)	7 (NR)

Adapted from Mandagere, 1986.

[1]Not detected [2]Not reported in study

lecithin, sarcosine, quartenary ammonium compounds, trimethylamines, and dimethylamines can form NDMA on reaction with nitrite (Ender and Ceh, 1971; Fiddler *et al.*, 1972; Eisenbrand *et al.*, 1976). The concentrations of dimethylamine in bacon have been reported to increase with storage from 200 μg immediately after processing, to 520 μg/kg after a period of storage (Patterson and Mottram, 1974). Compounds containing sarcosine and choline were proposed to be the most probable amine precursors of NDMA (Gray *et al.*,1978). The concentrations of compounds containing choline and sarcosine in pork bellies, however, have not been determined.

Formation of N-Nitrosopyrrolidine in Bacon

The consistent occurrence of NPYR in fried bacon has resulted in an intensive search for the precursors and mechanism(s) involved in its formation. Studies utilizing model food systems have implicated several compounds including proline (PRO), pyrrolidine (PYR), collagen, putrescine, and spermidine as possible precursors of NPYR (Gray, 1976). Of these potential precursors, PRO is considered to be the most likely candidate. Free PRO concentrations have been shown to range from 20-90 mg/kg in bacon (Fiddler *et al.*, 1974; Lakritz *et al.*, 1976; Nakamura *et al.* 1976; Gray and Collins, 1977; Bharucha *et al.*, 1979).

Although the exact mechanism for the formation of NPYR from PRO has not been determined, two pathways have been proposed (Gray, 1976; Bharucha *et al.*, 1979). The possible pathways of NPYR formation in bacon are illustrated in Figure 1. In the first pathway, PRO is nitrosated to N-nitrosoproline (NPRO) which then undergoes thermal decarboxylation to yield NPYR. The second pathway involves the initial thermal decarboxylation of PRO to PYR, followed by nitrosation to NPYR. Since decarboxylation of NPRO occurs at a much lower temperature than the decarboxylation of PRO to PYR, the initial pathway seems the more probable of the two (Bharucha *et al.*, 1979; Lee *et al.*, 1983b).

Bharucha *et al.* (1979) hypothesized a free radical mechanism for the formation of NPYR during the frying of bacon. NPYR is formed primarily towards the end of frying when most of the water in the bacon has vaporized. As the water is lost, the remaining lipid phase facilitates a more rapid heat transfer. The high temperature and the catalytic effect of the lipid hydroperoxides (Coleman, 1978) supports the involvement of a free radical mechanism in NPYR formation.

The free radical pathway for the formation of NPYR during the frying of bacon was proposed by Bharucha *et al.* (1979). Nitrous acid is converted to N_2O_3 as the water is vaporized during the frying process. At high temperatures (>100°C), N_2O_3 dissociates to NO and NO_2 radicals. A proton

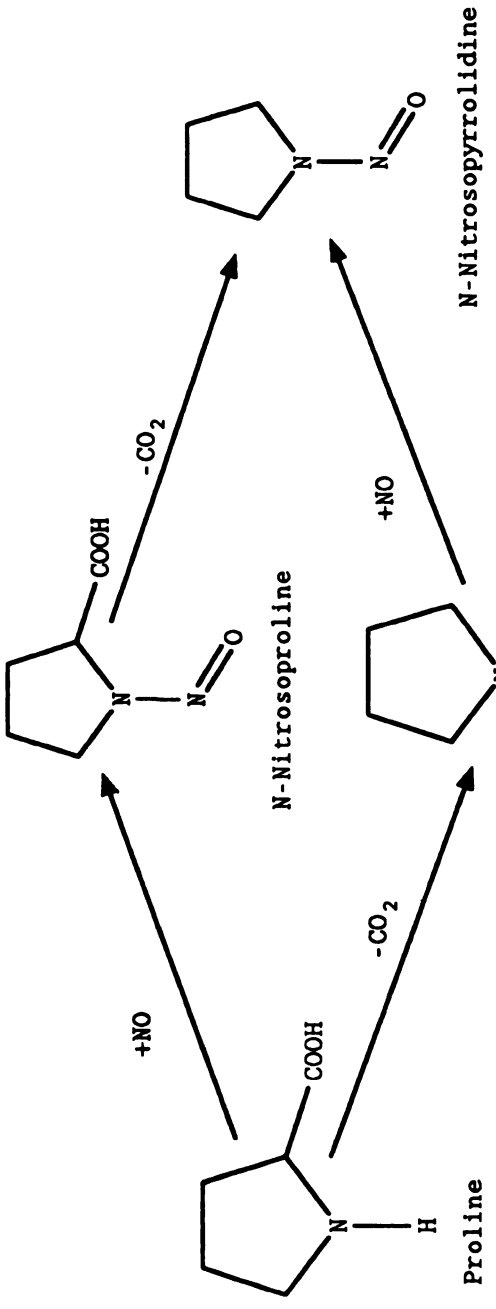


Figure 1. Possible pathways for the formation of NPYR in bacon. (Lee, 1981)

may then be abstracted from the nitrogen atom of PRO to form a PRO radical, which in turn, reacts with NO radical to form NPRO.

Factors Influencing N-Nitrosamine Formation in Bacon

As previously discussed, many factors influence the formation of N-nitrosamines in bacon. These factors include preprocessing procedures, fatty acid composition, added nitrite concentration, cooking methods and pH. Research delineating the contribution of each factor to N-nitrosamine formation in bacon is discussed below.

Preprocessing Procedures

It has been reported by Pensabene *et al.* (1980) that greater concentrations of NPYR are formed in fried bacon when aged bellies are used for processing as compared to concentrations found in bacon processed form fresh pork bellies. Increases in free amines and amino acids during aging may account for the observed increase in NPYR concentrations upon frying (Pensabene *et al.*, 1980; Amundson *et al.*, 1982). The free proline content in whole and lean tissue of pork bellies has been shown to increase by 50% after one week of storage at 2°C, while the free proline content in the adipose tissue increased by approximately 90%

(Gray and Collins, 1977).

Fatty Acid Composition of Adipose Tissue

During the frying of bacon, the majority of N-nitrosamines are formed in the adipose tissue (Pensabene *et al.*, 1974; Coleman, 1978; Bharucha *et al.*, 1979). Walters *et al.* (1979) studied the involvement of unsaturated fatty acids as transnitrosating agents. Nitrite (nitric oxide) may be capable of interacting with the double bonds found in unsaturated fatty acids. These investigators further demonstrated that α-nitroso-nitrite esters of unsaturated triglycerides can transnitrosate secondary amines. It was subsequently shown by Mirvish and Sams (1982) that the reaction of methyl linoleate with nitrogen dioxide resulted in a nitrosating compound. This reaction occurred at slower rates when the lesser unsaturated fatty acids, methyl stearate and methyl oleate, were used in place of methyl linoleate. Similar derivatives of unsaturated lipids in bacon adipose tissue may be involved with N-nitrosamine formation.

Ross *et al.* (1987) reacted methyl stearate, methyl oleate, methyl linoleate, and methyl linolenate with N_2O_3. The products of each of the above reactions were then individually reacted with 2,6-dimethylmorpholine. The products of the unsaturated fatty acids and N_2O_3 all

nitrosated 2,6-dimethylmorpholine to N-nitroso-2,6-dimethylmorpholine. The saturated methyl stearate did not convert 2,6-dimethylmorpholine to its N-nitrosoderivative, thus indicating that it was nonreactive with N_2O_3. High pressure liquid chromatography was used to quantify the products of the reaction of N_2O_3 with these fatty acid methyl esters. A minimum of ten major peaks were observed in the resulting chromatograms from the unsaturated fatty acid samples. No new peaks were observed when N_2O_3 was reacted with methyl stearate. These results further support the conclusion that N_2O_3 was unreactive with methyl stearate. Ross *et al.* (1987) further established that these reactions will occur under the conditions found in frying bacon (>80^{o}C), with a maximum yield occurring at 130^{o} C using N_2O_3 /methyl oleate products.

Animal feeding studies have been conducted to determine the influence of the fatty acid composition of bacon adipose tissue on N-nitrosamine formation (Skrypec *et al.*, 1985). Increased concentrations of NDMA and NPYR were noted in fried bacon in which the bacon analyzed prior to frying contained elevated concentrations of unsaturated fatty acids. The increase in unsaturated fatty acids were a result of the inclusion of highly unsaturated fats in the pig diet. In further support of the hypothesis that NPYR formation during frying could proceed through the intermediate formation of a nitroso-nitrite ester derivative of unsaturated lipids,

Mottram *et al.* (1977) reported that frying bacon in highly unsaturated corn oil resulted in a marked increase in the formation of NPYR. In addition, Hotchkiss and Vecchio (1985) found that frying ham, sausage, and other cured meats in corn oil, resulted in greatly enhanced N-nitrosamine formation. The transnitrosating capacity of unsaturated lipids in the cooking medium was demonstrated by these studies.

The Effect of Lipid Oxidation on N-Nitrosamine Formation in Model Studies

Model system studies have also implicated lipid oxidation products as catalysts of the nitrosation reaction. Kurechi and Kikugawa (1979) studied the effect of malondialdehyde, a common product of lipid peroxidation, on the formation of NDMA in model systems. NDMA was formed by reacting nitrite and dimethylamine for 3.5 hr at 37°C in the presence of malondialdehyde and acetal. This reaction was conducted at four different pH values. At pH 3.0, the formation of NDMA was inhibited, while at pH 4.0 there proved to be neither catalysis or inhibition of NDMA formation. However, at pH of 5.0 and 6.0, significant increases in the concentrations of NDMA were observed. At pH 6.0, an 8 fold increase in the concentration of NDMA was observed as compared to a control reaction system in which there was no aldehyde present. These researchers also demonstrated that

concentrations of NDMA formed were directly correlated with the concentration of malondialdehyde. As malondialdehyde concentrations increased, so did the concentration of NDMA produced in solution.

Coleman (1978) evaluated the influence of lipid hydroperoxides on the nitrosation reaction by using a model system consisting of 2-oleodistearin hydroperoxide, 400 mg/kg NO_2 and 800 mg/kg proline in methanol. Further reactions included replacing the 2-oleodistearin hydroperoxide with 2-oleodistearin and then omitting the lipid entirely. Coleman found a two-fold increase in NPYR formation when 2-oleodistearin hydroperoxide was included in the model system as compared to 2-oleodistearin or no lipid. Thus it appeared that lipid hydroperoxides dramatically increased the formation of NPYR. However, there have been no studies reported on the influence of oxidized pork fat on N-nitrosamine formation in bacon.

Nitrite Concentration

Nitrite has been used for many centuries as a curing agent and serves three major functions (Binkerd and Kolari, 1975). First, it inhibits toxigenesis by *Clostridium botulinum*. Secondly, nitrite produces the characteristic pink-red color in cured meats by reacting with myoglobin to form nitrosomyoglobin. Thirdly, nitrite prevents the

development of rancidity by acting as an antioxidant.

Bacon is normally processed with an ingoing nitrite concentration of 120 mg nitrite/kg meat. The nitrite concentration in cured meats, however, within a few days of processing, drops to less than 50% of the concentration originally added. Woolford and Cassens (1977) found that the initial pumped value of 156mg/kg dropped to 45mg/kg within a few days of processing in raw bacon. After seven weeks, the concentration of residual nitrite was almost undetectable. Ascorbic acid is required in curing brines by the USDA and has been shown to be very effective in reducing the residual nitrite concentrations in cured meats(Hotchkiss, 1987). The fate of nitrite in a brine cured meat system has been summarized by Cassens *et al.*, 1977 as: 5 - 10% as free nitrite, 1 - 5% bound to lipid, 1- 5% as NO gas, 5 - 15% bound to myoglobin, 1 - 10% oxidized nitrate, 5 - 15% bound to sulfhydryl groups, and 20 - 30 % bound to protein.

Cooking Methods

The highest concentrations of N-nitrosamine in fried bacon are formed when pan frying is used (Wasserman *et al.*, 1978). Microwave cooking produce lesser concentrations of N-nitrosamines (Herring, 1973; Pensabene *et al.*, 1978) and grilling (Bharucha *et al.*, 1979). In the case of microwave cooking, the lesser concentrations can be explained by the

lower internal temperatures attained within the slices during cooking as compared to pan frying (Wasserman *et al.*, 1978). Lesser concentrations of N-nitrosamines are formed during the grilling of bacon due to the draining of the cook-out fat which also serves to lower the internal temperature of the slice (Bharucha *et al.*, 1979).

Time and temperature of cooking also play an important role in the concentrations of N-nitrosamines formed. Bharucha *et al.* (1979) found that high concentrations of N-nitrosamines are formed when bacon is fried at 182°C for 12 min starting from a cold pan. The optimum temperature for N-nitrosamine formation was determined to be 185°C, which is near the normal frying temperature of bacon (Pensabene *et al.*, 1974). At 100°C, only 10% of NPYR was formed as compared to the concentration of NPYR formed at 180°C. As water volatilizes, the internal temperature of the slice increases from 110°C to 180°C. As discussed above, the majority of N-nitrosamines formed are produced after the bulk of the water has volatilized from the cooking pan (Pensabene *et al.*, 1974; Coleman, 1978; Bharucha *et al.*, 1979). Pensabene *et al.* (1974) further stated that the time of frying is important since N-nitrosamine formation increased after 4 min and reached a maximum concentration at 12 min of frying. After 12 min, a decline in the concentration of N-nitrosamines was found, which can probably be attributed to volatilization of N-nitrosamines over the course of cooking.

Inhibition of N-Nitrosamine Formation in Bacon

Lipophilic Inhibitors

Mirvish *et al.* (1978) reported that N-nitrosation occurs at high rates in lipophilic systems. Consequently, there was considerable interest in compounds capable of inhibiting the formation of N-nitrosamines in foods which would reside in the lipid phase.

Ascorbyl palmitate: A 90% reduction in NPYR formation was observed by Mottram and Patterson (1977) when ascorbyl palmitate was incorporated into a two phase system modeling adipose tissue. It was suggested that ascorbyl palmitate, which is incorporated into the nonpolar lipid phase, would be capable of competing with amines for any nitrosating species migrating to the nonpolar phase. Bharucha *et al.* (1980) applied ascorbyl palmitate to bacon slices as a slurry in soybean oil prior to frying. A 70% reduction in N-nitrosamines formation was observed, however, the effectiveness of ascorbyl palmitate was significantly reduced when the bacon was stored for several weeks.

α-Tocopherol: Vitamin E or α-tocopherol which is a lipophilic antioxidant has been shown to be an excellent

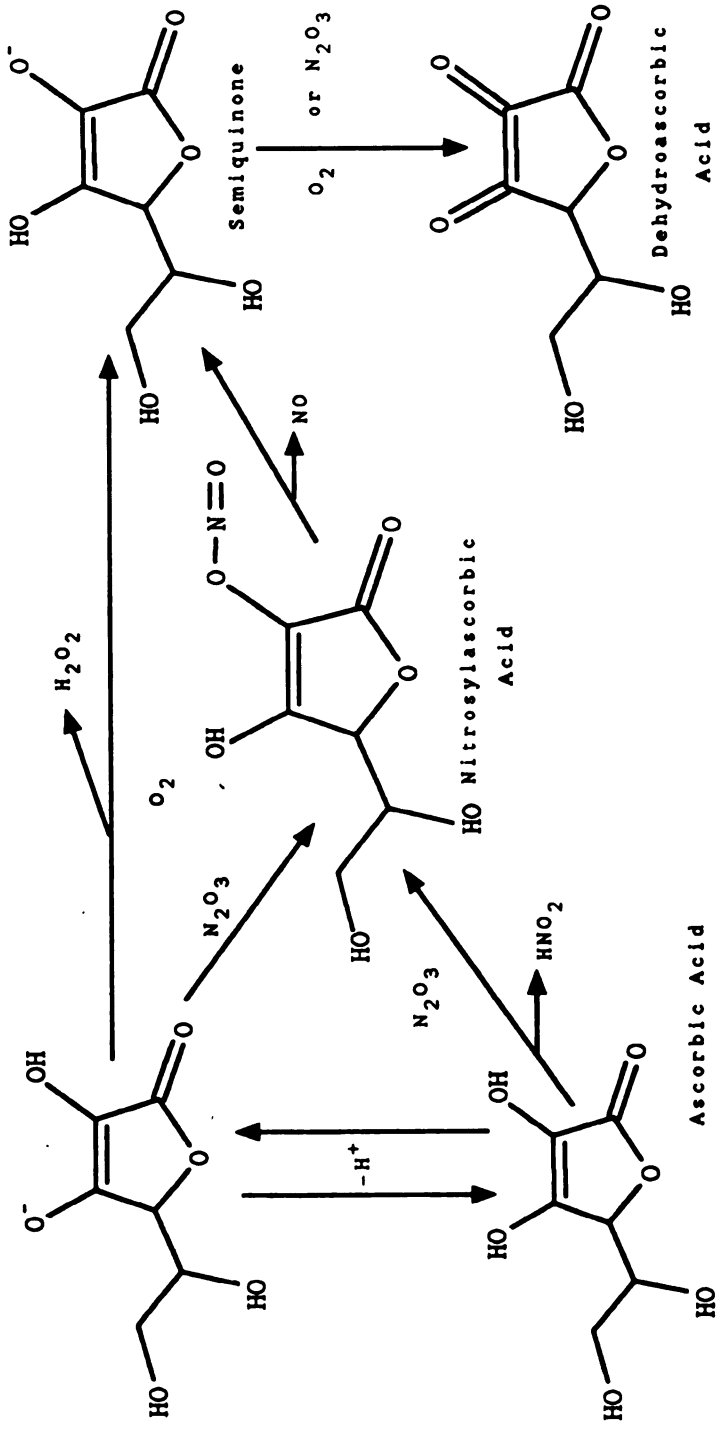


Figure 2. The reduction of dinitrogen trioxide to nitric acid by ascorbic acid. (Hotchkiss, 1987)

inhibitor of N-nitrosamine formation in bacon. It has been proposed that α-tocopherol reacts with nitrosating agents in a manner similar to ascorbic acid (Figure 2)(Newmark and Mergens, 1981). α-Tocopherol has the added benefit of not being capable of undergoing C-nitrosation due to its fully substituted ring (Walker *et al.*, 1979). Fiddler *et al.* (1978) found that a α-tocopherol/polysorbate cure reduced the concentrations of NPYR in fried bacon by approximately 70%. Gray *et al.* (1982) reported that α-tocopherol when introduced into bacon on the surface of salt, inhibited NPYR formation in bacon by 90% when present at the 500 mg/kg concentration. A 62% inhibition of NTHZ was also reported. In order to solubilize the α-tocopherol-coated salt in the brine solution, lecithin was added as an emulsifier(Gray *et al.*,1982). Lecithin was previously identified by Gray *et al.* (1978) as a possible precursor of NDMA in bacon. However, studies using the α-tocopherol-coated salt systems failed to demonstrate any significant increase in NDMA in the presence of lecithin (Bernthal *et al.*, 1986). Unlike ascorbyl palmitate, α-tocopherol has been found to maintain its inhibitory effect over storage time (Skrypec *et al.*,1985). Thus, it appears that any compound capable of competing with the amines for the available nitrite will generally function as an inhibitor of N-nitrosamine formation in foods. It is likely that naturally occurring phenolic compounds such as those that are present in rosemary extracts (Figure 3)

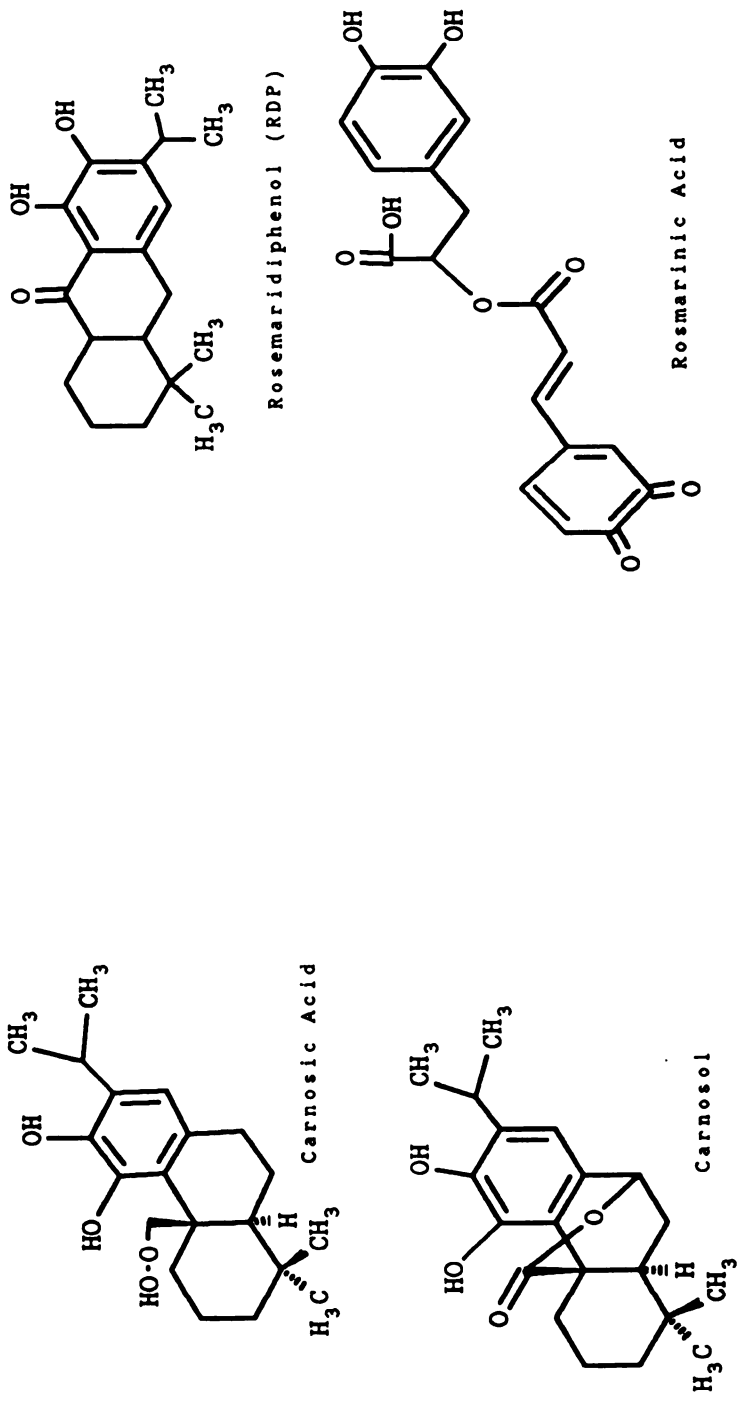


Figure 3. Structures of four antioxidants found in rosemary. (Loliger, 1983)

will function as inhibitors of the nitrosation reactions (Figure 4).

Wisconsin Process

Tanaka *et al.* (1985) developed a process which maintains adequate protection of the bacon against the outgrowth of *C. botulinum* spores, while reducing the concentration of nitrite added to the bacon. The system, known as the Wisconsin Process, is based on bacterial growth and competition. If the meat which has been inoculated with lactic acid-producing bacteria is heat-stressed, the bacteria begin to grow at a faster rate than *C. botulinum*, if present. As the bacteria grow, lactic acid is formed as a by-product of sucrose metabolism. Lactic acid lowers the pH of the meat and inhibits the outgrowth of the *C. botulinum* spores. Nitrite concentrations can be lowered from 120 mg/kg to less than 80 mg/kg and still maintain an equal or greater degree of protection against *C.botulinum* (Tanaka *et al.*, 1985). Sensory tests have shown no significant taste difference between traditionally processed bacon and that processed by the Wisconsin procedure.

Role of Smoke in N-Nitrosamine Formation in Bacon

The smoking of bacon serves two primary functions. First, the smoking process provides some preservative

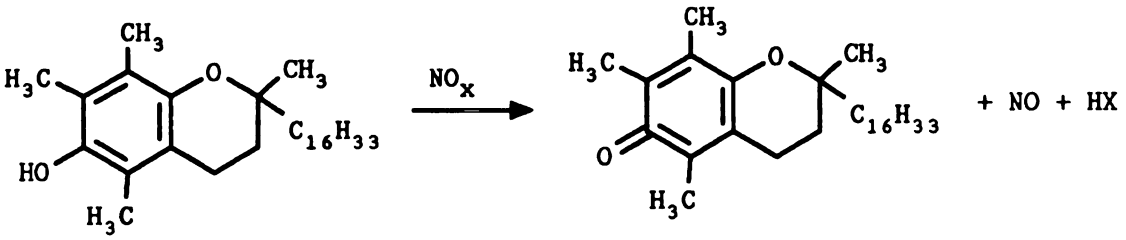


Figure 4. Reduction of dinitrogen trioxide to nitric oxide by α-tocopherol. (Hotchkiss, 1987)

activity for bacon. The second is to give the bacon a characteristic flavor and aroma. Woodsmoke is a complex mixture of many different compounds (Wistreich, 1979). The compounds found in smoke reside in three different phases (Wistreich, 1979). These are the particulate, gaseous, and condensable phases. Acids, esters, and polycyclic aromatic hydrocarbons are the primary components of the condensable phase (Hamm, 1977). Aliphatic acids were noted by Clifford *et al.*(1980) to exhibit antibacterial characteristics. These compounds and a low pH also contribute to color and flavor development (Toth and Potthast, 1984). The main acids found are acetic acid (3.7g/100g wood) and formic acid (0.8g/100g wood) (Toth and Potthast, 1984).

Presence of Carbonyls

Carbonyls such as aldehydes and ketones have been identified in woodsmoke (Toth and Potthast, 1984). These include formaldehyde, acetaldehyde, glycoaldehyde, and methyl glyoxal. These compounds take part in the Maillard Browning reaction and thus are involved in color development in foods.

Formaldehyde has been found in combusted wood at a concentration of 200 mg/ 100g (Toth and Potthast, 1984). Although formaldehyde reacts with amino groups, it tends to inhibit browning (Chen and Issenberg, 1972). Formaldehyde has been shown to react with sulfur-containing amines and

nitrite to form NTHZ (Mandagere *et al.*, 1984).

Presence of Phenols

Phenols are very important as flavor and aroma compounds (Wasserman, 1966). From softwoods, primarily guaiacol and its derivatives are produced, while hardwoods primarily produce syringol and its derivatives (Stahl *et al.*, 1973) With an increase in molecular weight of the phenols, color development is increased due to cross linking of collagen via H-bonding (Caurie *et al.*, 1974). Phenols can react with nitrite to form N-nitrosophenols which can act as catalysts in the formation of N-nitrosamines (Davies and McWeeny, 1977). N-Nitrosophenols have been found to exhibit some mutagenic potential (Gilbert *et al.*, 1980).

Phenols and polyphenols can function as both inhibitors and catalysts of the nitrosation reaction. Whether they act as catalysts or inhibitors is dependent upon the pH of the system, the concentration of both phenol and nitrosating agent, and the structure of the phenol. Phenol can be nitrosated up to 1000 times faster than dialkylamines (Challis, 1973). Thus, phenols could elicit an inhibitory effect by competing with amines for available nitrosating agents. However, in some instances the product, N-nitrosophenol, can further catalyze the formation of N-nitrosamines. High nitrite to phenol ratio favors

catalysis. In contrast, inhibition is favored by a high phenol to nitrite ratio.

N-Nitrosothiazolidine Formation in Smoked, Cured Meats

A mechanism for the formation of NTHZ and N-nitrosocarboxylic acid (NTCA) has been suggested by Mandagere *et al.* (1984). Formaldehyde , which can be found in concentrations up to 50mg/kg smoked meat product, can react with the sulfur-containing amino acids, cysteamine and cysteine, to form thiazolidine and thiazolidine-4-carboxylic acid, respectively (Figure 5). NTCA is decarboxylated more readily that thiazolidine-4-carboxylic acid (Mandagere *et al.*, 1984).

Traces of NTHZ (average of 6.3 μg/kg) and high concentrations of NTCA (average of 1,800 μg/kg) have been reported in raw bacon (Sen *et al.*, 1985). During the frying of bacon, NTHZ concentrations increased to an average of 19.4 μg/kg, while NTCA concentrations decreased to an average of 1,615 μg/kg. Thermal decarboxylation of NTCA occurred during the frying resulting in NTHZ formation Sen *et al.* (1985) also concluded that smoke house temperatures were not high enough to cause thermal decarboxylation of the NTCA; thus, NTHZ concentrations in raw bacon remained low.

In the same studies, Sen *et al.* (1985) conducted incubation experiments and found that formaldehyde,

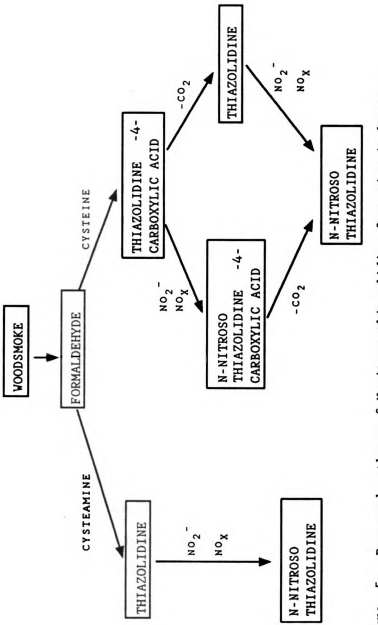


Figure 5. Proposed pathway of N-nitrosothiazolidine formation in bacon. (Mandagere et al., 1984)

cysteamine and nitrite must be present for the formation of large concentrations of NTHZ. If only two of these compounds are reacted in any combination, NTHZ is not formed.

Sen *et al.* (1986) analyzed both raw and fried bacon for NTCA and NTHZ. concentrations of NTCA in raw bacon ranged from 64 to 9,000 µg/kg, while NTCA concentrations in fried bacon ranged from 20 to 14,000 µg/kg. In contrast, NTHZ concentrations in raw bacon ranged from non-detectable to 7.5 µg/kg, and in fried bacon from 2.1 to 241 µg/kg. These authors observed the conversion of NTCA to NTHZ during frying. Raw bacon spiked with 10,300 µg/kg NTCA yielded 764 µg/kg NTHZ on frying, a 2.2% conversion of NTCA to NTHZ.

Liquid Smoke versus Woodsmoke

Advantages

It has been estimated that 65% of the smoked meat products produced in the U.S. are processed with liquid smoke (Hollenbeck, 1977). Liquid smokes give the food manufacturers four advantages: 1) decreased emissions into the environment during the smoking process; 2) increased efficiency; 3) smaller concentrations of carcinogenic compounds in the smoked product; and 4) increased control over the flavor and color characteristics of the smoked product. The first benefit, that of reduced emissions into

the environment, helps to reduce the pressure on the bacon processors from an increasingly environmentally conscious public and government agencies, while increasing the efficiency of the smoking process is of a economic benefit to the company. Many companies utilize continuous smoking processes. In this process, the product is sent through a tunnel on a conveyor and is sprayed with liquid smoke (Hollenbeck, 1977). The third benefit is the reduced concentrations of carcinogenic compounds in the smoke. This reduces consumer exposure to harmful compounds such as polyaromatic hydrocarbons (Gorbatov *et al.*, 1971). These compounds can be precipitated out of the smoke along with the tars during the aging of the liquid smoke. The liquid smoke also can be filtered through cellulose fibers to further reduce the concentrations of undesirable compounds (Hollenbeck, 1977). The fourth advantage is in the increased quality control that the use of liquid smoke allows the manufacturer. The manufacturer can precisely control the concentration of liquid smoke added from lot to lot of processed food product.

Disadvantages

Problems can arise from the use of nonbuffered liquid smoke and the premature reduction of available nitrite in the meat system (Sleeth *et al.*, 1982). As described previously, nitrite is an effective inhibitor against *C.botulinum* outgrowth (Tompkin *et al.*, 1978). Liquid smokes tend to be have a low pH due to the presence of high concentrations of acids and phenols (Sleeth *et al.*, 1982). Nitrite in a low pH system is quickly reduced to nitric oxide, thus lessening the concentration of nitrite available to inhibit the outgrowth of *C. botulinum.* One solution to this problem is to buffer the liquid smoke system (Sleeth *et al.*, 1982).

Production of Liquid Smoke

A common method of producing liquid smoke in the U.S. is to extract the primary compounds of interest out of the smoke using water (Hollenbeck, 1977). The smoke is produced in an oxidative atmosphere using smoldering saw dust. The primary compounds responsible for flavor and color are then extracted using a countercurrent extraction procedure. The concentration of the solution is governed by the degree of recycling of the water through the smoke. The liquid smoke is then aged. During the aging process, tars polymerize and

precipitate out of solution. Particulate is then removed by filtering through cellulose fibers. Another method of liquid smoke production involves exposure of fine wood chips to super heated steam and then condensing the steam distillate (Fessman, 1976).

Addition of Liquid Smoke to the Food Product

There are several methods used to add liquid smoke to food products. The liquid smoke can be added directly to the product, or the product can be dipped into the liquid smoke (Wasilewski and Kozlawski, 1977). A third method used in continuous processing is to spray the liquid smoke onto the surface of the product. The spray can also be atomized (Hollenbeck, 1977). A newer method of application is the regeneration of the smoke from liquid smoke concentrates (Wistreich, 1979). The concentrate is atomized and air containing the atomized smoke is then heated and introduced into the smoke house. The concentration of the air/smoke mixture is governed by the degree of recirculation. The time required to smoke a product can be cut to 1/16 the time required to traditionally smoke a product.

The Effects of Liquid Smoke on N-Nitrosamine Formation

Theiler *et al.* (1984) found that liquid smoke could

significantly lessen NPYR concentrations in a ground pork model system when incorporated into the curing brine. However, surface application onto pork bellies did not produce significant reductions in concentrations of NPYR. When liquid smoke was added to a curing brine used in pork bellies, a 60% reduction in NPYR was noted in the fried bacon. However, it has been reported that significant reductions of NPYR and NTHZ could be obtained when liquid smoke was atomized onto the surface of cured pork bellies (Ikins *et al.*, 1986). These researchers also found that inclusion of liquid smoke into the curing brine resulted in even greater reductions in the concentrations of NPYR and NTHZ in fried bacon, with an 82% reduction of NPYR as compared to that in bacon produced from pork bellies which were traditionally smoked. It was reported earlier by Pensabene and Fiddler (1985b) that NTHZ formation was reduced in bacon which had been smoked through atomization of liquid smoke.

Toxicological Concerns of N-Nitrosamine Compounds

The concern over N-nitroso compounds in food centers around effects due to long term exposure and not effects due to short term exposure. The environmental/food exposure rates of N-nitroso compounds to humans is less than the concentration which is required to produce acute toxic

effects as a result of short term exposure (Hotchkiss, 1987). However, while the concentrations are not high enough to produce acute effects due to short term exposure the concentrations are high enough to produce tumors in rats though long term exposure (Crampton, 1980). Ninety percent of the 300 N-nitroso compounds tested have been found to produce carcinomas in animals (Hotchkiss,1987). However, there is little direct evidence of N-nitroso compounds producing carcinomas in humans.

Target organs in rats for N-nitroso compounds for NDMA include the liver, kidney, and nasal cavity, while NPYR produces carcinomas in the liver and nasal cavity in rats (Ember,1980). A structure activity relationship exists between the target organ and the active N-nitrosamine. Symmetrical dialkylnitrosamines primarily target the liver, whereas nonsymmetrical dialkylnitrosamines generally target the esophagus. Thus, small changes in the structure of the N-nitrosamine can change which organ is targeted Hotchkiss, 1987).

N-Nitrosamine concentrations comparable to those found in foodstuffs have been shown to promote tumor development in animals. Concentrations of 130 ug/kg of NDMA has been found to produced tumors in rats when the rats were exposed to this concentration of N-nitrosamine on a daily basis (Crampton, 1980). Significant tumor development has been noted in animals two generations after exposure to the N-nitroso

compound (Tomatis *et al.*, 1975).

It is generally thought that N-nitroso compounds must be metabolically activated to the carcinogen (Archer, 1982). The mechanism involves hydroxylation of a carbon atom α to the N-nitrosamino nitrogen. The hydroxyl compound then spontaneously rearranges to an aldehyde and a primary alkyldiazohydroxide. Loss of a hydroxide ion from the latter compound produces an alkyldiazonium ion. This ion decomposes to molecular nitrogen and a carbonium ion. The carbonium ion acts as an alkylating agent which reacts with water, nucleophiles, nucleic acids, and proteins (Archer, 1982). Lijinsky *et al.* (1968) furthered this theory by substituting the α hydrogens with deuteriums. This significantly decreased the potency of the N-nitrosamine.

Mihara and Shibamoto (1980) found NTHZ to be mutagenic, in contrast to Fiddler *et al.* (1984), who demonstrated that NTHZ was nonmutagenic. It should be noted, however, that the two groups formed NTHZ by two different methods. Mihara and Shibamoto synthesized the NTHZ by reacting cysteamine, formaldehyde, and nitrite, while Fiddler *et al.* formed NTHZ by direct nitrosation of thiazolidine. A possible explanation for this discrepancy could be that a mutagenic contaminant, side product, or residual precursor is formed when the three precursors are reacted. Using HPLC to separate the various fraction, a mutagenic fraction did elute after the NTHZ fraction (Fiddler et al, 1984).

METHODS AND MATERIALS

Materials

Dichloromethane was purchased from Mallinckrodt Inc. (Paris, KY) and was redistilled prior to use. All chemicals, which were analytical grade and used without further purification, were purchased from Mallinckrodt Inc. N-Nitrosamine standards were purchased from Thermedics Inc., Woburn, MA..

Alberger Fine Flake salt and α-tocopherol-coated Alberger Fine Flake salt were donated by the Diamond Crystal Salt Co. (St. Clair, MI). Rosemary oleoresin was supplied by Kalsec Inc. (Kalamazoo, MI) and coated onto Alberger Fine Flake salt by the Diamond Crystal Salt Co. Bac-N-Phos, a system of sodium polyphosphate, sodium hexametaphosphate, and sodium bicarbonate, was acquired from Stauffer Chemical Co. (Westport, CT). The lactic acid-producing bacteria, *Pediococcus acidilactici*, were donated as a frozen concentrate from ABC Research Laboratories (Gainsville, FL). All other brine ingredients were purchased from Michigan State University Food Stores.

N-Nitrosamine Reduction in Brine-Cured Bacon

Preparation of Bacon

Bacon was prepared under carefully controlled processing conditions at the Meat Laboratory, Michigan State University. Thirty pork bellies (approximately 4-5kg) were obtained from a local supplier within 24 hr of slaughter, randomly divided into five groups of six bellies, and immediately processed into bacon. The bellies were pumped to 110% of their green weight with brines listed in Table 2. The bellies were held at 2°C for 24 hr and then smoked for 8 hr by a standard smoking process using hickory wood sawdust (Reddy *et al.*, 1982). The smoked bellies were transferred to a holding cooler (-2°C) where they were held overnight before slicing (30mm) and vacuum packaging. The bacon samples were held for 1 week at 4°C prior to frying. This process was replicated four times.

Frying of Bacon

The bacon was fried in a calibrated preheated Sunbeam teflon-coated electric frying pan at a thermostat setting of 171°C (340°F) for 6 min (3 min per side)(Lee *et al.*, 1983a), drained of cooked-out fat, and then frozen until analyzed.

Table 2. Brine formulations used in a study of N-nitrosamine reduction in brine-cured bacon.

Treatments	Salt (20%)			Acid phosphate (3.5%)	Phosphate (3.5%)	Other[a]
	Regular	α-Tocopherol coated	Rosemary oleoresin coated			Ascorbate Sucrose Nitrite
Control	X				X	X
Acid phosphate	X			X		X
α-Tocopherol		X			X	X
Rosemary oleoresin			X		X	X
Wisconsin process	X					X[b]

[a] nitrite added at 1200 mg/kg; ascorbate added at 5500 mg/kg; sucrose added at 5%.

[b] was inoculated with 800 mg/kg nitrite using *Pediococcus acidilactici*.

N-Nitrosamine Analysis

The frozen bacon samples were ground with dry ice and analyzed for their N-nitrosamine content by the mineral oil distillation method as described in detail by Ikins *et al.*, (1986). Five microliters of the sample extract were then injected in a gas chromatograph (Varian 3700, Walnut Creek, CA.) - Thermo Energy Analyzer system (Thermo Electron Model 502, Waltham, MA.). The TEA gain was adjusted to 3.0, with a transfer line temperature of 250° C and a pyrolyzer temperature of 470 ° C. The GC was temperature programmed from 140° C to 180° C at a rate of 15°C degrees per min with a one min initial hold time at 140°C and a nine min final hold time at 180°C. The column used for separation was a 10% Carbowax 20M TPA 80-100 mesh Chromosorb WHP packed in a glass chromatographic column (3m x 2 mm ID)(Supelco, Bellefonte, PA). Peak areas of the eluted N-nitrosamines were calculated by a Hewlett Packard Model 3390A (Walnut Creek, CA.). Attenuation was set at 6 with a chart speed of 1.0 cm/hr. Peak area of an external standard containing NDMA and NPYR was compared to the peak area of the sample for quantitation. The following equation was utilized to determine N-nitrosamine concentration:

Analytical efficiency was monitored by the addition of 10 ug

$$\left[\frac{\text{Area of Sample}}{\text{Area of 1 µg of Corresponding Standard}}\right]\left[\frac{\frac{\text{1000 µl of sample}}{\text{5 µl of injected}}}{\frac{\text{sample}}{\text{25 g meat sample}}}\right] = \text{µg/kg meat}$$

of an internal standard, N-nitrosoazetidine (NAZET), at the start of the analytical procedure.

Sodium Nitrite Analysis

Residual sodium nitrite content was determined in a 5 g sample of bacon sample prior to cooking, by the AOAC procedure (1984). Quantitation was based on a standard curve plotting absorbance versus concentration. The standard curve was made up of 7 standards (1, 3, 5, 7, 10, 20, 30, and 40 µg). The following equation was used to determine concentration of nitrite per gram of meat:

$$\left[\text{Amount Read from Standard Curve (µg)}\right]\left[\frac{\frac{\text{500 ml Total}}{\text{25 ml Aliquot}}}{\text{5 g Sample}}\right] = \text{mg/kg meat}$$

N-Nitrosamine Formation in Bacon Processed in Ireland and the United States

Processing of Irish bacon: Irish bacon was processed at the University College Cork meat processing facility. Eight backs and bellies were obtained from a local commercial supplier approximately 48 hr after slaughter, held overnight at 2° C and then randomly divided into two groups. The backs and bellies in each group were cut in half. One half of each back and belly was processed using a curing brine containing regular Alberger Fine Flake salt while the other half was cured using α-tocopherol coated-salt, as described by Gray *et al.* (1982). The target concentration of α-tocopherol in the finished bacon was 500mg/kg. The backs and bellies were pumped to 110% of their green weight with brines containing 20% salt, 4% sodium tripolyphosphate, 5,500 mg/kg sodium ascorbate and sodium nitrite. Two concentrations of nitrite were used. The target concentration in the first group of backs and bellies was 120 mg/kg, while that for the second group was 200 mg/kg. Following pumping, the backs and bellies were tumbled for one min under high vacuum and then held overnight at 2°C before restoring to their green weight at 60°C in a smokehouse without the application of smoke. The cured backs and bellies were held for one week at 2°C before slicing (30mm) and frying.

Processing of U.S. Bacon: Bacon was processed as described previously. Brines contained 20% salt (regular Alberger Fine Flake salt or α-tocopherol-coated salt), 5.0% sucrose, 3.5% sodium tripolyphosphate, 5500 mg/kg sodium ascorbate, and 1200 mg/kg or 2000 mg/kg sodium nitrite.

Bacon Frying

Representative portions of bacon produced in Ireland were fried in a preheated teflon-coated electric frying pan at a thermostat setting of 177°C (350°F). The slices were fried for eight min (4 min per side) in beef drippings, removed from the pan and drained on paper towels. The fried slices were quickly frozen, vacuum packaged and air-freighted to Michigan State University for N-nitrosamine analysis. Bacon processed in the U.S. was fried as previously discussed. Nitrite and N-nitrosamine analysis was carried out as previously discussed.

The Effect of Lipid Oxidation in Pork Bellies on N-Nitrosamine Formation in Fried Bacon.

Procurement of Pork Bellies

Thirty crossbred pigs (approximately 3 months old), raised at the Michigan State University Swine Research Farm,

were divided into five groups of six pigs. Each group was balanced with respect to litter mate, body weight, and sex by a restricted randomization technique, and each group was subjected to different dietary treatments by complete randomization. The feed formulation treatments are shown in Table 3. At the end of the feeding trial, all animals were slaughtered by a standard commercial procedure. The bellies were removed from the carcass for immediate bacon manufacturing. The processing and frying of the bacon was carried out as previously described. The extent of oxidation of the raw pork bellies was determined by the thiobarbituric (TBA) assay (Tarladgis *et al.*, 1960). Residual nitrite analysis in the uncooked bacon and the N-nitrosamine concentrations in the fried bacon were determined as described previously.

Effect of Liquid Smoke Components on N-Nitrosamine Formation in Restructured Bacon Product

Determination of Formaldehyde in Liquid Smokes

Formaldehyde concentrations in liquid smokes were determined using a method based on the reaction of carbonyls with cysteamine and nitrite to form N-Nitrosothiazolidine derivatives (Mandagere *et al.*, 1984). Three liquid smokes (0.05 g) shown to produce three different known

Table 3. Pig diets used to study the effects of lipid oxidation on N-nitrosamine formation in fried bacon.

Group #	Treatment[a]
1	Control Diet
2	Short-term α-tocopherol supplementation (control diet + 200mg/kg feed of α-tocopherol for last 4 weeks)
3	Long-term α-tocopherol supplementation (control diet + 200mg/kg feed of α-tocopherol for duration of feeding trial (10 weeks)
4	Mixed tocopherol supplementation (control diet + 200mg/kg feed of mixed tocopherols for duration of feeding trial (10 weeks)
5	Received oxidized corn oil (PV 9 meq/kg feed) representing 3% of feed for duration of feeding trial (10 weeks)

[a]All groups (except group 5) received a standard feed formula containing 3% corn oil

concentrations of NTHZ in fried bacon (Jaffer, unpublished data) were reacted with 50 mg cysteamine and 100 mg of nitrite (Table 4). The total volume of the reaction mixture was brought to 20 ml with distilled water. The pH was adjusted to 5.5 using 0.1 N NaOH and the components in the model system were reacted at room temperature for 2 hr. The reaction mixture was extracted with 3x 10 ml aliquots of redistilled dichloromethane and the extracts dried over sodium sulfate. The final volume was brought to 40 ml. Five µl of the samples were then injected into the GC-TEA system as described previously. GC and TEA conditions were set as previously described. The analytical efficiency of this process was monitored by spiking known concentrations of formaldehyde into duplicate liquid smoke samples.

A linear standard curve was constructed by reacting 0.01, 0.05, and 0.10 mg of formaldehyde with 50 mg cysteamine and 100 mg nitrite and analyzed in the same manner as the liquid smoke samples. Peak area versus amount of formaldehyde was then plotted to produce a standard curve. The formaldehyde contents of the liquid smokes were then calculated using the following formula:

$$\left[\frac{\left[\frac{\text{NTHZ Found (ng)} \times \text{Sample (ml)}}{\text{SAMPLE INJECTION (µl)}}\right]\left[\frac{\text{MW NTHZ}}{\text{MW HCHO}}\right]\left[\frac{1\,g}{1\times10^{9}}\right]}{\left[\frac{\text{LIQUID SMOKE SAMPLE}}{(g)}\right]\left[\frac{1\,Kg}{1000\,g}\right]}\right] = g/kg$$

Processing of Restructured Bacon

Restructured bacon was utilized to eliminate belly to belly variation normally encountered in pork bellies from different pigs (Pensabene *et al.*, 1979). Frozen belly trimmings (43.6 kg), not more than a month old and taken from previous studies, were chopped (Hobart Model VCM 40E, Troy, OH.) and mixed in a mixer (Keebler Engineering Co., Chicago, IL.). From this, 1.5 kg portions of the chopped, mixed meat were taken for sample formulation. A known volume (150 ml)of a brine containing salt (20% w/w), sugar (5%), phosphate (3.5%), and nitrite (1200 ppm) was added to meat to produce a 10% weight gain. Just prior to processing, ascorbate, nitrite and the specific treatment ingredients were added to the brine (Table 4). The liquid smokes were added at the 0.18% concentration. Three different concentrations of formaldehyde comprised the next three treatments, the concentrations of formaldehyde corresponding to those in the liquid smokes. A single concentration of malondialdehyde was

Table 4. Variables used to study the effect of liquid smoke components on the formation of N-nitrosamine compounds.

Group #	Treatment[a]
C	Control
M	Malondialdehyde (≅1.05g/kg liquid smoke)
LS1	Liquid smoke #1
LS2	Liquid smoke #2
LS3	Liquid smoke #3
F1	Formaldehyde #1 (≅0.36 g/kg liquid smoke)
F2	Formaldehyde #2 (≅1.56 g/kg liquid smoke)
F3	Formaldehyde #3 (≅1.05 g/kg liquid smoke)

[a] all groups were treated with a standard brine consisting of 120 ppm nitrite, 550 ppm ascorbate, 3.5% tripolyphosphate, 5.0% sucrose, and 20.0% salt.

arbitrarily chosen and used as a separate treatment. A control treatment consisting of only the stock brine plus ascorbate was also formulated. The ascorbate was added to the brine just prior to mixing with the meat so as to prevent any premature reaction of the ascorbate with the nitrite.

Mixing was accomplished using an Kitchen Aid K5-A mixer (Hobart, Benton Harbor, MI). Both the mixing bowls and mixing paddles were stored in a cooler (-1.0°C). This was done to minimize the leaching of the fat from the meat during mixing. After mixing for two min, the samples were stuffed into 6 x 30" fibrous casings (Viskase, Chicago, IL.) using a hand operated stuffer (Vogt, W. Germany). The resulting restructured bacon was then tempered overnight in a -1.0 ° C cooler and then cooked the following day. The cooking schedule was four hr at 58° C followed by three hr at 52° C. No traditional smoke was employed. The yield after cooking was calculated to verify that the restructured bacon was at 100% of the original weight. The restructured bacon was then sliced, packaged, fried, and analyzed for N-nitrosamines. The restructured bacon was fried as previously described. The nitrite and N-nitrosamine concentrations were quantitated as described previously.

Statistical Analysis of Data

Two way analysis of variance statistics were conducted

to determine significant differences occurring within groups of treatments. Bonferoni t statistics were applied to determine significant differences between treatments (Gill, 1978).

RESULTS AND DISCUSSION

Reduction of N-Nitrosamine Formation in Fried Bacon

Residual Nitrite Concentrations in Raw Bacon

The means and standard deviations of residual nitrite concentrations in raw bacon prior to frying for each treatment are presented in Table 5. The results of the analysis of variance (ANOVA) for the effect of brine treatments on residual nitrite concentrations are given in Table A in the Appendix.

The ANOVA indicated that there were significant differences among treatments. Bonferoni t values established that the residual nitrite concentrations of bacon processed with α-tocopherol-coated salt and rosemary oleoresin-coated salt were significantly greater ($\alpha<0.05$) than the nitrite concentration in the control bacon. Although nitrite residues in bacon processed with acid phosphates and by the Wisconsin process were not significantly lower ($\alpha<0.10$) in concentration from the control, a decreasing trend could be observed.

Although no other study has been conducted using rosemary oleoresin in the processing of bacon, the similarity between the molecular structure of α-tocopherol and rosemary oleoresin constituents may be an indication that rosemary

Table 5. Means and standard deviations of nitrite, recovery of internal standard, and N-nitrosamine concentrations in bacon processed with various curing adjuncts.

Treatments	Residual Nitrite mg/kg	Recovery[a] %	NDMA µg/kg	NPYR µg/kg	NTHZ µg/kg
Control	12.4 ± 9.4	99 ± 17	3.6 ± 4.1	5.3 ± 3.0	4.3 ± 3.0
Bac-N-Phos	8.4 ± 7.7	98 ± 23	2.1 ± 0.8[1]	4.0 ± 2.2[1]	4.2 ± 2.0
α-Tocopherol	25.1 ± 16.7[1]	101 ± 19	2.5 ± 1.0[1]	4.8 ± 5.3	4.8 ± 2.3
Rosemary Oleoresin	21.5 ± 9.8[1]	95 ± 15	3.2 ± 1.7	4.7 ± 3.4	3.9 ± 2.9
Wisconsin process	6.8 ± 5.0	96 ± 16	2.2 ± 1.5[1]	3.3 ± 2.4[1]	3.2 ± 1.1

n = 24

[a] Internal standard NAZET.

[1] significantly different from the control at α = .1 (Bonferoni t test).

oleoresin could reduce N-nitrosamine formation in bacon. Both α-tocopherol and the phenolic compounds present in rosemary oleoresin have hydroxyl groups which can readily reduce nitrite to nitric oxide, which readily escapes to the atmosphere. Thus, lesser concentrations of residual nitrite might be expected in bacon treated with these compounds. Previous studies have shown that bacon cured with ascorbic acid (Mirvish *et al.*, 1972) have lesser concentrations of residual nitrite. However, Fiddler *et al.* (1978) reported that α-tocopherol had little effect on the residual nitrite concentrations in bacon. The data presented in this study finds residual nitrite concentrations greater in both bacon treated with α-tocopherol and rosemary oleoresin as compared to bacon treated only with ascorbic acid. Nitrite concentrations in bacon have been shown to lessen dramatically in raw bacon over time. (Hotchkiss, 1987). Figure 6 illustrates the change in nitrite concentrations over time for the various treatments of this study. Day 0 residual nitrite concentration was analyzed immediately after processing. In all of the treatments, there was a marked decrease in the concentration of residual nitrite over time. The Wisconsin process had the lowest concentrations of nitrite throughout the hold period. This is not suprising since the ingoing concentration of nitrite for the Wisconsin process was 80 mg/kg as compared to the greater ingoing concentration (120 mg/kg) of the other treatments. The

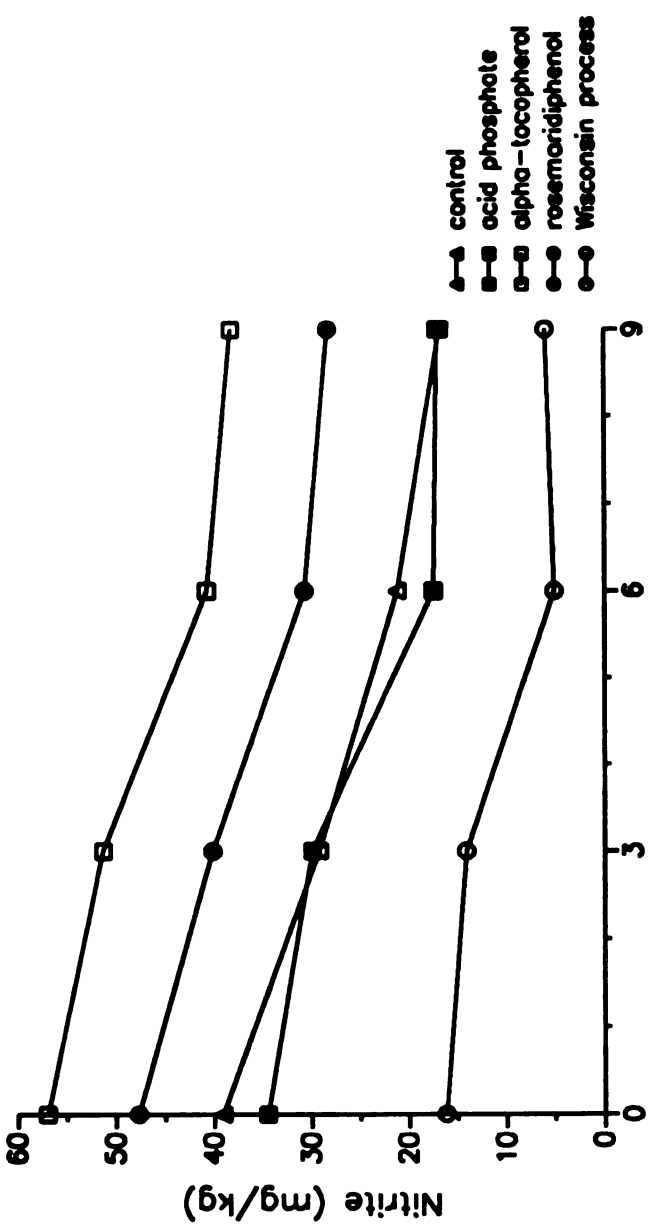


Figure 6. Average residual nitrite levels in bacon prepared with various curing adjuncts stored at 4°C for nine days.

residual concentrations of nitrite in bacon produced by the Wisconsin process were also reported by Tanaka *et al.* (1985) to be less than concentrations found in bacon produced by traditional methods. These researchers reported residual nitrite concentrations in bacon produced by the Wisconsin process to range between 4-22 mg/kg, while those in bacon processed using traditional methods ranged between 32-48 mg/kg.

N-Nitrosamine Concentrations in Bacon

Results of the ANOVA for the effect of brine treatments on NDMA formation in fried bacon are given in Table A in the Appendix. Means and standard deviations for NDMA, NPYR, and NTHZ concentrations in fried bacon and the corresponding recovery of the internal standard are given in Table 6. The average recovery of the internal standard, N-nitrosoazetidine (NAZET), ranged from 95% ± 15 to 101% ± 19. The N-nitrosamine concentrations were not corrected for recovery of the internal standard.

NDMA: The ANOVA indicated that there were significant differences among treatments with respect to only NDMA. When NDMA concentrations in the control bacon samples were compared to those in bacon samples processed using the other curing treatments (acid phosphates, α-tocopherol-coated salt,

and Wisconsin process), the NDMA concentrations in bacon processed with adjuncts were determined to be significantly lower ($\alpha<0.05$) than that of the traditionally processed control samples. Although the rosemary oleoresin treatment did inhibit NDMA formation the level of inhibition was not significant at the $\alpha<0.05$ level. α-Tocopherol also inhibited NDMA formation. This trend is similar to that of Gray *et al.* (1982) who reported reductions of 76 to 92%. Fiddler *et al.* (1978) also found that cure-solubilized α-tocopherol inhibited NDMA formation. The Wisconsin process produced NDMA concentrations in bacon less than those of the control samples. This observation supports the data of Tanaka *et al.* (1985) who reported up to 50% reduction in NDMA concentrations in bacon produced by the Wisconsin process. The acid phosphate exhibited the greatest degree of inhibition of the treatments.

NPYR: Significant differences ($\alpha<0.05$) in concentrations of NPYR in bacon were found between the following treatments: control and acid phosphate treatment ($\alpha<0.10$), control and Wisconsin process. The rosemary oleoresin-coated salt appeared to slightly increase the concentration of NPYR in fried bacon relative to the control samples, but the increase was not statistically significant ($\alpha<0.10$). NPYR concentrations in fried bacon produced by the Wisconsin process were less than the concentrations in the control

samples. These results are similar to those reported by Tanaka *et al.* (1985) who also detected lesser concentrations of NPYR in bacon produced by the Wisconsin process as compared to bacon produced by the traditional process. The highest concentration of NPYR detected by these researchers in bacon produced by the Wisconsin process was 8.5 μg/kg, while traditionally processed bacon NPYR concentrations were in excess of 10 μg/kg. Bacon processed with the acid phosphates and α-tocopherol-coated salts had NPYR concentrations which were less than the control samples. The trend of α-tocopherol in inhibiting NPYR formation follows that reported previously by Gray *et al.* (1982). However, generally greater inhibition (90%) has been previously observed. These researchers found that NPYR formation was inhibited to a greater degree than the formation of NDMA. Pensabene *et al.* (1978) also observed that use of α-tocopherol in the curing brine inhibited NPYR formation.

NTHZ: Significant differences in NTHZ concentrations among treatments were found with the ANOVA. However, when the individual treatments were compared to one another by the Bonferoni's t test, only the NTHZ concentrations in bacon produced by the rosemary oleoresin-coated salt treatment and the Wisconsin process were determined to be significantly different ($\alpha<0.05$). These data contrast with results reported by Gray *et al.* (1982) which shows significant

inhibition of NTHZ formation. However, Pensabene and Fiddler (1985b) reported that α-tocopherol, when used as a cure addition or spray, did not significantly inhibit NTHZ formation in raw bacon processed without ascorbate.

With the exception of NDMA, the Wisconsin process, which had a smaller ingoing concentration of nitrite, was most effective in reducing the concentrations of N-nitrosamines formed (Figure 7). The acid phosphate treatment was the most effective in reducing the concentrations of NDMA formed. Alpha-tocopherol was also very effective in reducing NDMA concentrations in cured bacon. This treatment, however, did not reduce the concentrations of NPYR or NTHZ greater over those concentrations found in the control cured bacon.

The degree of inhibition of N-nitrosamine formation was not as great as the author expected due to the low concentrations of N-nitrosamines formed in the traditionally processed bacon. Concentrations of NPYR found in a later study were approximately 10 μg/kg (see page 61) as compared to concentrations of approximately 5 μg/kg found in the control samples in this study. Studies conducted by Gray *et al.* (1982) also reported concentrations of NPYR greater than 5 μg/kg for control bacon. Thus, if NPYR concentrations in the control bacon samples were less than normally detected, the degree of inhibition by the various treatments would be less than anticipated. Therefore, further replication of the control treatment may be required to substantiate the actual

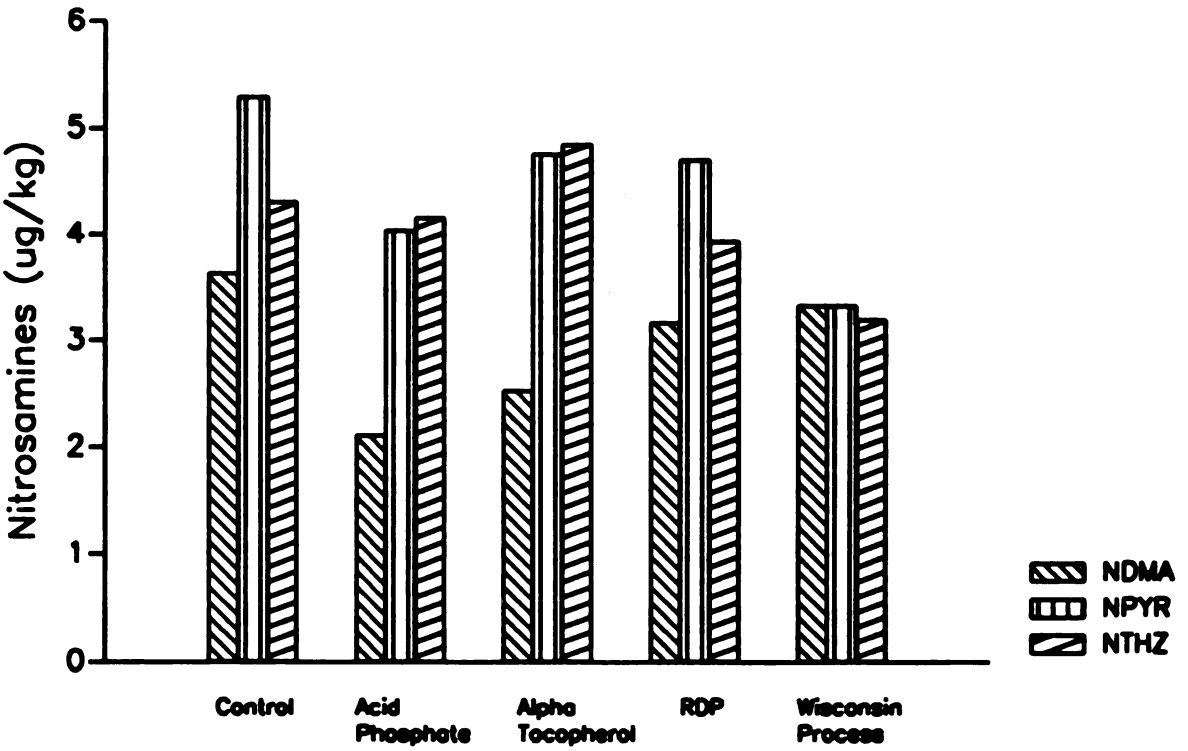


Figure 7. Average N-nitrosamine levels in fried bacon using various curing adjuncts.

degree of inhibition provided by the curing adjuncts.

N-Nitrosamine Concentrations in Bacon Processed in Ireland and the United States as Influenced by Nitrite Concentration and the Presence of α-Tocopherol

Residual nitrite concentrations in the uncooked bacon samples were determined after storage for one week at 4°C (Table 6). Lesser residual concentrations were observed in the bacon processed in the U.S. when compared to the Irish belly (streaky) bacon, while residual nitrite concentrations in the back bacon were much smaller than those in the Irish streaky bacon. Of interest is the apparent reduction of the residual nitrite concentration by the presence of α-tocopherol in the cure. However, these results are somewhat different than those obtained for the streaky bacon and to those reported by Pensabene and Fiddler (1985b). These investigators showed that α-tocopherol, when added to the curing brine, had no effect on the residual nitrite concentrations in uncooked bacon samples.

N-Nitrosamine concentrations in the bacon samples fried after storage at 4°C for one week were quantitated using mineral oil distillation procedure modified to prevent artifactual N-nitrosamine formation during sample extraction (Hotchkiss and Vecchio, 1985; Ikins *et al.*, 1986). As

Table 6. Means and standard deviations for nitrite concentrations (mg/kg) in various bacon samples processed with two levels of nitrite.

Treatments	Bacon Process		
	Irish Back	Irish Belly	U.S. Belly
120 mg/kg NO_2 + α-tocopherol	28 ± 2	68 ± 6	30 ± 8
120 mg/kg NO_2	9 ± 1	48 ± 6	30 ± 5
200 mg/kg NO_2	66 ± 13	93 ± 37	54 ± 23
200 mg/kg NO_2 + α-tocopherol	22 ± 4	97 ± 27	43 ± 11

expected from literature reports/ N-nitrosamine concentrations in bacon processed in the U.S. were significantly ($\alpha<0.01$) greater than the concentrations found in bacon processed in Ireland (Table 7). The average NPYR concentrations in bacon processed in the U.S. prepared with 120 and 200 mg/kg nitrite were 10.7 and 12.1 μg/kg, respectively, whereas average values of less than 4.0 μg/kg were obtained for the equivalent Irish streaky bacon samples. NPYR concentrations for the back bacon were even lesser. Although many factors influence the formation of NPYR in fried bacon, the results obtained in the present study further demonstrate that the fat content of the bacon plays a major role in N-nitrosamine formation. N-Nitrosamine formation occurs primarily in the fat portion of the fried bacon (Fiddler *et al.*, 1974; Mottram *et al.*, 1977; Amundson *et al.*, 1982), so bacons with greater fat to lean ratios are likely to form more NPYR. This was demonstrated by Amundson *et al.*(1982) who compared the N-nitrosamine concentrations in two bacon samples having initial fat contents of 51 and 57%. Bacon with the greater fat content, when held for 10 days under refrigeration, produced approximately 3 to 4 times more NPYR than did the leaner bacon when fried under similar conditions. A correlation between N-nitrosamine concentrations in fried bacon and bacon composition (fat content) has been established by Pensabene *et al.* (1979). However, these researchers also indicated the NPYR and NDMA

Table 7. N-Nitrosamine concentrations (μg/kg) in bacon processed in Ireland and the U.S. with two levels of nitrite, and percent inhibition of N-nitrosamine with α-tocopherol.

Treatment	Irish back			Irish belly			U.S. belly		
	NDMA	NPYR	NTHZ	NDMA	NPYR	NTHZ	NDMA	NPYR	NTHZ
120 mg/kg NO_2	0.7±0.4	0.6±0.1	0.5±0.4	1.7±0.6	3.8±1.1	0.8±0.6	2.0±0.7	10.7±3.3	4.2±1.1
120 mg/kg NO_2 + α-tocopherol	0.8±0.3 (-)	0.6±0.2 (-)	0.1±0.1 (80%)	0.7±0.1 (59%)	0.7±0.4 (81%)	0.3±0.1 (63%)	1.5±0.4 (25%)	2.2±1.2 (79%)	2.9±0.9 (31%)
200 mg/kg NO_2	1.8±0.9	3.0±2.2	0.9±0.3	1.8±0.4	3.9±1.0	1.1±0.3	2.7±0.4	12.1±4.7	11.5±6.8
200 mg/kg NO_2 + α-tocopherol	1.1±0.1 (39%)	0.7±0.3 (75%)	0.5±0.8 (44%)	1.7±0.4 (<1%)	0.7±0.2 (82%)	1.0±0.8 (9%)	2.5±0.8 (<1%)	5.1±2.3 (57%)	7.0±4.4 (41%)

[a]Figures in parentheses represent percent inhibition by α-tocopherol.

concentrations are more highly correlated with residual and added nitrite.

Bacon samples processed in the present study were not analyzed for fat content. However, visual observations revealed that the U.S. bellies were much fatter that the Irish counterparts. Chant *et al.* (1976) selected one hundred fresh pork bellies from a commercial processor and classified them by a subjective leanness score. Bacon processed from these bellies had fat contents ranging from 59 to 74%. Pearson and Tauber (1984) cited a value of 69.37% for the fat content of uncooked, slab or sliced bacon. The fat content of Irish streaky bacon is somewhat lower (Bogue, private communication), while the fat content of bacon made from fresh pork backs is generally less than 20% (23). As the major precursor (free proline) for NPYR formation in fried bacon resides in the adipose tissue, the difference in the NPYR contents in the three bacon types can be explained on the basis of their fat contents. Furthermore, recent studies have indicated that the nitrosating agents in bacon reside in the lipid fraction and that these lipid-derived compounds are likely responsible for the nitrosation of proline during frying (Hotchkiss *et al.*, 1986; Ross *et al.*, 1987).

N-Nitrosamine concentrations for bacon processed in the U.S. in Table 7 are comparable to those cited in the literature (Hotchkiss, 1987). As expected, there was a small increase in NPYR and NDMA contents with the higher ingoing

concentration of nitrite. NPYR concentrations in the Irish back bacon samples were also influenced by the processing concentration of nitrite, with an average of 3.0 μg/kg being determined for the greater nitrite concentration bacon. This value lies within the range cited by Crosby *et al.* (1972), Mottram *et al.* (1977) and Webb and Gough (1980). NDMA concentrations in the bacon processed in Ireland were generally less than 2 μg/kg.

In recent year, the presence of NTHZ in bacon processed in the U.S. has received considerable attention (Hotchkiss, 1987). This N-nitrosamine is found as a consequence of the smoking process and arises from the interaction of formaldehyde in the wood smoke with cysteamine/cysteine in the bacon (Skrypec *et al.*, 1985; Sen *et al.*, 1986). N-Nitrosamine data clearly demonstrates the involvement of smoke in the formation of NTHZ in bacon processed in the U.S. Bacon samples prepared with the legal concentration of ingoing nitrite (120 mg/kg) had an average NTHZ content of 4.2 μg/kg when fried. Similar values have been reported by Sen *et al.* (1986) for commercial bacon samples. Greater NTHZ concentrations (average 11.5 μg/kg) were obtained for the bacon samples processed with the greater concentration of nitrite. Only trace concentrations of NTHZ (<1 μg/kg) were detected in the unsmoked bacon processed in Ireland and likely arise from the residual smoke in the smoke house. A similar conclusion was made by Mandagere *et al.* (1987) who

detected low concentrations of N-nitrosothiazolidine carboxylic acid (NTCA) in raw bacon which had been cooked to its green weight in a smoke house without the smoke cycle.

A major focus of the present study was to evaluate the effectiveness of α-tocopherol in blocking N-nitrosamine formation in bacon processed in Ireland. This compound has previously been shown to be very effective in reducing NPYR concentrations in bacon processed in the U.S. (Gray *et al.*, 1982; Fiddler *et al.*, 1978). N-Nitrosamine data in Table 3 confirm the efficacy of α-tocopherol as an inhibitor of NPYR formation in both bacon processed in Ireland and the U.S. The inclusion of α-tocopherol in the curing brine significantly ($a<0.01$) reduced NPYR concentrations in the U.S. and Irish belly bacons processed with the two concentrations of nitrite and in back bacon processed with the greater concentration of nitrite. The percent inhibition achieved in this study was comparable to those previously reported by Gray *et al.* (1982) and Reddy *et al.* (1982). The apparent lack of inhibition of NPYR in the back bacon produced with the lesser concentration of nitrite was due to the low concentrations of NPYR in these samples. Similarly, the lesser percentage inhibition achieved for NDMA was again due to the low concentrations of NDMA in the bacon samples as compared to NPYR.

NTHZ concentration in the bacon processed in the U.S. were also significantly ($a<0.01$) reduced by the inclusion of

α-tocopherol as a curing adjunct. The effect of α-tocopherol has been previously reported by Gray *et al.* (1982). α-Tocopherol used at an ingoing target concentration of 500 mg/kg in combination with sodium ascorbate effectively reduced NTHZ formation in fried bacon by 60-70%.

Results of this study clearly demonstrate that bacon processed in Ireland using two concentrations of nitrite (120 and 200 mg/kg) contained N-nitrosamine concentrations which conformed to USDA regulations, i.e. the N-nitrosamine concentrations were less than 10 μg/kg. Such information, although somewhat preliminary in nature, should assist Irish processors in their efforts to introduce bacon products into the U.S. market. However, a more extensive survey of bacon processed in Ireland should be conducted to gain additional evidence that bacon processed in Ireland, primarily because of its leanness, contains smaller concentrations of N-nitrosamines than bacon produced in the United States from pork bellies.

Effect of Lipid Oxidation in Pork Bellies on N-Nitrosamine Formation.

Another phase of the study evaluated the effect of oxidized lipids in pork belly adipose tissue on N-nitrosamine formation in bacon. Lipid oxidation occurring in lipids of pork bellies was initiated by feeding oxidized

fat to pigs as described by Buckley *et al.* (1988).

Means and standard deviations for TBA values, residual nitrite and N-nitrosamine concentrations, as well as the recoveries of the internal standard, NAZET, are reported in Table 8. ANOVA data are reported in Tables C and D in the Appendix. The average recovery of the internal standard ranged from 82% ± 23 to 99% ± 27. N-Nitrosamine contents were not corrected for recovery. The smokehouse yield for the bellies used in this study averaged 97%.

Lipid Oxidation in Pork Bellies and Processed Bacon

TBA values are reported in Table 9 for pork bellies which had been stored at -20°C for three months before processing into bacon. TBA data clearly show that the feeding of α-tocopherol supplements to pigs for both five and ten weeks reduced the degree of lipid oxidation as compared to that found in pork bellies from pigs fed a control diet. The bellies of pigs fed a diet containing mixed tocopherols for ten weeks had lower TBA values than bellies from the control pigs; however, the level of reduction in lipid oxidation was somewhat lower than that achieved when the pigs were fed α-tocopherol-supplemented diets. The bellies from pigs fed a diet containing oxidized vegetable oil had the highest TBA values of 1.3. Thus, the feeding of antioxidants to the pigs did reduce the degree of lipid oxidation which

Table 8. Means and standard deviations of TBA, nitrite, internal standard recovery, and N-nitrosamine data for the study of the effects of lipid oxidation on N-nitrosamine formation.

Treatment	TBA[a]	Residual nitrite (mg/kg)	Recovery[b] (%)	NDMA (μg/kg)	NPYR (μg/kg)	NTHZ (μg/kg)
1	0.8 ± 0.4	20.6 ± 4.7	99 ± 27	2.3 ± 0.7	8.4 ± 2.8	3.7 ± 1.2
2	0.8 ± 0.3	25.4 ± 8.1	89 ± 26	2.1 ± 0.6	9.2 ± 4.3	4.1 ± 1.6
3	0.7 ± 0.1	22.6 ± 9.8	97 ± 36	2.5 ± 0.9	6.6 ± 2.5	3.1 ± 1.4
4	0.7 ± 0.0	13.5 ± 3.8	84 ± 24	1.8 ± 0.6	5.2 ± 2.2	3.4 ± 1.2
5	0.9 ± 0.2	12.4 ± 3.2	82 ± 23	1.7 ± 0.7	4.4 ± 1.6	3.7 ± 1.7

n = 12 for TBA and nitrite data.
n = 24 for recovery and nitrosamine data.
[a] expressed as mg malonaldehyde/kg tissue.
[b] internal standard, NAZET, was used for recovery.

Table 9. Thiobarbituric acid values (TBA) of pork bellies from pigs fed diets of varing degrees of lipid oxidation.

#	Treatment	TBA Value[a]
1	Control	0.70
2	α-Tocopherol (4 weeks)	0.24
3	α-Tocopherol (10 weeks)	0.23
4	mixed Tocopherols (10 weeks)	0.59
5	Oxidized fat	1.30

[a] Expressed as mg malonaldehyde/kg bacon.

occurred during storage. Moreover, the bellies of pigs fed an oxidized feed had the highest degree of lipid oxidation after three months of storage. α-Tocopherol has been reported by Buckley *et al.* (1988) to stabilized microsomal and mitochrondial lipids toward metmyoglobin/hydrogen peroxide-initiated peroxidation in both pork chops and restructured pork roasts prepared from meat from these pigs.

In contrast to the degree of variation in lipid oxidation in the stored pork bellies, ANOVA showed no significant differences (α=0.05) among the TBA values of the raw bacon produced from the pigs fed the various diets. A key difference between bacon and pork bellies is the addition of nitrite to bacon during the curing process. Nitrite has been reported by Asghar *et al.* (1987) to be a very effective inhibitor of lipid oxidation in pork muscle during storage. Therefore all the bacon, regardless of the feed formulation fed the respective pigs, exhibited similar degrees of lipid oxidation. The effectiveness of nitrite as an antioxidant was attributed by Asghar *et al.* (1987) to the fact that it stabilizes the heme pigment, thereby minimizing the release of iron during processing. Free heme iron is a potent catalyst of lipid oxidation in muscle foods (Pearson and Tauber, 1984). Similar results had been reported by Buckley and Connolly (1980) who found that, while α-tocopherol reduced TBA values in raw pork, it did not influence TBA values significantly in cured bacon.

N-Nitrosamines

Oxidation of lipids in pork bellies only slightly affected the formation of NPYR in the corresponding fried bacon. Fried bacon from pork bellies from pigs fed both mixed tocopherols for ten weeks and oxidized feed had significantly lower ($\alpha<0.05$) NPYR concentrations than the fried bacon produced from pork bellies from pigs fed the control diet. Fried bacon from pork bellies from pigs fed a ration containing α-tocopherol for ten weeks contained less NPYR than fried bacon from pork bellies of pigs fed control diets. However, fried bacon from pork bellies from pigs fed α-tocopherol for five weeks had NPYR concentrations greater than that of the control.

Feeding pigs oxidized feed resulted in bacon which had the lowest NPYR concentrations. Model system studies have shown that oxidation products of lipids such as malondialdehyde can promote the formation of NPYR and NDMA at pH values generally encountered in bacon (Kurechi and Kikugawa, 1979). Coleman (1978) reported that the addition of hydroperoxides in model systems containing proline/nitrite in methanol increased NPYR formation by a factor of two. TBA values have been reported to indicate the degree of lipid oxidation in fat (Gray and Pearson, 1987). However, the corresponding TBA values previously reported for the current

study were not significantly different ($\alpha<0.10$). Therefore the differences in concentrations of NPYR formed must be attributed to other factors aside from the lipid effects.

The pigs fed oxidized feed exhibited 17% suppression in growth (Buckley, unpublished data). The bellies taken from these pigs were on the average lesser in weight than those bellies taken from pigs fed the other diets. Visual inspection of the bellies from pigs fed the oxidized feed revealed a greater lean-to-fat ratio than that of the pigs fed the other diets. It has been reported by Amundson *et al.* (1982) that as the lean-to-fat ratio increased in bacon, the concentration of NDMA and NPYR formed decreased. Thus, lesser concentrations of NPYR would be expected.

Bacon produced from the pigs fed α-tocopherol did not show a significant decrease in NPYR formation during frying. This can be attributed to the low concentrations of α-tocopherol found in the adipose tissue, in the range of 10mg/kg (Buckley, unpublished data). Previous studies demonstrated that α-tocopherol could reduce N-nitrosamine formation in fried bacon when used at a concentration of 500 mg/kg α-tocopherol or greater (Gray *et al.*, 1982). Therefore, feeding α-tocopherol to pigs in the concentrations used in the current study did not result in the deposition of sufficiently high concentrations of α-tocopherol in the tissue to inhibit nitrosamine formation.

The Effect of Liquid Smoke Components on N-Nitrosamine Formation in Restructured Bacon

This study focused on the effects of selected components in liquid smokes on the formation of N-nitrosamines in restructured bacon. Restructured bacon was prepared as described in the experimental section. Treatments consisted of three different liquid smokes, three concentrations of formaldehyde, a treatment containing malonaldehyde and a control treatment to which no type of smoke or aldehyde was added. Each concentration of formaldehyde corresponded to the concentration found in one of the liquid smokes. Restructured bacon was used instead of intact pork bellies to eliminate inherent belly-to-belly variation (Theiler *et al.*, 1981). The average smokehouse yield for the restructured bacon was 84% ± 2. This yield was lower than the average yields reported for the bellies used in the other studies. Therefore, the restructured bacon product lost more moisture with time in the smokehouse than anticipated. The smaller weight of the restructured bacon, use of a casing, and direct mixing of brine into the product may have been factors responsible for this lesser yield.

Means and standard deviations for the nitrite and N-nitrosamine data are presented in Table 10. ANOVA data are presented in Table E in the Appendix. The use of the ANOVA established significant differences among treatments with

Table 10. The effect of liquid smoke and individual smoke components on the formation of N-nitrosamines in fried restructured bacon.

Treatments	Nitrite (mg/kg)	Recovery[a] (%)	NDMA (μg/kg)	NPYR (μg/kg)	NTHZ (μg/kg)
Control	71.0 ± 1.0	74 ± 13	12.0 ± 10.3	36.0 ± 18.0	2.8 ± 0.7
Malon-aldehyde	67.8 ± 2.2	84 ± 5	13.4 ± 8.4	35.4 ± 8.4	3.5 ± 0.5
LS 1	70.0 ± 2.0	83 ± 11	4.0 ± 1.0	25.6 ± 4.7	3.2 ± 0.4
LS 2	58.0 ± 18.0	88 ± 3	3.0 ± 0.4	27.3 ± 2.9	6.8 ± 1.6
LS 3	67.0 ± 1.0	81 ± 10	4.7 ± 0.3	36.8 ± 1.4	3.0 ± 0.3
F 1	59.0 ± 15.0	87 ± 8	10.0 ± 10.2	44.7 ± 2.1	6.2 ± 1.5
F 2	73.0 ± 3.0	86 ± 8	4.7 ± 0.2	34.6 ± 17.7	9.1 ± 1.8
F 3	73.0 ± 3.0	85 ± 11	9.3 ± 5.8	29.8 ± 9.9	7.8 ± 2.0

[a] n = 3

respect to nitrite and NTHZ only. The average analytical recoveries for the internal standard, NAZET, ranged from 74% ± 13 to 88% ± 3. The N-nitrosamine data are not corrected for recovery of the internal standard (NAZET).

NDMA

The concentrations of NDMA in fried restructured bacon processed with liquid smoke 1 (LS1), liquid smoke 2 (LS2), and liquid smoke 3 (LS3) were less than the NDMA concentration of the control bacon samples. The control bacon samples received no smoke. However, the concentrations of NDMA found in the liquid smoke treatments were not significantly different ($\alpha<0.05$) from the concentrations determined in the control bacon. The concentrations of NDMA found in the bacon processed with liquid smoke treatments as described were also less than the concentrations of NDMA detected in bacon spiked with formaldehyde. Previous studies have shown a reduction in NDMA concentrations when liquid smoke was used in place of traditional smoke (Ikins *et al.*,(1986).

NPYR

NPYR concentrations in fried restructured bacon with LS1 and LS2 were less than the corresponding concentrations in

fried restructured formaldehyde spiked bacon treatments, formaldehyde 1 (F1) and formaldehyde 2 (F2). The concentrations of NPYR in fried restructured bacon with LS1 and LS2 were also less than the control samples. These data suggest that the liquid smokes may inhibit the formation of NPYR. Dramatic reductions in the concentrations of NPYR in bacon cured with liquid smoke were reported by Ikins *et al.* (1986). It has been reported by Potthast and Eigner (1986) that the addition of formaldehyde at the same concentration found in liquid smoke to a meat system incorporating nitrite and pyrrolidine resulted in greater concentrations of NPYR in the fried meat product compared to those found in a fried meat product with the corresponding liquid smoke added. In the same study, formaldehyde addition was found to produce a two fold increase in NPYR formation over that found when no formaldehyde was added. Thus, the catalytic activity of formaldehyde must be inhibited by other components in the liquid smoke. It has been proposed that phenolic compounds in liquid smokes can inhibit formation of N-nitrosamines (Davies and McWeeny, 1977).

NTHZ

The concentrations of NTHZ in fried restructured bacon with F1, F2, and formaldehyde 3 (F3), were all greater than the concentrations found in restructured bacon processed with

LS1, LS2, and LS3. However, the differences between NTHZ concentrations in the restructured bacon processed with the corresponding formaldehyde concentrations were not found to be significant ($\alpha<0.10$). Visual observation of the NTHZ concentration indicates that there are large apparent differences between treatments. However, since only three replicates were analyzed for each treatment, n is very small. Therefore with such a small n value, no differences could be detected using the ANOVA or the Bonferoni's t test. The concentrations of NTHZ in the control fried restructured bacon was less than the concentrations determined in liquid smoke treatments, formaldehyde treatments, and malonaldehyde treatment. The concentration of NTHZ found in the malonaldehyde treatment apparently was not different from the concentration of NTHZ found in the control treatment. This supports earlier data discussed in this thesis which also indicated that lipid oxidation did not influence the formation of N-nitrosamines in bacon. However, model system studies have apparently shown this not to be the case (Kurechi *et al.*, 1979). All three liquid smoke treatments had lesser concentrations of NTHZ than the formaldehyde treatments. Past researchers have attributed lower concentrations of NTHZ in bacon when liquid smoke was utilized to the presence of phenolic compounds (Ikins *et al.*, 1986). NTHZ would not have been expected in the control since the source of the formaldehyde, smoke, was not present.

This indicates that carryover of smoke residues must have occurred in the smokehouse during cooking, resulting in subsequent contamination of all the restructured bacon treatments.

NTHZ formation in the fried restructured bacon was related to the concentration of formaldehyde present in the various treatments. This was expected as formaldehyde has been shown to be a precursor of NTHZ (Mandagere *et al.*, 1984).

SUMMARY AND CONCLUSIONS

Various parameters influencing N-nitrosamine formation in fried bacon were evaluated to determine their catalytic or inhibitory effect.

Four different curing adjuncts were evaluated for their inhibitory effect on the formation of NDMA, NPYR, and NTHZ in fried bacon. The adjuncts added to the curing brine were α-tocopherol, acid phosphates, rosemary oleoresin, and lactic acid-producing bacteria, also known as the Wisconsin process. The Wisconsin process proved to be the most effective method of reducing N-nitrosamines in fried bacon when comparing NPYR, NDMA, and NTHZ together.

A comparison of bacon produced from pork bellies and backs in Ireland to bacon produced from pork bellies in the U.S. was conducted. Nitrite was added at 120 mg/kg and 200 mg/kg, with and without α-tocopherol. Results indicated that the leaner bacon processed in Ireland from both pork bellies and backs, when fried, had lesser concentrations of N-nitrosamines as compared to those concentrations found in bacon processed in the U.S. For all bacon samples, the α-tocopherol-treated bacon had significantly lesser concentrations of N-nitrosamines as compared to bacon processed without α-tocopherol. However, further studies are indicated to confirm these preliminary findings.

The effect of lipid oxidation products in the adipose

tissue of pork bellies on N-nitrosamine formation in bacon was studied. Fried bacon from pigs fed an oxidized diet did not produce greater concentrations of N-nitrosamines over bacon processed from pigs fed regular diets or diets containing antioxidants. This can be related to nitrite being an excellent inhibitor of lipid oxidation in adipose tissue and to the fact that the bellies taken from the animals fed oxidized feed were leaner than bellies taken from the other treatments.

Finally, the effect of liquid smoke components on the formation of N-nitrosamines in fried bacon was studied. A model system consisting of restructured bacon was treated with three different liquid smokes and three different formaldehyde concentrations mimicing those found in the liquid smokes. A seventh treatment of malondialdehyde along with a control treatment were formulated. The liquid smokes were effective in reducing concentrations of NPYR and NTHZ over those found in restructured bacon containing corresponding concentrations of formaldehyde. It is likely then that the liquid smokes did contain components which effectively inhibited N-nitrosamine formation.

Processing variables have been shown to effect the concentrations of N-nitrosamines formed in fried bacon. Curing adjuncts and the use of liquid smokes in place of traditional smoking can effectively inhibit N-nitrosmine formation. Lipid oxidation of the adipose tissue, however,

did not effect the concentrations of N-nitrosamines formed in the fried bacon as previous model system studies have indicated. Bacon processed in Ireland contained substantially lower levels of N-nitrosamines as compared to bacon processed in the United States. In conclusion, controlling certain processing variables can effectively lessen the human exposure to N-nitrsoamines in fried bacon.

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Appendices

Table A. Analyses of variance table of nitrite and N-nitrosamine data for bacon processed with various curing adjuncts.

Source	df	Mean Squares			
		Nitrite	NDMA	NPYR	NTHZ
Total	89				
Trial	2	665.8773***	29.8527***	314.4519***	31.7975**
Treatment	4	1406.9716***	4.3255	9.7394*	10.2834
Trial x Treatment	8	190.1196	2.0256	9.5677*	5.4141
Residual	75	98.6222	1.4696	2.8887	5.4893

* $\alpha<0.05$ ** $\alpha<0.01$ *** $\alpha<0.001$

Table B. Analyses of variance of nitrite and N-nitrosamine data in various bacon samples processed with two levels of nitrite.

Source	df	Mean Squares			
		Nitrite	NDMA	NPYR	NTHZ
Total	47				
Bacon Processing	2	9390.7634***	4.7915***	188.3649***	177.5152***
Treatments	3	3807.7356***	2.5374***	62.1675***	25.2027**
BP X T	6	593.7856	.03132	19.3487***	16.4141*
Residual	36	253.9283	.2662	3.8252	5.5080

* $\alpha<0.05$ ** $\alpha<0.01$ *** $\alpha<0.001$

Table C. Analyses of variance table for TBA & nitrite data on the effect of lipid oxidation on N-nitrosamine formation.

Source	df	Mean Squares	
		TBA	Nitrite
Total	59		
Trial	1	0.011207***	205.7201
Treatment	4	0.001154	391.5635
Trial x Treatment	4	0.004809*	85.3485
Residual	50	0.001111	34.6132

*** $\alpha < 0.001$ ** $\alpha < 0.01$ * $\alpha < 0.05$

Table D. Analyses of variance table for nitrosamine data on the effect of lipid oxidation on N-nitrosamine formation.

Source	df	Mean Squares		
		NDMA	NPYR	NTHZ
Total	119			
Trial	1	0.045630	30.552521 *	2.511413
Treatment	4	3.080284	96.002653 ***	2.881841
Trial x Treatment	4	0.974726	24.219958 **	3.248872
Residual	103	0.518822	6.592299	1.91868

*** $\alpha < 0.001$ ** $\alpha < 0.01$ * $\alpha < 0.05$

Table E. Analyses of variance for nitrosamine data on the effect of liquid smoke components on N-nitrosamine formation.

Source	df	Mean Squares		
		NDMA	NPYR	NTHZ
Total	17			
Liquid smoke/ Formaldehyde	1	75.047584	187.31447	49.570924***
Treatments	2	19.949752	26.285185	17.786210***
LS vs T	2	6.840202	258.41414	2.424736
Residual	12	23.306845	74.557165	1.983577

* $\alpha<0.05$ ** $\alpha<0.01$ *** $\alpha<0.001$