FURTHER FACTORS INFLUENCING N-NITROSAMINE FORMATION IN FRIED BACON

By

Daniel J. Skrypec

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Food Science and Human Nutrition

1984

ABSTRACT

FURTHER FACTORS INFLUENCING N-NITROSAMINE FORMATION IN BACON

By

Daniel J. Skrypec

The influence of pig diet, and subsequently the fatty acid composition of bacon adipose tissue, frying atmosphere, liquid smoke preparations, selected phenols, and nitrogen oxides on the formation of N-nitrosamines in fried bacon and model systems was investigated. Bacon prepared from swine fed corn oil-supplemented diets contained significantly higher levels of N-nitrosopyrrolidine (NPYR) and N-nitrosodimethylamine (NDMA) than control bacon samples. Bacon prepared from swine fed coconut fat-supplemented diets contained significantly lower levels of NPYR. Fatty acid analyses of the adipose tissue of the bacon samples indicated that NPYR levels in bacon correlated well with the degree of unsaturation of the adipose tissue. Bacon samples, fried in atmospheres of air and nitrogen were analyzed for the presence of NDMA and NPYR. N-Nitrosamine levels in the cooking vapors, bacon, and cook-out fat of bacon fried in nitrogen were much lower than those of bacon samples fried in air.

The effect of liquid smoke preparations on N-nitrosamine formation in fried bacon was investigated by applying these smokes either to the surface of pork bellies by atomization or by incorporating them into the brine. Bacon prepared by the surface application of liquid smoke showed no significant difference in N-nitrosamine levels when compared to the control bacon samples. Bacon prepared with pickle-solubilized liquid

smokes also tended to not affect N-nitrosamine levels when compared to the control bacon. Additionally, selected phenolic compounds when added to bacon did not affect N-nitrosamine formation.

The influence of unsaturated fatty acids on the nitrosation of pyrrolidine by nitrogen oxides was investigated. Results of model system studies indicated that linoleate provided an environment more conducive to NPYR formation than did oleate. Similarly, when triglycerides, extracted from the fat of pigs fed various supplemented diets, were used in these model systems, the formation of NPYR paralleled the level of unsaturation present.

This study has shown that the adipose tissue serves as a reservoir for a number of potential nitrosating agents. The extent of nitrosation is directly related to the presence of oxygen and also to the amount of unsaturation of the component fatty acids.

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to Dr. J.I. Gray for his abundant patience, guidance and never ending sense of humor during the course of my study. The decision to become associated with Dr. Gray was truly a pivotal one in my academic career. The knowledge I received from his professional guidance is indeed immeasurable.

I would like to thank my committee members, Drs. M. Bennink, S. Aust, M. Yokoyama, and A. Pearson for their helpful suggestions.

I would also like to thank Barb Zylema, Rhonda Skidmore and Sue Cuppett for their technical assistance and Loraine Stephens for her help in the preparation of this dissertation. My appreciation is also expressed to Tom Ryan for the level of insanity he interjected into the "sane" graduate student lifestyle.

I would like to sincerely thank my parents for their love and supportive guidance through my academic career.

Most of all I would like to express my unreserved love and gratitude to Mary Kay, who through her love and the ability to bring me "down to earth" allowed me to reach heights thought to be unattainable.

TABLE OF CONTENTS

LIST OF TABLES

Table | Page

LIST OF FIGURES

INTRODUCTION

The occurrence and ubiquitous nature of N-nitroso compounds in the environment are well documented. These compounds have been identified in many food systems including cured meat products, non-fat dried milk, and dried malt and beer (Gray, 1981). They are formed principally from the reaction of secondary amino compounds with nitrite that may be intentionally added to foods or produced by bacterial reduction of nitrates (Mirvish, 1975). Data from animal studies indicate that many of these compounds are carcinogenic and in addition, many demonstrate mutagenic, embryopathic, and teratogenic properties (Druckrey et al., 1967; Magee and Barnes, 1977; Olajos, 1977). Therefore, it is highly probable that this class of compounds is hazardous to man.

The food items of major concern are cured meat products, especially bacon. N-Nitrosopyrrolidine (NPYR), and to a lesser extent, N-nitroso-dimethylamine (NDMA) have been consistently isolated from cooked bacon. These compounds have been detected in bacon itself, the cook-out fat and the vapors produced during the frying process. Subsequently, there have been many investigations into the mode of formation of these compounds in bacon (Gray, 1981).

Elucidation of the mode of N-nitrosamine formation has provided the means to develop possible ways to prevent their occurrence in bacon. The formation of NPYR during the frying of bacon occurs essentially, if not entirely, in the fat phase after the bulk of the water has been removed (Bharucha et al., 1979). It was concluded that N-nitrosamine formation in bacon occurs by a free radical rather than an ionic mechanism. Potential N-nitrosamine blocking agents should therefore possess the ability to trap NO. radicals. Ascorbyl palmitate (Sen et al., 1976), α-tocopherol (Fiddler et al., 1978) and long chain acetals of ascorbic acid (Bharucha et al., 1980) have been shown to be very effective in this respect.

The mechanisms pertaining to N-nitrosamine formation in bacon are still not fully elucidated. It has been reported that an oxygen-dependent mechanism may be responsible for most of the N-nitrosamines produced during the frying process. Dennis et al. (1982) postulated that the oxidation of nitric oxide is a key step in the reaction and that N-nitrosamine formation results from nitrosation reactions occurring during the frying process. Mottram et al. (1977) have shown that nitrosation occurs mainly in the adipose tissue and that the non-polar lipids provide an environment conducive to N-nitrosamine formation. Similarly, Goutefongea et al. (1977) suggested that nitrite can react with unsaturated carbon-carbon bonds present in adipose tissue. It appears that the level of unsaturated fatty acids present in adipose tissue may play a role in the nitrosation reactions occurring in the frying of bacon.

Oxides of nitrogen have also been implicated as possible N-nitrosating agents. For example, Hanst et al. (1977) have indicated that nitric oxide, nitrogen dioxide, dinitrogen trioxide, and dinitrogen tetroxide can under certain circumstances convert secondary amines to N-nitrosamines.

Nitrogen oxides are also present in woodsmoke produced by the combustion of hickory sawdust (National Academy of Sciences, 1981). However, there is a paucity of data regarding the role of these potential N-nitrosating agents in N-nitrosamine formation in bacon.

Similarly, the effect of phenols present in woodsmoke and liquid smoke on N-nitrosamine formation in bacon has not been fully investigated. Model system studies have indicated that suitable concentrations of selected phenols can lead to an enhancement of the rate of N-nitrosamine formation (Davies et al., 1980). It remains to be determined, however, whether such catalysis will occur during the smoking of cured meat products.

The foremost element of the present study is to investigate further several factors that may influence N-nitrosamine formation in bacon. Specific objectives of the study are:

1. to investigate the effect of the fatty acid composition of adipose tissue on N-nitrosamine formation in bacon
2. to investigate the oxygen-dependency of N-nitrosamine formation in bacon;
3. to ascertain the effect of different liquid smoke preparations on N-nitrosamine formation in bacon
4. to determine the role of phenolic compounds present in liquid smoke in N-nitrosamine formation in bacon, and
5. to investigate the nitrosating potential of oxides of nitrogen in N-nitrosamine formation in unsaturated fatty acid systems.

LITERATURE REVIEW

N-Nitroso Compounds

The presence and ubiquitous nature of carcinogenic N-nitroso compounds in the environment have generated much concern in recent years. Approximately eighty percent of the 130 N-nitroso compounds tested have been shown to be carcinogenic in experimental animals. Some of the N-nitroso compounds have also exhibited mutagenic, teratogenic, or embryopathic properties (Preussmann et al. 1976; Olajos, 1977). Therefore, it is highly probable that this class of compounds is potentially hazardous to man.

Traditionally, human exposure to N-nitroso compounds is believed to be confined to those associated with cured meat products. However, these compounds are also present in many chemical, agricultural and consumer products (Fine, 1977). N-Nitroso compounds can also be formed under the physiological conditions existing in the stomach and intestine when ingested amines and nitrite are present (Sander, 1967; Sen et al., 1969; Lijinsky et al., 1970). Exposure to these carcinogenic compounds may originate from many different sources. However, nitrite-preserved foods, especially bacon, have continued to be the center of many research activities.

The toxicological and human health implications surrounding N-nitroso compounds have been adequately documented (Scanlan, 1975, Crosby and Sawyer, 1976; Gray and Randall, 1979, Sen, 1980). N-Nitroso compounds can be divided into two groups according to their chemical reactivity: (1) N-nitrosamines, and (2) N-nitrosamides. However, in this review, the main focus will be on the formation, occurrence, and inhibition of N-nitrosamines in cured meat products.

Chemistry of N-Nitrosamine Formation

N-Nitrosamines are formed principally from the reaction between secondary amines and nitrous acid. N-Nitrosamines can be also formed, although to a lesser extent, from primary and tertiary amines, quaternary ammonium compounds and primary polyamines (Gray and Randall, 1979). Many N-nitrosamines have been identified in various food systems including cured meat products, non-fat dried milk and dried malt and beer (Gray and Randall, 1979). In addition, the formation of less volatile and non-volatile N-nitroso compounds in foods has been suggested by model system studies (Mirvish, 1971; Gray and Randall, 1979; Kakuda and Gray, 1980).

Kinetics of N-nitrosation reactions

Mirvish (1975) reported, that for nitrosation to occur, nitrite must first be converted to nitrous acid (HNO_2, pKa 3.36), indicating that the reaction is catalyzed by acid. Nitrous acid then becomes an active nitrosating species. The actual nitrosating species can be one of the following, depending on the reaction conditions: nitrous anhydride (N_2O_3), nitrous acidium ion ($H_2NO_2^+$), free nitrosonium ion (NO^+), nitrosyl halide (NOX) or nitrosyl thiocyanate (NOCNS) (Mirvish, 1970). The reactions and equilibrium equations for the nitrite ion in aqueous solution as reported by Mirvish (1970) are shown in Figure 1.

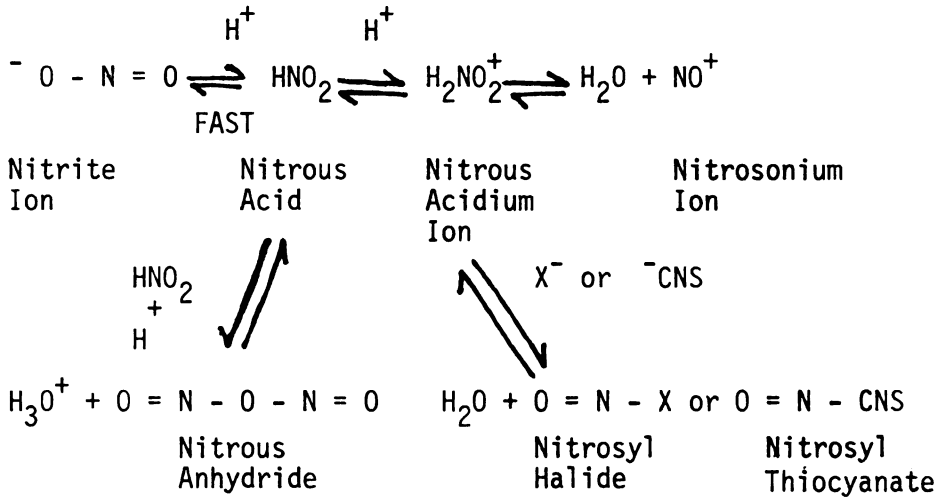


Figure 1. Reactions and equilibrium equations for the ion in aqueous solution (Mirvish, 1971).

The kinetics of N-nitrosamine formation from secondary amines and nitrous acid have been extensively studied by Mirvish (1972; 1975). The nitrosation reaction for most secondary amines is believed to proceed via the active nitrosating species, nitrous anhydride (Mirvish, 1975). He proposed that the reaction proceeds according to the overall third order rate equation as shown below:

$$\text{Rate of N-nitrosamine formation} = k\,(\text{total amine})\,(\text{nitrite})^2$$

where k is the rate constant.

The above equation illustrates that the reaction rate is proportional to the concentration of the free amine and to the square of the nitrite concentration. Mirvish (1975) reported that because it is the free amine and not the protonated amine that is nitrosated, both pH and amine basicity may affect N-nitrosamine formation. Data indicate that there is an inverse relationship between the basicity of amines and the ease of nitrosation (Sander et al., 1975). More clearly stated, the lower the basicity of a secondary amine, the easier it is to achieve nitrosation in an acid environment. Sander et al., (1975) stated that at increasing acid concentrations, a higher proportion of the nitrite is converted to the

nitrosating agent, nitrous anhydride. Salt formation by the secondary amines is also enhanced by increasing acid concentrations. Thus, there is an optimal pH value for the nitrosation of secondary amines which is approximately pH 3.

Precursors of N-Nitrosamines in Food Systems

Nitrates, nitrites and oxides of nitrogen

The major sources of nitrate and nitrite for the human are food and water. Nitrate constitutes the primary source of fixed nitrogen in green plants and occurs in high concentrations (up to 3,000 mg/kg) in vegetables such as cabbage, carrots, cauliflower, raddish, beets, spinach and others (Ashton, 1970; White, 1975; Lin and Lue, 1979; Lin and Yen, 1980). The levels of nitrate present in vegetables depends on the nitrate concentration in the soil, the genetic makeup of the vegetable, and the growing conditions (Maynard et al., 1976). Nitrate also occurs in water, especially rural well waters (Comly, 1945; Burden, 1961). Fruits and fruit juices, baked goods, cereals, milk and other dairy products also contain nitrate (White, 1975, 1976). The nitrite concentration in water and vegetables, on the other hand, is usually very low, although fairly high levels have been detected in storage-abused spinach and beets (Heisler et al., 1974). Tannenbaum et al. (1974) reported that nitrite is a normal constituent of human saliva, originating from the reduction of nitrate by microorganisms inhabiting the mouth. They reported that the level of salivary nitrate in healthy individuals is fairly constant and averages about 6-10 mg/liter.

In addition to the above sources, nitrate and nitrite can originate in foods as intentional food additives. Many countries use these chemicals for the preservation of fish, meat, cheese and other food products. These

chemicals are primarily used for their role in inhibiting the outgrowth of Clostridium botulinum spores and subsequent botulinal toxin development (Christiansen et al., 1973; Tompkin et al., 1978; Lucke and Leistner, 1979). Although nitrate itself is not active against C. botulinum, the reduction of nitrate to nitrite by microorganisms present in foods provides an inhibitory property. Aside from its antibotulinal property, nitrite serves several other functions during meat curing, including production of the characteristic cured meat color (Brooks et al., 1940), and flavor (Bailey and Swain, 1973) and providing antioxidant effects and eliminating the problem of warmed-over flavor (Bailey and Swain, 1973; Pearson et al., 1977; Fooladi et al., 1979; MacDonald et al., 1980). White (1976), Birdsall (1981), and Hartman (1982) have estimated the average daily ingestion of nitrate and nitrite for U.S. residents, and calculated the relative significance of various dietary sources. Data from these studies indicate that vegetables are the major sources (87%) of nitrate in the average American diet; the remainder originates from salivary secretion and cured meats (9.4%). The intake of nitrite, however, is provided from cured meats (39%), and vegetables (16%). White (1976) reported that saliva can account for a major portion (76.2%) of the total body burden of nitrite and comes from the bacterial reduction of nitrate.

Various studies have shown that the nitrate level in saliva can increase dramatically after consumption of nitrate-rich foods such as vegetables (Spiegelhalder et al., 1976; Tannenbaum et al., 1976). These results suggest that the ingested nitrate is converted in vivo to nitrite and then excreted in the saliva. Since the volume of daily secretion of saliva can be quite high (up to 1,000 ml), the high concentration of

nitrite (as observed after a nitrate-rich diet) in saliva can be an important source for formation of N-nitrosamines in the human stomach. According to the results obtained by Tannenbaum (1978) and Tannenbaum et al. (1978), nitrate can also be produced in the upper and lower gastrointestinal tract, thus complicating the situation further. However, Witters et al. (1979) questioned the validity of these findings and concluded that the nitrite found in the ileum, urine and feces could be due to the depletion of body stores of nitrite and nitrate and the passage of nitrate down the gut.

Nitrogen oxides have been found primarily in polluted air (in the ambient atmosphere, in indoor environments, and in the work place) and in tobacco smoke (World Health Organization, 1978; Ehrneberg et al., 1980; Erlandsson, 1981). They are generated by the chemical and microbial reduction of nitrite and nitrate salts and are common environmental pollutants produced by combustion. Four of these compounds have been implicated in the formation of N-nitroso compounds: nitrogen dioxide, dinitrogen tetroxide, dinitrogen trioxide and nitric oxide (Challis et al., 1978; National Academy of Sciences, 1981). The first three react unaided, but nitric oxide requires either oxidation to nitrogen dioxide or the presence of certain metal salts, iodine or hydrogen iodide in order to become a nitrosating agent. The formation of N-nitrosamines from oxides of nitrogen is usually faster and more extensive than from aqueous nitrous acid (Challis and Kyrtopoulos, 1979).

Inhaled nitrogen oxides could play an important role in the exposure of humans to nitrate and nitrite (Ehrenberg et al., 1980; Erlandsson, 1981). Oda (1981) reported that inhalation of nitrogen dioxide leads to the appearance of large amounts of nitrate and nitrite in the blood of

rats. Pryor and Lightsey (1981) reported that nitrogen dioxide reacts with unsaturated lipids to produce nitrite and that this could occur in vivo. Parks et al. (1981) have found that substantial amounts of nitrate and nitrite may be accumulated in the lungs under certain circumstances and that these ions may be rapidly distributed in the body as nitrate.

The average concentration of nitrogen oxides in the atmosphere is approximately 90 $\mu g/m^3$, although their concentration in the air of smog laden cities may reach 1 mg/kg or approximately 1,888 $\mu g/m^3$ (National Academy of Sciences, 1981). Estimates of the daily intake of nitrite and nitrate from this source range considerably. An atmosphere concentration of nitrogen oxides of 114 $\mu g/m^3$ in Gothenburg, Sweden was found to result in an average daily exposure of 1.2 mg nitrate and 0.9 mg nitrite (Erlandsson, 1981). Newmark and Mergens (1981) reported that the intake of nitrogen oxides can be as high as 1 mmol (average of 54 mg nitrite plus nitrate) in cities during smog formation. Cigarette smoke also contains nitrogen oxides, primarily nitric oxide. Bokhoven and Niessen (1961) reported that almost all of the nitrogen oxides inhaled in cigarette smoke are retained in the body. Estimation of the human exposure to nitrate originating from nitrogen oxides is conjectural since data on the actual conversion of nitrogen oxides to nitrate are incomplete. However, nitrogen oxides may contribute to the exposure of humans to nitrate, especially in polluted atmospheres and from tobacco smoke (National Academy of Sciences, 1981). A summary of the various figures used for the average concentration of nitrate, nitrite and nitrogen oxides are given in Table 1.

Table 1. Average concentrations of nitrate, nitrite, and nitrogen oxides used to estimate human exposure.

Sources	Nitrate	Nitrite	Nitrogen Oxides
Cured Meats	40 mg/kg	10 mg/kg	
Fresh Meats	10 mg/kg	1 mg/kg	
Vegetables	86 mg/kg	0.2 mg/kg	
Fruits	20 mg/kg	negligible	
Baked goods and cereals	12 mg/kg	2.4 mg/kg	
Milk and dairy products	0.5 mg/liter	negligible	
Water	1.3 mg/liter	negligible	
Ambient atmosphere			1 $\mu g/M^3$
Tobacco smoke			513 μg/cigarette

[a] Adapted from the National Academy of Sciences report (1981)

Amines in foods

As previously discussed, N-nitrosamines can be formed from the nitrosation of secondary, tertiary and certain primary amines and quarternary ammonium compounds. The reactivity of these compounds with nitrosating agents varies considerably, especially in their respective environmental media (e.g., air, drugs, food, etc.). Most secondary amines and N-alkylureas react readily with nitrite to produce N-nitroso compounds, whereas primary, tertiary and quaternary amines, simple N-alkylamides, and N-alkylguanidines usually react much more slowly to form N-nitroso compounds (Mirvish, 1975; National Academy of Sciences, 1981).

Amines in foods are formed by both biological and chemical pathways (Maga, 1978). These include: (a) amino acid decarboxylation, which is responsible for the formation of spermidine from methionine (Lakritz et al., 1975), putrescine from ornithine (Tabor et al., 1958), cadaverine from lysine (Tabor et al., 1958), tyramine from tyrosine (Kristoffersen, 1963), and histamine from histadine (Dierick et al., 1974); (b) trimethylamine oxide conversion such as the enzymatic conversion of trimethylamine oxide to trimethylamine (Tarr, 1940); (c) aldehyde amination as in the amination and transamination of aldehydes, which is the potential pathway for the formation of most monoamines associated with foods (Hartmann, 1967; Maier, 1970); (d) phospholipid decomposition, such as the formation of ethanolamine from the splitting of cephalin (Herdlicka and Janicek, 1964); and (e) thermal amine decomposition, which accounts for the appearance of a wide variety of amines, such as ethanolamine, methylamine, propylamine, and either iso- or pentylamine, which are formed during heating of cysteine (Mulders, 1973). Amines might also be formed in foods during the non-enzymatic browning process (Velisek and Davidek, 1974).

Fairly high levels of dimethylamine, trimethylamine and trimethylamine oxide have been detected in various fish, especially those of marine origin (Shewan, 1951; Castell et al., 1971; Golovnya, 1976). Ruiter (1973) reported that similar amines were present at the mg/kg level in mature Gouda cheese. Additionally, a wide range of simple aliphatic amines and monoamines such as tyramine, histamine, and tryptamine have been detected in cheeses (Golovnya and Zhuravleva, 1970; Voight et al., 1974; Gray et al., 1979). Kawamura et al. (1971) conducted a survey of secondary amines in commercial foods and found that modified powdered milk contains about five times as much dimethylamine as milk, while the amounts in cheese and butter were only traces.

Polyamines such as putrescine, cadaverine, spermidine and spermine are present in the germs of various cereals including barley, rice, oats, wheat, corn, and sorghum (Morruzzi and Caldavera, 1964). Spices such as black pepper, cayenne pepper and paprika were found to contain fairly high levels of cyclic amines, specifically pyrrolidine and piperidine (Marion, 1950; Gough and Goodhead, 1975). Since spices are used in the preparation of various foods throughout the world, they may contribute significantly to the total intake of amines in the human diet.

Volatile amines such as methylamine, dimethylamine, trimethylamine, ethylamine, n-propylamine, and isopropylamine have been reported in pork belly by Patterson and Mottram (1974). These authors reported that the concentrations of dimethylamine, trimethylamine, n-propylamine, and isopropylamine increased during the manufacture of bacon. Data indicate that dimethylamine and trimethylamine contribute to the formation of NDMA in cured pork (Patterson and Edwards, 1975; Gray et al., 1978). Rice et al. (1976) reported the presence of histamine, putrescine, tyramine, and

2-phenylethylamine in dry and semi-dry sausages. A number of amines including dimethylamine, di-n-propylamine, pyrrolidine, morpholine, and piperdine have been detected at levels of 2 μg/kg or less in baked hams (Singer and Lijinsky, 1976).

Various meat products have been reported to contain low levels of simple amines (Landmann and Batzer, 1966; Cantoni et al., 1969; Patterson and Mottram, 1974). Spinelli et al. (1974) reported that monoamines (histamine, tryptamine, tyramine, and ethanolamine) and polyamines (spermine, spermidine, putrescine, and cadarverine) are present in fresh pork bellies at concentrations ranging from 0.03 mg for cadaverine to 8.1 mg for spermine per 100 g of tissue. These authors reported that processing these bellies into bacon did not significantly alter the amine content. Fresh hams contain similar amines with concentrations ranging from 0.5 mg for tyramine to 180 mg for putrescine per 100 g fresh tissue (Lakritz et al., 1975). They also demonstrated that cooking resulted in a substantial decrease in amine concentration which probably was due to volatilization, while significant increases in spermine, spermidine, putrescine, and cadaverine occurred during putrefaction.

A number of free amino acids including proline, alanine, isoleucine, leucine, methionine, phenylalanine, tyrosine, valine, glutamic acid, cysteine and aspartic acid as well as hydroxyproline and sarcosine have been quantitated in pork bellies (National Academy of Sciences, 1981). The concentration of most of the amino acids increase upon storage, especially that of proline (Lakritz et al., 1976). Proline, hydroxyproline, and sarcosine can be nitrosated to form N-nitrosamines. A study by Janzowski et al. (1978) indicates that N-nitrosohydroxyproline is probably formed in foods from hydroxyproline during processing with nitrate and/or nitrite. However, there is no evidence that N-nitroso compounds formed from amino acids are carcinogenic.

Fish and meat products normally consumed in the Japanese diet have been found to contain detectable levels of N-methylguanidine (Fujinaka et al., (1976), a compound when nitrosated is highly carcinogenic. These authors speculated that the precursor of N-methylguanidine might be creatinine or creatine, both of which are abundant in meats. Mirvish and Cairnes (1981) reported that creatinine was the major precursor to N-methylurea formed in Japanese dried bonito fish. They postulated that the nitrosation-denitrosation of creatinine produced N-methylurea, possibly via N-nitrosomethylurea. High concentrations of creatine (3-6 g/kg) and its dehydration product creatinine (150-200 mg/kg) are found in fresh pork and beef (Velisek et al., 1975). The nitrosation products of the reaction of creatinine with sodium nitrite under acidic conditions was identified as creatinine -5-oxime, and N-nitrososarcosine was formed from creatine (Archer et al., 1971). Additionally, fried bacon and a dried fish product were found to contain 2-3 g/kg creatinine (Mirvish and Cairnes, 1981). Since these nitrosation reactions occurred with a very large excess of sodium nitrite, it is doubtful whether these compounds could be formed in cured meat products.

In light of the above information on the precursors of N-nitrosamines, the occurence of amines and nitrate or nitrite in our daily diet is unavoidable, even without the consumption of cured meat products.

N-Nitrosamines in Cured Meat Systems

The majority of the published results on the occurrence of N-nitrosamines in various foodstuffs have been reviewed by Scanlan (1975), Havery et al. (1978), the International Agency for Research on Cancer (1978), Preussman et al. (1979), Kawabata et al. (1979), Schmahl (1980), and Gray (1981). The food items of major importance as far as the

formation of N-nitrosamines are concerned are the cured meat products. Summaries of the occurrence of N-nitrosamines in cured meats have been published by Sen (1980) and Walters (1980). It should be pointed out that except in a few cases, the levels of N-nitrosamines detected were generally low and even these were detected only in a small percentage of the samples tested. One very important consideration is that the main contributors of N-nitrosamines in our diet are cooked bacon, nitrate or nitrite-treated smoked fish, and, certain types of salted and dried fish. The major N-nitrosamines detected in these foods are NDMA, NPYR, N-nitrosodiethylamine (NDEA), and N-nitrosopiperidine (NPIP).

Cured meats other than bacon

Many cured meat products other than bacon have been examined for the presence of volatile N-nitrosamines. Early studies indicated that fairly high levels (sometimes as high as 25,000 μg/kg) of NDMA, NPYR, and NPIP were sometimes found in corned beef, luncheon meat, smoked beef, frankfurters, pork, continental sausages and salami. The sporadic occurrence of these N-nitrosamines was unclear until Sen et al. (1973a, 1974) related this phenomenon to the use of curing premixes which contained both sodium nitrate and nitrite. These findings were later verified by other laboratories (Gough and Goodhead, 1975; Havery et al., 1976). Components of black pepper and paprika can react with nitrite to form NPIP and NPYR, respectively. Subsequently, regulations now require that the curing agents and spices be packaged separately. This procedure has resulted in marked decreases in the levels of N-nitrosamines in various cured meat products (Sen and McKinley, 1974; Gough et al., 1977).

Recently, several surveys dealing with the presence of N-nitrosamines in cured meats other than bacon have been published (Nitrite Safety Council, 1980). In general, the majority of the positive samples

contained extremely low levels of N-nitrosamines, usually less than 1 μg/kg (Sen et al., 1979; Nitrite Safety Council, 1980). Gray et al. (1981) detected the formation of N-nitrosomorpholine (NMOR) in heated chicken frankfurters prepared with various levels of nitrite. However, it has been suggested that the detectable levels of NMOR are due in part to the use of morpholine as an anti-corrosion agent in the steam supply.

Bacon systems

The cured meat item of major importance as far as the formation of N-nitrosamines is concerned is bacon. NPYR and to a lesser extent, NDMA, have been isolated consistently from cooked bacon (Table 2). Although NPYR is not detected in raw bacon, it is found invariably after cooking, the levels depending on cooking conditions and other less well defined factors (Pensabene et al., 1980). Interestingly, the cooked bacon or rendered fat contain only a portion of the total quantity of N-nitrosamine is formed. A substantial portion of these compounds volatilized in the fumes during frying. Several studies have reported a wide range of values for the percentages of N-nitrosamines found in the vapor (Table 3). In addition to the mode of cooking, the moisture content and ratio of lean to adipose tissue in the bacon samples influence the amount of N-nitrosamine in the cooking vapor.

Recent studies on the levels of NPYR in cooked bacon have suggested that much progress has been made in decreasing the formation of this N-nitrosamine (Greenberg, 1976; Sen et al., 1977; Havery et al., 1976, 1978). The trend towards lower NPYR levels in cooked bacon is partially explained by the use of reduced levels of nitrite and increased levels of the nitrosation inhibitor, sodium ascorbate, in the bacon curing mixture (Havery et al., 1978). The amounts of NPYR formed in cooked bacon is also influenced by the method of cooking, frying temperature (Gray, 1981),

Table 2. N-Nitrosamine formation (μg/kg) in fried bacon[a]

Investigators	N-Nitrosopyrrolidine		N-Nitrosodimethylamine	
	Bacon	Cook-out fat	Bacon	Cook-out fat
Crosby et al. (1972)	tr-40	---	tr	---
Sen et al. (1973b)	4-25	---	2-30	---
Fiddler et al. (1974)	2-28	6-24	---	---
Pensabene et al. (1974)	11-38	16-39	---	---
Gray et al. (1977)	tr-23	tr-41	---	---
Pensabene et al. (1979)	2-45	5-55	2-9	2-34
Sen et al. (1979)	2-22	15-34	tr-17	3-12
Pensabene et al. (1980)	2-6	11-34	---	---

[a] Adapted from Gray (1981).

Table 3. Percentages of N-nitrosamines in the cooking vapors produced during the frying of bacon[a]

Investigators	N-Nitrosamine (%)		
	NPYR	NDMA	Sample
Gough et al. (1976)	60-95	75-100	bacon
Hwang and Rosen (1976)	14-37	---	bacon
Wartheson et al. (1976)	20-40	---	pork belly[b]
Sen et al. (1976b)	28-82	28-92	bacon
Gray and Collins (1977a)	27-49	---	pork belly[b]
Mottram et al. (1977)	57-75	73-80	bacon
Gray et al. (1978)	---	56-80	pork belly[b]
Bharucha et al. (1979)	up to 31	up to 62	bacon

[a]Adapted from Gray (1981)

[b]Contained added nitrite.

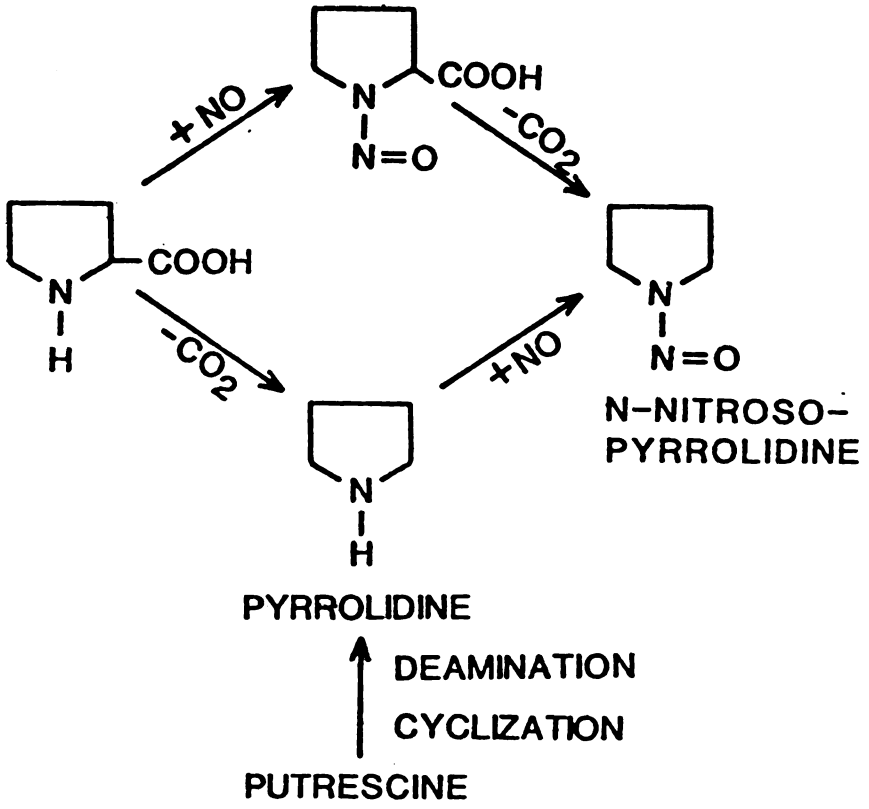
slice thickness (Theiler et al., 1981a), curing solution ingredients (Theiler et al., 1981b), belly composition (Amundson et al., 1982a) and handling (Amundson et al., 1982b).

Mechanism of NPYR formation in bacon

The consistent occurrence of NPYR in fried bacon and cook-out fat has led to an intensive search for both the precursors and mechanisms that could account for its formation. Free proline is generally regarded as the most probable precursor of NPYR in bacon (Huxell et al. 1974; Bharucha et al., 1979; Gray and Randall, 1979), although a number of other compound such as collagen, putrescine, spermidine, pyrrolidine (PYR) and glycyl-L-glycine, have been suggested (Gray, 1976). Free proline is present in pork belly at a concentration of approximately 20-80 mg/kg (Lakritz et al., 1976; Nakamura et al., 1976; Gray and Collins, 1977a; Bharucha et al., 1979). Spinelli-Gugger et al. (1981) have postulated that peptides contained in pork belly adipose tissue may be responsible for NPYR formation. In another study by Spinelli-Gugger et al. (1980), it was reported that the proline content of pork belly adipose tissue increased after processing due to the decomposition of collagen.

The mechanism by which NPYR is produced from proline is not fully elucidated but two theories have been proposed (Figure 2). One pathway involves the initial nitrosation of proline, followed by decarboxylation, while the other pathway indicates that proline is first decarboxylated to PYR followed by N-nitrosation to NPYR. Since the conversion of N-nitrosoproline to NPYR occurs at a much lower temperature than the transformation of proline to pyrrolidine, the pathway involving intermediacy of N-nitrosoproline appears to be the more likely route (Bharucha et al., 1979; Lee et al., 1983). It is also believed that preformed N-nitrosoproline in raw bacon is not the primary precursor of

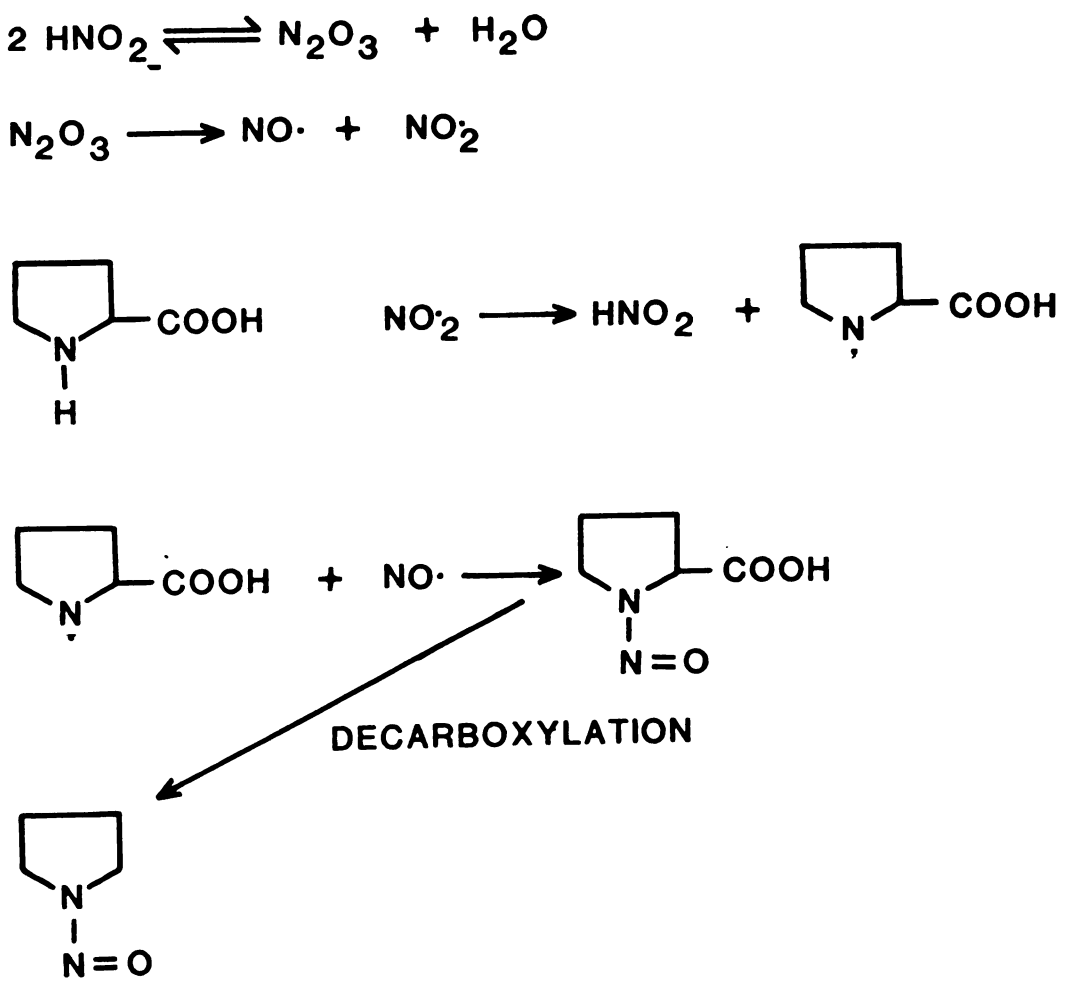
Figure 2. Possible pathways of N-nitrosopyrrolidine formation in bacon


+NO
COOH
N
N=O
-CO2
COOH
N
H
-CO2
+NO
N
N=O
N-NITROSO-
PYRROLIDINE
N
H
PYRROLIDINE
DEAMINATION
CYCLIZATION
PUTRESCINE

NPYR in cooked bacon (Sen et al., 1976c; Hansen et al., 1977; Bharucha et al., 1979) as evidenced by the inhibition of NPYR formation when ascorbyl palmitate is added to bacon (Sen et al., 1976a). However, this by no means rules out the intermediacy of N-nitrosoproline which can be formed at the higher temperatures attained during the frying process (Bharucha et al., 1979). A study by Lee et al. (1983) also indicates that the major pathway of NPYR formation in fried bacon is via the nitrosation of proline followed by the decarboxylation of N-nitrosoproline. The yield limiting step is believed to be the decarboxylation reaction.

The mechanism involved in NPYR formation has been studied in depth by Coleman (1978). He reported that the requirement of a high temperature, the inhibitory effects of water and antioxidants, and the catalytic effect of lipid hydroperoxides are consistent with the involvement of a free radical in the formation of NPYR. Similarly, Bharucha et al. (1979) suggested that since both NPYR and NDMA increase substantially towards the end of the frying process, N-nitrosamine formation during bacon frying occurs essentially, if not entirely, in the fat phase after the bulk of the water is removed, and therefore by a radical rather than an ionic mechanism. These authors postulated that, during the frying of bacon, nitrous acid is converted essentially into N_2O_3 by the continuous removal of water, and N_2O_3, in turn undergoes dissociation at higher temperatures ($>100^{o}C$) to the nitric oxide and NO_2. The NO_2 radical can then act as the chain initiator and abstract the amino proton from proline to give a radical which combines with the NO radical to give N-nitrosoproline as shown (Figure 3).

Figure 3. Free radical mechanism of N-nitrosopyrrolidine formation in bacon


2 HNO2 ⇌ N2O3 + H2O
N2O3 → NO· + NO2·
COOH
N
H
NO2· → HNO2 +
COOH
N
COOH
N
+ NO· →
COOH
N
N=O
DECARBOXYLATION
N
N=O

Mechanism of NDMA formation

While there is a great deal of recent N-nitrosamine research centered on NPYR and its precursors in bacon, there is a paucity of information dealing with the formation and precursors of other volatile N-nitrosamines (Gray and Randall, 1979). NDMA has been consistently detected in fried bacon; however, there are very few reports as to the actual precursors and mechanism of formation of this compound. Model system studies have implicated several compounds including dimethylamine and trimethylamine (Ender and Ceh, 1971; Fiddler et al., 1972; Scanlan et al., 1974), quaternary ammonium compounds (Fiddler et al., 1972), sarcosine (Ender and Ceh 1971; Eisenbrand et al., 1976), and lecithin (Mohler and Hallmayer, 1972; Pensabene et al., 1975). Gray et al. (1978) examined various compounds, all of which were endogenous to bacon, as possible precursors of NDMA in bacon. They reported that the choline-containing compounds and sarcosine produced measurable quantities of NDMA under conditions normally encountered in the pan frying of bacon. Patterson and Mottram (1974) reported that the levels of dimethylamine in pork loin eye muscle increases during the curing process. Values of dimethylamine below 200 μg/kg were found before curing, while up to 520 μg/kg of dimethylamine were found in vacuum stored bacon. Studies on the sarcosine content of pork belly are lacking.

Factors Influencing NPYR Formation

The principal factors which influence the formation of NPYR in cooked bacon have been well documented (Gray 1976; Gray and Randall, 1979; Sen 1980). These include the method of cooking, frying temperature and time, nitrite concentration, ascorbate concentrations, preprocessing procedures, presence of lipophilic inhibitors, and possibly smoking.

Cooking methods

It has been firmly established that pan frying results in greater NPYR formation than other cooking procedures such as microwave cooking (Herring, 1973; Pensabene et al., 1974) and grilling (Bharucha et al., 1979). The reduced levels of N-nitrosamines found in grilled bacon is thought to be due to the cook-out fat being somewhat removed from the heating area. Consequently, the bacon slices never reach the higher temperatures that can occur during pan frying. The level of NPYR in cooked bacon is clearly influenced by both the frying temperature and frying time. Pensabene et al. (1974) showed that bacon samples from one belly formed no NPYR when fried for 105 minutes at 99°C, while samples taken from the same belly when fried to the same degree of "doneness" at 204°C for 4 minutes contained 17 μg/kg of NPYR. Bharucha et al. (1979) reported that the maximum amount of N-nitrosamine was produced when the bacon was fried for 12 minutes at 360°C, after starting with a cold frying pan. Minimal levels of N-nitrosamines were found in the cook-out fat after 4 minutes of bacon frying; however, the N-nitrosamine level increased sharply with time and reached a maximum at approximately 12 minutes and then began to decline. In regards to the initial low formation of N-nitrosamines, two explanations were given: (a) the N-nitrosamines were actually formed at about 100°C, but being steam volatile, were removed with the water vapor; or (b) the nitrosation reaction occurred at temperatures greater than 100°C, after the major portion of the water was removed.

Nitrite concentration

The kinetics of N-nitrosamine formation *in vitro* has been studied extensively (Mirvish, 1970; Mirvish, 1975). In moderately acidic media, the reaction rate is directly proportional to the concentration of the free amine (non-protonated) and to the square of the concentration of the undissociated nitrous acid. Consequently, it is not surprising that the amount of nitrite permitted in bacon has received considerable attention. Sen et al. (1974) suggested that the initial nitrite concentration has a greater influence on N-nitrosamine formation in bacon than the residual nitrite concentration. However, recent evidence indicates that the lowest residual nitrite gives the least probability of N-nitrosamines being formed (Dudley, 1979; Sebranek, 1979). Therefore, it has been recommended that the in-going nitrite levels for bacon be reduced from 156 to 120 mg/kg, with the simultaneous inclusion of 550 mg/kg of sodium ascorbate (Federal Register, 1975). Similarly, in Canada, the amount of nitrite to be used in the preparation of side bacon has been reduced from 200 to 150 mg/kg, calculated before any smoking or cooking (Gray, 1976).

In a recent study by Robach et al. (1980), the effects of various concentrations of sodium nitrite and potassium sorbate on N-nitrosamine formation in bacon was investigated. Bacon, processed with 40 mg/kg of nitrite and 0.26% sorbate contained an average of 8.7 μg/kg of NPYR, whereas samples prepared with 120 mg/kg of nitrite contained an average of 28.1 μg/kg of NPYR. Undoubtedly, the marked reduction in NPYR levels is due to the reduced levels of nitrite. However, Tanaka et al. (1978) reported that sorbic acid possesses anti-N-nitrosamine activity. Further studies by Amundson et al. (1982a) also revealed that N-nitrosamine formation was suppressed, though not eliminated, by the same nitrite-sorbate cure in both lean and fat bellies.

Preprocessing

Storage of pork bellies also has a definite effect on NPYR formation in fried bacon (Pensabene et al., 1980). Bacon, made from fresh bellies produced significantly less NPYR than that made from bellies that had been either stored for 1 week in a refrigerator or frozen for 3 months and then thawed before using. It was suggested that the higher levels of NPYR results from the increase in both amines and amino acids that occurs during extended storage (Pensabene et al., 1980; Amundson et al., 1982b). Several studies have shown that the free proline content in whole and lean tissue of green pork bellies increased approximately 50% after storage at $2^{o}C$ for 1 week (Lakritz et al., 1976; Gray and Collins, 1977b). Additionally, the free proline content in the adipose tissue increased approximately 90% over the same period.

Smoking

The effects of smoking on the formation of N-nitrosamines in bacon have been investigated by Bharucha et al. (1980). They reported that unsmoked bacon samples generally tended to contain greater N-nitrosamine levels, presumably due to their higher nitrite content at the time of frying. Sink and Hsu (1977) cited a reduction in residual nitrite in frankfurters that were dipped in liquid smoke, which was presumed to be due to a lowering of the pH. The effect of smoke appears to be a combination of pH decrease and direct C-nitrosation of phenolic compounds to lower the residual nitrite in the product (Knowles, 1974). A more detailed review is included later in the text.

N-Nitrosamine Blocking Agents or Inhibitors

The formation of N-nitroso compounds can be reduced or even completely prevented by the presence of blocking agents when the potential for

nitrosation exists. A blocking agent is essentially a substance capable of rapidly reducing nitrous acid to the non-nitrosating nitric oxide or oxides of nitrogen of lower oxidation state (Mergens and Newmark, 1980). Thus, any compound that could compete successfully with the secondary amine for the available nitrosating agent would reduce the possibility of N-nitrosamine formation (Gray and Dugan, 1975).

Ascorbic acid was first reported by Mirvish et al. (1972b) to be an effective inhibitor of the nitrosation reaction in aqueous systems. Since then, many compounds have been investigated as potential blocking agents. These have been adequately reviewed by Douglas et al. (1978) and include phenolic compounds (phenol, gallic acid, propyl gallate, tannic acid, α-tocopherol, butylated hydroxyanisole, butylated hydroxytoluene, tertiary butylhydroxyquinone, ethoxyquinone), sulfur compounds (bisulfite, sulfamate, cysteine, glutathinone, methionine), urea, as well as ascorbic acid and its derivatives. The efficiency of these compounds to act as nitrosating blocking agents is somewhat dependent on the nature of the reaction medium. The most widely studied of these compounds have been ascorbic acid and α-tocopherol.

Ascorbic acid has been shown to be particularly effective against nitrosation, particularly in weakly acidic conditions (Mirvish et al., 1972b). Nitrite, or more correctly dinitrogen trioxide (N_2O_3), the nitrosating species, is reduced to the non-nitrosating nitric oxide (NO), while ascorbic acid is oxidized to dehydroascorbic acid. The ascorbate anion also reacts with N_2O_3 thus, secondary amines and ascorbic acid compete directly for the nitrosating species (National Academy of Sciences, 1982). According to Dahn et al. (1960), the ascorbate anion reacts with N_2O_3 approximately 230 times faster than ascorbic acid, presumably as a result of its greater nucleophilic activity. The anion

predominates at a pH of 3-5 and its reaction with nitrite is so rapid that the formation of N_2O_3 is rate limiting. The properties and utility of ascorbic acid as an inhibitor of the nitrosating reaction have been adequately described in the literature (Kamm et al., 1973, 1975, 1977; Gray and Dugan, 1975; Archer et al., 1975; Mirvish, 1977, 1981; Mergens et al., 1978; Newmark and Mergens, 1981).

The effectiveness of ascorbate (or its isomeric form, erythorbate) in blocking nitrosation reactions has three major determinants. First, the binding between nitrite and ascorbate, which elicits the inhibitory response, is pH sensitive (Fan and Tannenbaum, 1973). Ascorbate is much more effective at a pH of 3-5 since the ascorbate anion (pKa 4.29) which predominates is nitrosated more rapidly. Secondly, the rate at which a given amine is nitrosated will determine the inhibitory efficacy of ascorbate. The faster the amine is nitrosated, the less effective ascorbate would be at blocking the reaction because both the amine and the reductant would compete for the nitrosating agent. Thirdly, since ascorbate is water-soluble, it has no effect on the nitrosation reactions in the adipose tissue, which is the major site of N-nitrosamine formation in bacon (Mottram et al., 1975; Mottram and Patterson, 1977).

Ascorbic acid is not an effective blocking agent for nitrosation reactions occuring in a two-phase model systems composed of aqueous and non-polar components (Mottram and Patterson, 1977). Bacon may represent such a two-phase system, in which enclosed fat globules interface with cytoplasmic protein layers and extracellular space (Cassens et al., 1979). Mottram and Patterson (1977) reported increased yields of N-nitrosamines in a two-phase model system containing sodium ascorbate. These authors postulated that this resulted from rapid nitrosation in the

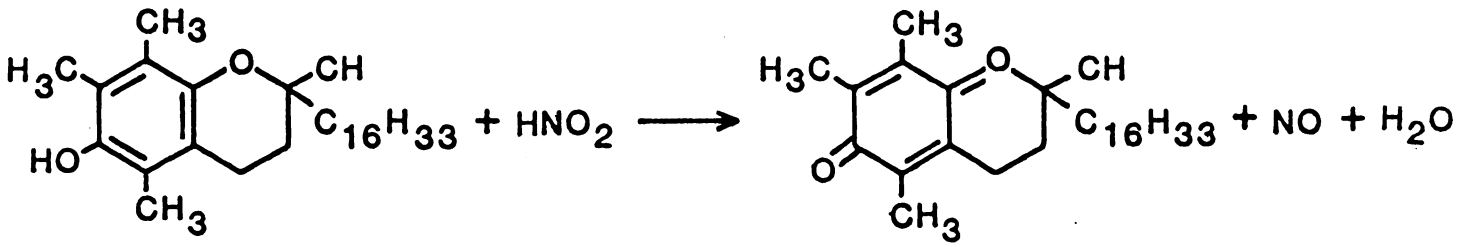
non-polar phase by oxides of nitrogen derived from the reduction of nitrite by ascorbate in the aqueous phase.

The complex nature of food composition and its effect upon N-nitrosamine formation have been reviewed by Mergens and Newmark (1980). Lipids, when present, can act simultaneously as a ready solvent for the unprotonated free base substrate and nitrous anhydride (N_2O_3), resulting in a extremely rapid nitrosation reaction. Removal of the water phase of a food, as in the frying of bacon, typifies this phenomenon. Residual nitrite present in the aqueous phase at the time of frying is dehydrated to give nitrous anhydride and driven into the fat layers where the actual nitrosation occurs. Consequently, lipid soluble compounds, particularly α-tocopherol, have been shown to be very effective in inhibiting the nitrosation reaction (Pensabene et al., 1978).

The structure of α-tocopherol permits it to be particularly effective in inhibiting N-nitrosamine formation. α-Tocopherol functions as an inhibitor through its ability to reduce nitrite to nitric oxide. During this reaction, the tocopherol is oxidized to a quinone (Figure 4). Since α-tocopherol does not have unsubstituted carbon atoms in the phenolic ring, it cannot form C-nitroso derivatives that might promote transnitrosation. However, β ,α , and δ-tocopherols have unsubstituted positions in the phenol ring and are not as effective as α-tocopherol in inhibiting N-nitrosamine formation, perhaps because they produce nitrosating C-nitroso compounds (Mirvish, 1981; Newmark and Mergens, 1981).

The majority of studies on blocking the nitrosation reaction have centered on the use of ascorbic acid or its derivatives and α-tocopherol; however several other compounds have been investigated. Polyphenolic compounds such as gallic acid as well as simple phenols can function as

Figure 4. Reaction of nitrite with α-tocopherol



α-TOCOPHEROL

α- TOCOQUINONE

blocking agents under certain conditions. The mechanism is based on the fact that phenols can consume the nitrite either by formation of C-nitrosophenols, or in the case of polyphenols, by nitrite reduction to nitric oxide coupled with oxidation of the phenols to quinones (Challis, 1973; Challis and Bartlett, 1975; Mirvish, 1981). Pignatelli et al. (1980) have reported that 1,2 - and 1,4 - dihydroxyphenols (including naturally occurring flavonols) inhibit N-nitrosamine formation at pH 4.0. However, 1,3-dihydroxyphenols, such as resorcinol, are powerful catalysts under similar conditions (Pignatelli et al., 1980).

Certain sulfur compounds can also function as N-nitrosamine blocking agents. Bisulfite reduces nitrite in two steps (Histatune, 1961), first to nitric oxide and then to nitrous oxide. Sulfamate reduces nitrite to molecular nitrogen (Jones, 1973). The thiols, cysteine and glutathione also inhibit N-nitrosamine formation (Sen and Donaldson, 1974; Gray and Dugan, 1975). Thiols react with nitrite to form S-nitroso compounds. However, these compounds can act as nitrosating agents in the absence of nitrite (Davies et al., 1978).

The Influence of Adipose Tissue Composition on N-Nitrosamine Formation in Bacon

Recently, a considerable amount of work has been performed to elucidate the mechanism(s) involved in the formation of N-nitrosamines within the adipose tissue of fried bacon. Mottram et al. (1977) have shown that nitrosation occurs mainly in the adipose tissue and have postulated that the non-polar lipid provides an environment conducive to N-nitrosamine formation. Similarly, Walters et al. (1979) reported that the obvious nitrosating species and amine precursors to NPYR are available within the fat portion of unfried bacon. Goutefongea et al. (1977)

studied the interaction of nitrite with adipose tissue and suggested that nitrite reacts with unsaturated carbon-carbon bonds. Walters et al. (1979) also reported that unsaturated lipids can react with nitric oxide in a similar manner to simpler olefins such as cyclohexenes. They demonstrated that pseudonitrosites of unsaturated triglycerides transnitrosate to secondary amines and suggested that similar derivatives of unsaturated lipids may be involved in N-nitrosamine formation in adipose tissues. Further studies are needed to confirm the formation and intermediacy of pseudonitrosites originating from unsaturated fatty acids in N-nitrosamine formation in bacon.

The Effect of Oxygen in the Frying Atmosphere on N-Nitrosamine Formation in Bacon

One of the major problems in determining the mechanism of N-nitrosamine formation in bacon is the large number of potential nitrosating species present. Frouin (1976) has suggested that much of the nitrite added to cured meats is rapidly converted to various forms of bound nitric oxide. The formation of N-nitrosamines from bound nitric oxide may in principle occur either by direct transnitrosation to the secondary amines or by an indirect process involving the initial release of the nitric oxide moiety (Dennis et al. 1982). Free nitric oxide itself is known to be a very poor nitrosating agent (Challis and Kyrtopoulos, 1977); however in the presence of oxygen, it is rapidly oxidized to higher oxides of nitrogen which are powerful nitrosating species. Dennis et al., (1982) have shown that when bacon is fried in an inert atmosphere, N-nitrosamine formation is markedly reduced. These authors suggested that this oxygen-dependent activity is consistent with a mechanism in which the oxidation of nitric oxide is a key step in N-nitrosamine formation in bacon.

Role of Smoking Procedures in N-Nitrosamine Formation in Bacon

Woodsmoking is one of the oldest methods of food preservation; however little is known about the chemical interactions involved in this process. A majority of the information pertains to the composition of the smoke and the components that affect the texture and flavor of the smoked products (Gilbert and Knowles, 1975; Clifford et al. 1980). Woodsmoke consists of two phases - a disperse, liquid phase containing smoke particles and a dispersing gaseous phase or vapor (Foster and Simpson, 1961). The direct deposition of smoke particles on the food is believed to be negligible compared to the absorption of vapors by surface and interstitial water.

Smoking involves the combustion of organic material, so it is highly probable that nitrogen oxides produced from this combustion are absorbed by the food being smoked (National Academy of Sciences, 1981). These nitrogen oxides can act as nitrosating agents and may subsequently form N-nitrosamines (Challis et al., 1978; Challis and Kyrtopoulos, 1978, 1979). It has been established that smoking produces up to a 55% reduction in the basic amino acid content, especially lysine, and that this could be possibly due to deamination by nitrogen oxides (Clifford et al., 1980). In any event, the nitrogen oxides will undergo hydrolysis by surface and interstitial water, and nitrite and nitrate ions will be deposited in the food (National Academy of Sciences, 1981).

The relationship between nitrogen oxides and N-nitrosamine formation was further substantiated by Pryor and Lightsey (1981). They reported that nitrogen dioxide will react with unsaturated fatty acids to produce nitrous acid via allylic hydrogen abstraction. The nitrous acid can then react with secondary amines to produce N-nitrosamines. These authors also reported that the rate of nitrosation of the amine increases directly with

the number of double bonds present in the reaction medium. These results are indicative that nitrous acid formed by the NO_2-unsaturated ester reaction is participating in the nitrosation of the amine. The formation of nitrous acid by NO_2-alkene reactions adds credence to the postulate that the unsaturated fatty acids along with nitrogen oxides are involved in N-nitrosamine formation in bacon. Further studies are needed to elucidate the mechanisms surrounding the nitrogen oxide interaction with unsaturated fatty acids and the role they play in N-nitrosamine formation in bacon.

Traditionally, cured bellies used in the production of bacon are smoked by exposing them to direct smoke in a smokehouse. However, direct smoking of meat has a number of disadvantages which include the amount of time spent in the smokehouse and the deposition of carcinogenic polycyclic aromatic hydrocarbons on the surface of the bacon. The meat industry has seen the development of a variety of liquid smoke preparations which are in wide use (Gorbatov et al., 1971). These liquid smoke preparations contain virtually no carcinogenic compounds and they allow the time involved in bacon production to be reduced.

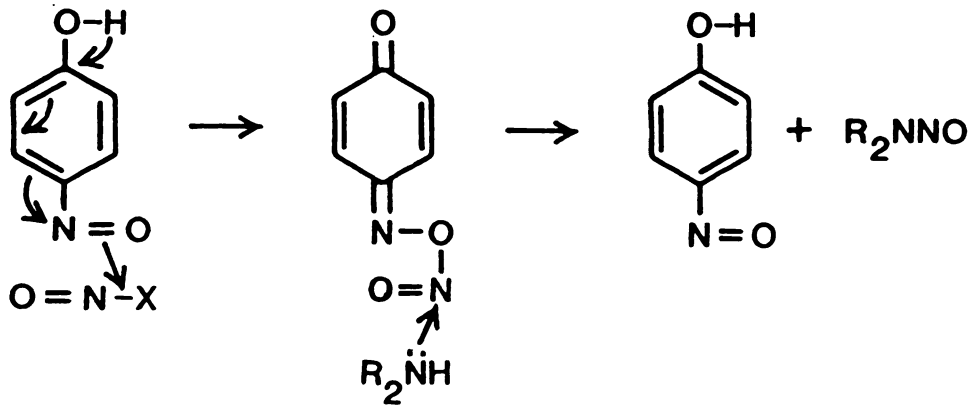
The composition of several liquid smoke preparations has been extensively investigated and it is reported that they contain three basic classes of components: acids, phenols, and carbonyl compounds, all of which contribute to the flavor and color characteristics of smoked products (Sleeth et al., 1982). The phenols, which are acidic in nature, and the acids account for the low pH of liquid smoke, which is generally on the order of 2 to 3. Furthermore, phenols contribute greatly to the smokey flavor imparted by liquid smoke (Wasserman, 1966). The carbonyl compounds, which are more neutral, constitute a major portion of the

color-forming components (Hollenbeck et al., 1973). These compounds give the meat surface a desirable brownish smoke color when the liquid smoke is applied by spraying or atomization.

The phenolic compounds present in liquid smoke react rapidly with nitrosating agents (Challis and Lawson, 1971), and should therefore inhibit the formation of N-nitroso compounds (Challis, 1973). Inhibition by some of the phenolic constituents has been reported by Pignatelli et al. (1980), but the effect might be partly counteracted by the ability of C-nitrosophenols to catalyze the formation of the N-nitrosamines (Davies and McWeeny, 1977; Walker et al., 1979).

Phenol, in the presence of nitrous acid, is rapidly transformed in p-nitrosophenol (Challis, 1973). Walker et al. (1979) reported the catalytic effect of this compound on the formation of NDEA from nitrous acid and diethylamine. Similarly, Davies et al. (1980) found that p-nitroso-N-dialkylanilines catalyze the nitrosation of pyrrolidine and morpholine. Upon examination of the structures of those compounds which enhance nitrosation they found that all are capable of tautomerism to quinone monoximes or quinonemonoxime imines. The nitrosating ability of these compounds, therefore, correlates well with the ability to undergo this tautomeric change. Davies et al. (1980) reported that, although two mechanisms for this catalysis may be possible, the general scheme involves an initial reversible reaction between the nitrosophenol and nitrous acid (or species NOX in equilibrium with nitrous acid) to form an intermediate nitrosating agent. A slower reaction follows in which the amines attacks the intermediate, resulting in the formation of the N-nitrosamine and the regeneration of the nitrosophenol. The most plausible mechanism for this reaction is shown in Figure 5.

Figure 5. Possible mechanism of nitrosophenol catalysis of N-nitrosamine formation


O–H
N = O
O = N – X
O
N – O
O = N
R2NH
O–H
N = O
+ R2NNO

Knowles et al. (1975) reported that both nitroso- and nitrophenols are found in bacon. These compounds are present in traditionally smoked bacon and liquid smoke treated bacon. These authors concluded that nitrite interacts with a wide variety of smoke phenols during the production and frying of bacon. Sleeth et al. (1982) found that when liquid smoke was pumped into bellies via the pickle, an inhibition of N-nitrosamine formation occurred.

These authors reported that an internal application of the liquid smoke is required to achieve the derived reduction of N-nitrosamines. However, liquid smoke contains significant levels of phenols which may enhance the rate at which organic amines are nitrosated (Davies and McWeeny, 1977). The use of liquid smoke in bacon processing and its ability to enhance or inhibit N-nitrosamine formation lends itself to further investigations.

Toxicology of N-Nitroso Compounds

N-Nitrosamines and, in general most N-nitroso compounds constitute the most extensive series of known chemical carcinogens. Most N-nitrosamines can initiate tumors in at least one animal species, and all animal species which have so far been tested are susceptible to nitrosamine carcinogenesis (Wishnok, 1979). NDMA has been shown to be carcinogenic in six species and NDEA in about twenty animal species including some subhuman primates (Magee et al., 1976; Sen, 1980). The most sensitive animal to the toxic and carcinogenic actions of N-nitrosamines appears to be the mink (Koppang and Rimeslatten, 1976). NDMA, the simplest and the most widely occurring N-nitrosamine in foods, causes mostly liver tumors and occasionally kidney tumors, wherease methylbenzylnitrosamine and N-nitroso-n-butyl-(4-hydroxy-butyl) amine causes cancer of the esophagus and bladder, respectively (Druckrey et al.,

1969; Magee et al., 1976). NPYR, a cyclic N-nitrosamine which occurs most commonly in bacon, was most recently studied for its toxicity by Hecker et al., (1979). Their investigation confirmed the earlier studies (Preussmann et al., 1976; Cottrell et al., 1979) showing that NPYR produces hepatocellular tumors in rats.

N-Nitrosamines are considered to be indirect acting carcinogens and therefore require metabolic activation to exert their carcinogenic effect (Lijinsky, 1977). N-Nitrosodialkylamines, such as NDMA and NDEA, are highly potent carcinogens, whereas N-nitrosamines with branching and consequently fewer hydrogens at the α-carbon (e.g. N-nitrosdiethanolamine, N-nitroso-sarcosine) generally have lower carcinogenic potency. The α-position of N-nitrosamines has been shown to be associated with the carcinogenic action of these compounds (Wishnok, 1979). The ultimate toxic and carcinogenic species resulting from the metabolism of N-nitrosamines are electrophiles, which are capable of covalently reacting with cellular macromolecules including DNA, RNA, and proteins (Pegg, 1977). Although tumor induction occurs only in organs in which alkylation takes place (Magee et al., 1976), the nature of the alkylating molecule is not known for any given N-nitrosamine.

Carcinogenic N-nitrosamines are also generally mutagenic, although the correlation between carcinogenicity and mutagenicity is not perfect. N-Nitrosamines require microsomal or host-mediated metabolism to become mutagenic, whereas the carcinogenic N-nitrosamides do not require metabolic activation for mutagenesis and are therefore direct-acting mutagens.

EXPERIMENTAL

Safety Precautions

Important safety note: Caution should be exercised in the handling of N-nitrosamines since they are potential carcinogens. Direct contact with these chemicals should be avoided. Safety gloves should be worn when N-nitrosamines are being handled. All experimental work should be done under a hood or well-ventilated area.

The Experimental Section will be divided into five distinct phases, each corresponding to the individual objectives of the study.

I. Effect of Pig Diet on N-Nitrosamine Formation in Bacon

Preparation of the diet

Diets consisting of 15% added corn oil, coconut fat, or tallow were prepared by adding the respective fat to a basic (control) ration (Table 4). The basic diet was a commercial type corn soybean meal-based diet supplemented with vitamins and minerals to meet nutritional requirements. The four different diets were transferred separately to a stainless steel Wenger horizontal mixer (Wenger Mixing Mnfg. Co., Sabetha, KS) and thoroughly mixed with vitamins and mineral supplements. The four lots of feed each weighed approximately 700 kg. The feed was packaged in 22 kg plastic-lined bags and stored at room temperature, which was cold.

Table 4. Composition of supplemented diets.

Ingredients (lbs)	Control	Tallow	Corn Oil	Coconut Fat
Ground shelled maize	1300.0	1069	1069	1069.0
Soybean meal	193.13	193.13	193.13	193.13
Ground limestone	15.45	15.45	15.45	15.45
Mono-dicalium phosphate	17.0	17.0	17.0	17.0
M.S.U. vitamin - trace mineral mix	7.73	7.73	7.73	7.73
Salt	3.91	3.91	3.91	3.91
Selenium pre-mix[a]	0.78	0.78	0.78	0.78
Fat source	---	231	231	231
Total	1538	1538	1538	1538

[a]Contains 200 mg of Se/kg

Feeding trial

Twenty pigs weighing 62-81 kg were allotted at random into four groups of five and fed the previously described diets for 5 weeks. The pigs were a mixed breed, predominently sired by Duroc boars from a sow herd which was Yorkshire Chester White-Landrace based. The pigs were housed in pens with cement floors in a heated barn. Each pen was equipped with a self-feeder and a nipple-type waterer. After the first week, the self-feeders were replaced with trough feeders due to the solidification of fat in the diets in colder weather. Feed and water were offered ad libitum. The pigs were weighed initially and at the end of the feeding trial. The pigs consumed an average of 2.17 to 3.38 kg/day and on average gained 0.75 to 0.89 kg/day.

Preparation of bacon from pork bellies

The pigs were taken to the MSU Meat Laboratory at approximately 6 p.m. on the day preceding slaughter. Feed was withheld from the animals until approximately 7 a.m. the following day when they were slaughtered. After slaughtering, the tissues were examined for possible gross lesions by a United States Department of Agriculture Meat Inspector. Bellies from the right side of the carcass and fat samples from six different anatomical locations were collected for subsequent future analyses.

Twenty pork bellies (approximately 8-11 lbs.) were obtained from the pigs, which represented 5 bellies per treatment. After 5 days in a $2^{o}C$ cooler, the bellies were stitch-pumped to 111% of their green weight with a brine containing 15% sodium chloride, 5.0% sucrose, 3.5% sodium tripolyphosphate, sodium nitrite (1,200 mg/kg) and sodium ascorbate (5,500 mg/kg). The bellies were equilibrated for 48 hours at $2^{o}C$ and smoked, tempered, sliced and packaged as described by Reddy et al. (1982). The

bacon manufactured from these pigs was stored for 2 and 4 weeks at refrigerated conditions (4°C). The bacon was analyzed for N-nitrosamines, residual nitrite, salt, and adipose tissue fatty acid composition. Iodine values were also determined for the fat in the bacon adipose tissue.

Bacon frying conditions

The electric skillet (Sunbeam Corp., Chicago, IL.) was turned on at least 10 minutes before frying. The skillet thermostat was set to give a temperature of approximately 171°C. The temperature of the skillet was checked by placing cooking oil (250 ml) in the skillet and checking the temperature of the oil with a thermometer. High and low temperatures were noted and the setting of the thermostat was adjusted to produce a minimum temperature of 171°C.

Four to five strips of bacon were placed into the skillet making sure overlapping did not occur. The bacon was fried on each side for 3 minutes, removed and allowed to drain on paper towels. The cook-out fat was collected and the excess fat in the skillet removed by thoroughly wiping with paper towels which were discarded. This was repeated if the same treatment of bacon was to be cooked. If a different treatment of bacon was to be fried, the skillet was cleaned and the calibration steps repeated. The fried bacon was tightly wrapped in aluminum foil and was stored overnight in a freezer at approximately -20°C. The cook-out fat samples were stored in covered beakers at -20°C for further analysis.

Extraction of N-nitrosamines from fried bacon

The frozen, fried bacon was ground in a Waring blender with crushed dry ice until a fine, homogeneous mixture was obtained. N-Nitrosamine levels were determined using the gas chromatography-thermal energy analyzer (GC-TEA) method as outlined by Reddy et al. (1982). This method

employs the addition of 1 g of ammonium sulfamate to eliminate possible artifactual formation of N-nitrosamines during the distillation procedure.

Extraction of N-nitrosamines from bacon cook-out fat

N-Nitrosamines in the cook-out fat were determined by the method of Owens and Kinast (1980), except that 1 g of ammonium sulfamate was added to the distillation flask prior to distillation.

GC-TEA analysis of N-nitrosamines

Quantitative determination of NPYR and NDMA was carried out using a GC-TEA system comprised of a Varian 3700 gas chromatograph coupled to a TEA model 502 LC (Thermal Electron Corp., Waltham, MA.) via a 1/8" glass-lined stainless steel transfer line. The GC column was 6' x 1/8" i.d. stainless steel column packed with 10% Carbowax 20M + 5% KOH on 80/100 mesh Chromosorb W (Supelco Inc., Bellefonte, PA). The operating conditions for the system included: temperature programming, 140-180^{o}C at 15^{o}C/min; carrier gas (nitrogen) flow rate, 30 ml/min; TEA pyrolyzer temperature, 475^{o}C; oxygen flow rate, 10 ml/min; TEA attenuation, 256; and cold trap temperature, -196^{o}C.

The raw data were collected and processed by a Hewlett Packard Model 3390A reporting integrator. The response of the GC-TEA system was established by injecting standard N-nitrosamines (Aldrich Chemical Co., Milwaukee, WI.) and calibrating the area to the known concentration. Concentrations of the N-nitrosamines were calculated from the following equation:

$$\mu g/kg = \frac{1000 \times V \times A \times C}{S \times W}$$

where V = final volume of extract after concentration (ml); A = sample TEA detector response of peak area; C = standard concentration (μg/ml); S = standard TEA detector response of peak area; W = weight of bacon sample analyzed (g).

Recovery studies were performed by spiking known amounts of NPYR and NDMA into the distillation flask containing 25 g of fried pork belly prior to distillation. Previous analyses indicated that fried pork belly did not contain detectable amounts of the N-nitrosamines. Average recoveries for the spiked samples were 77 and 82% for NPYR and NDMA, respectively. Similar recoveries of NPYR and NDMA were obtained from the pork belly drippings.

Nitrite analysis

Nitrite determinations were performed according to the standard AOAC procedure (1975), with the exception that N-1-naphthylethylene diamine was used instead of the reportedly carcinogenic α-naphthylamine (Usher and Telling, 1975).

Fatty acid composition of adipose tissue

The fatty acid composition of the triglycerides of the adipose tissue taken from the pigs fed the various diets was determined by gas chromatographic analysis of their fatty acid methyl esters. Transestimfication of the fatty acids was achieved by the procedure of Morrison and Smith (1964) using BF_3-methanol as the methylating agent.

A Hewlett Packard gas chromatograph (Model 5840A) equipped with a flame ionization detector and Hewlett Packard 18850A GC integrator was used for the analysis of the fatty acid methyl esters. The glass GC column (2m x 2mm i.d.) was packed with 15% diethyleneglycol succinate (DEGS) on Chromosorb W 60/80 mesh (Supelco Inc., Bellefonte, PA.).

Operating conditions for the system were: GC carrier gas and flow rate, nitrogen at 30 ml/min; GC injection port temperature 210°C; GC column temperature 190°C isothermal; FID temperature, 350°C; attenuation, 8; and chart speed, 1 cm/min. Identification and quantification of the fatty acids in the samples was determined by preparing standard fatty acid methyl esters under identical conditions.

Iodine values

Iodine values were determined on the bacon adipose tissue by the A.O.C.S. official method Da 15-48 (1973).

Statistics

Means were tested for significance by a students t-test as described by Gill (1978). The analysis consisted of two samples per belly and five bellies per treatment.

II. The Effect of Oxygen on N-Nitrosamine Formation in Bacon

Two systems were employed to study the effect of frying atmosphere on N-nitrosamine formation in bacon.

Frying procedures

In the initial study, equal portions of commercially prepared bacon were fried in a commercial aluminum electric skillet enclosed in a glove box (Labconco Corp., Kansas City, MO.) to which was attached an exhausting pump. The glove box was flushed several times with either air or nitrogen depending on the frying atmosphere to be investigated. A cylinder of air (or nitrogen) was connected to the glove box and a steady stream of gas introduced during the frying procedure (171°C, 3 minutes per side). The vapors emitted from the frying pan were collected in three traps attached in series to the glove box outlet. The first trap contained 10 ml of a

solution of ammonium sulfamate (20 g/l) in 1.0 M sulfuric acid at 0°C, while the two subsequent traps were immersed in liquid nitrogen. After frying, the glove box was exhausted for an additional 10 minutes. The levels of N-nitrosamines present in the cooking vapor were determined as described below.

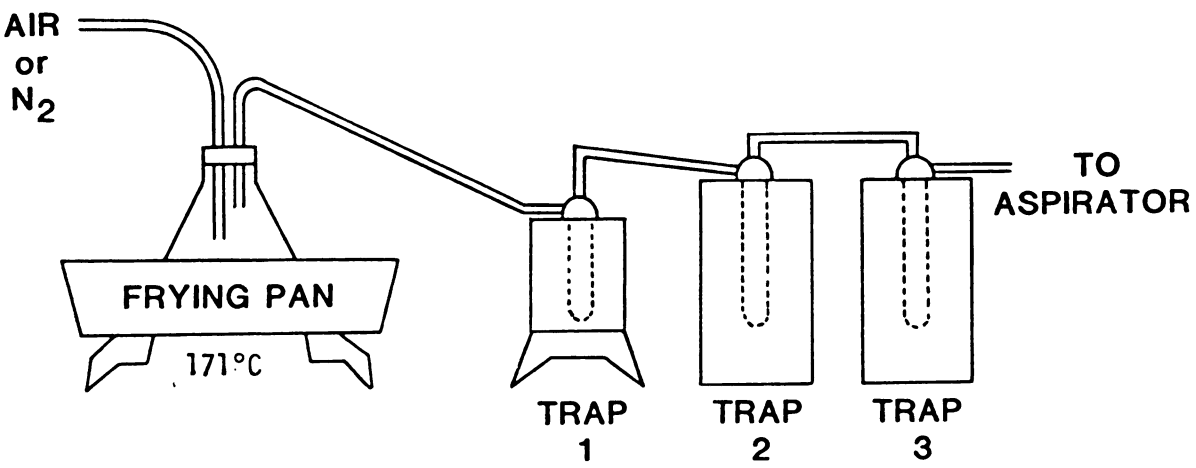
The second series of experiments utilized a different frying system (Figure 6). A glass Fernbach culture flask (2800 ml) was fitted with a cork containing two glass tubes and placed on top of a conventional electric skillet. Approximately 250 ml of cooking oil were placed in the flask to calibrate the skillet temperature. The thermostat was adjusted to produce a minimum temperature of 171°C. Commercial bacon was cut into small strips to fit into the flask and to facilitate turning of the strip during frying. To attain approximately the same level of "doneness," as compared to bacon fried by the recommended skillet procedure (Reddy et al., 1982), a frying time of 14 minutes was used.

Air was flushed from the frying system at different times by purging the system with nitrogen through an inlet positioned near the bottom of the flask. The cooking vapors were collected in a similar manner to the glove box frying experiments, except that the fumes were pulled through the three traps via water aspiration. The bacon and cook-out fat were collected and analyzed for N-nitrosamines as previously described. The cooking vapors were analyzed for N-nitrosamines as described below.

N-Nitrosamine analysis of the cooking vapors

The combined aqueous condensates from the three traps were extracted with dichloromethane (3 x 25 ml). The solvent extracts were washed with 10 ml of 1.0 M sodium hydroxide, dried over anhydrous sodium sulfate, and concentrated and analyzed as before.

Figure 6. Bacon frying system for evaluating the oxygen-dependant mechanism of N-nitrosamine formation


AIR
or
N_2
FRYING PAN
171°C
TRAP
1
TRAP
2
TRAP
3
TO
ASPIRATOR

III. Effect of Liquid Smoke Preparations on N-Nitrosamine Formation in Bacon Materials

Four liquid smoke preparations were obtained from two different suppliers. Two samples, Charsol C-10 and Aro-Smoke P-50, were purchased from Red Arrow Products Company, Manitowac, Wisconsin. The other two samples, Royal Smoke H (neutralized) and Royal Smoke A, were purchased from Griffiths Laboratories U.S.A., Alsip, Illinois.

Preparation of bacon using pickle-solubilized liquid smoke

Aro-Smoke P-50 and Royal Smoke H were introduced into pork bellies via the brine, the composition of which was similar to that previously described. The concentrations of smoke incorporated are listed in Table 5. After equilibrating for 48 hours at $2^{o}C$, the bellies were placed in the smokehouse for the duration of the cook cycle. No natural smoke was introduced into the smokehouse. The bellies were tempered, sliced, and packaged as previously described.

Preparation of bacon using atomized liquid smoke

The bellies were stitch-pumped with the curing brine solution and equilibrated for 48 hours at $2^{o}C$ prior to liquid smoke exposure. Royal Smoke A and Charsol C-10 liquid smokes were atomized onto the surface of pork bellies. This was performed in a still smokehouse, i.e., the circulating fans were turned off and all the dampers were closed. The liquid smoke preparations were applied to the pork bellies in two applications. The amounts of liquid smoke used depended on the dwell time of each application, i.e., the time at which the smoke cloud remained in the smokehouse. By equating the dwell times for the two smoke preparations the amount of liquid smoke exposure the pork bellies received would approximately be equal. After application of the liquid smoke, the

Table 5. In-going concentrations of liquid smoke preparations into pork bellies

Treatment	Type of Liquid Smoke	% in Brine	No. of Bellies
H 1	Royal Smoke H	0.94	3
H 2	Royal Smoke H	1.88	3
H 3	Royal Smoke H	2.81	3
P 1	Aro-Smoke P-50	1.25	3
P 2	Aro-Smoke P-50	1.56	3
P 3	Aro-Smoke P-50	2.50	3

bellies were placed back in the smokehouse for the remainder of the cook cycle.

Additional pork bellies, similarly stitch-pumped, were not exposed to liquid smoke and were cooked in the smokehouse. These bellies served as the control samples. The bellies were tempered, sliced, packaged as previously described.

N-Nitrosamine analysis

The bacon obtained from the liquid smoke studies were fried, ground, extracted, concentrated, and analyzed for N-nitrosamines by GC-TEA as previously described.

IV. The Effect of Phenol to Carbonyl Ratios on N-Nitrosamine Formation in Bacon

Materials

All chemicals were analytical reagent grade unless otherwise specified. Resorcinol was purchased from Fisher Scientific Co. (Fair Lawn, N.J.). Catechol was obtained from Eastman Organic Chemicals (Rochester, N.Y.) while methyl ethyl ketone was supplied by Mallinckrodt, Inc. (St. Louis, MO).

Preparation of bacon from pork bellies

Eight trimmed pork bellies were cut in half and each half was stitch-pumped with a curing solution similar in composition to that used in the previous studies. The pork bellies were pumped to a targeted 11% pumping gain with curing brines containing methyl ethyl ketone and two different levels of resorcinol and catechol. The initial concentration pumped into the bellies approximated the phenol (resorcinol or catechol) to carbonyl (methyl ethyl ketone) ratio normally found in commercial

liquid smoke preparations (Sleeth et al., 1982). The second concentration of phenol pumped was double the level initially used. The number of bellies per treatment and the levels of phenol and carbonyls used are detailed in Table 6. After stitch-pumping with the appropriate brine solutions, the bellies were allowed to equilibrate for two days at 2°C prior to cooking. The bellies were placed in the smokehouse for the duration of the cook cycle, but no external smoke was applied. The bellies were tempered, sliced and packaged as previously described.

N-Nitrosamine analysis

The bacon was fried, ground, extracted, and analyzed as in previous experiments.

V. N-Nitrosamine Formation in Model Systems by Nitrogen Oxides Materials

All chemicals and reagents used were of analytical grade. Pyrrolidine (PYR) was purchased from Aldrich Chemical Co. (Milwaukee, WI.). Oleic acid and linoleic acid were obtained from Fisher Scientific Co. (Fair Lawn, N.J.). Matheson Company (Joliet, IL) provided the nitrogen dioxide (200 ppm NO_2 in nitrogen) and nitric oxide (200 ppm NO in nitrogen) used in these model systems.

Model system

A 50 ml-filtering flask served as the reaction vessel. The flask was fitted with a cork through which a disposable pipet was placed with its tip reaching the bottom of the flask. Two flow meters (Roger Gilmont Instruments Inc., Great Neck, N.Y.) were connected to the pipet via a glass connecting tube (Y-shaped). Nitric oxide or nitrogen dioxide was passed, through one flow meter, while the other flow meter controlled the

Table 6. In-going concentrations of selected phenols and carbonyl compounds into pork bellies

Treatment	Carbonyl and level in brine (%)	Phenol and level in brine (%)	No. of Bellies
C 1	2-butanone: 0.14	catechol: 0.1	3
C 2	2-butanone: 0.14	catechol: 0.2	3
R 1	2-butanone: 0.14	resorcinol: 0.1	3
R 2	2-butanone: 0.14	resorcinol: 0.2	3

passage rate of air or nitrogen into the flask. The flow meters were both adjusted to deliver at a constant rate of 115 ml/minute. This system facilitated the dilution of the nitric oxide or nitrogen dioxide to 100 ppm by mixing with either air or nitrogen (Figure 7).

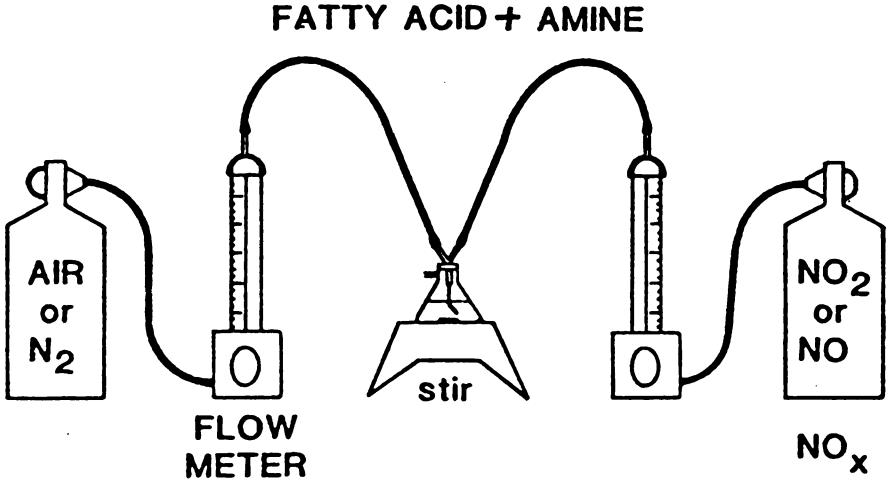
The flask contained 18 g of the appropriate fatty acid and 2 g of a 10^{-2}M PYR solution made up in ethanol. The final fatty acid/ethanol solution was 10^{-3} M with respect to PYR. The gaseous mixture was bubbled through the fatty acid solution for 30 minutes at room temperature. A magnetic stir bar constantly mixed the solution. The reaction was stopped by addition of 10 ml of 1N NaOH. The contents of the flask was then transferred to a 50 ml beaker and frozen at -20^{o}C until analysis.

In a related experiment, the leaf fat obtained from the swine feeding trials were extracted to remove their component triglycerides. Sub-samples of the leaf fat were compiled for each dietary treatment and blended with hexane. After filtering, the solvent was removed on the Buchi Rotovapor R rotary evaporator (Buchi Inc. Switzerland). An 18 g aliquot of the remaining fat was mixed with 2 g of 10^{-2} M PYR solution in the reaction vessel. Only the nitric oxide/air mixture was bubbled through these fat solutions for 30 minutes. The solution in the flask was stirred and heated to a constant temperature of 45^{o}C during the reaction period. The reaction was stopped by addition of 10 ml of 1N NaOH and the mixture was frozen at -20^{o}C until analysis.

N-Nitrosamine analysis

Extraction of the N-nitrosamines from the reaction mixture was performed by the mineral oil distillation procedure previously described except that the entire contents of the reaction vessel was used. To

Figure 7. Model system used to investigate the role of unsaturated fatty acids in the nitrosation of pyrrolidine by nitrogen oxides


FATTY ACID + AMINE
AIR
or
N_2
FLOW
METER
stir
NO_2
or
NO
NO_x

prevent artifactual N-nitrosamine formation, 1 g of ammonium sulfamate was added to the reaction mixture before distillation. N-Nitrosamine levels were determined by the GC-TEA method.

RESULTS AND DISCUSSION

I. Effect of Diet on N-Nitrosamine Formation in Bacon

The influence of diet on the formation of N-nitrosamines in fried bacon was investigated by feeding pigs regular (control), corn oil, tallow, and coconut fat-enriched diets for five weeks. Results of the N-nitrosamine analyses indicate that NPYR concentrations fell in the range of 4-9 μg/kg (average 5.7 μg/kg) for the control samples (Table 7). These values are consistent with those recently reported in other bacon studies where NPYR levels were generally below 10 μg/kg (Mergens and Newmark, 1979). Bacon from the group of pigs fed coconut fat contained significantly ($P<0.05$) lower levels of NPYR, with an average concentration of 3.8 μg/kg being obtained. On the other hand, the bacon from the corn oil group averaged 10.6 μg/kg of NPYR indicating that corn oil supplementation resulted in a significant increase ($P<0.05$) in NPYR formation. Feeding tallow to the pigs for five weeks did not influence NPYR levels in the fried products. Similar trends were observed for NPYR levels in the cook-out fat.

The effect of diet on NDMA levels was not as pronounced as for NPYR, possibly due to the smaller quantities of this N-nitrosamine in cooked bacon. Significantly ($P<0.05$) higher concentrations of NDMA were found in fried bacon produced from pigs fed the corn oil-enriched diet. Unlike NPYR, there was no signficant difference between NDMA levels in the control bacon samples and bacon from the coconut fat-supplemented group.

Table 7. N-Nitrosamine concentrations (μg/kg) and residual nitrite (mg/kg) in fried bacon and cook-out fat from pigs fed various oil-supplemented diets

Diet/Treatment	Fried bacon[a]		Cook-out fat[b]		Residual nitrite
	NPYR	NDMA	NPYR	NDMA	
Control	5.7 (4-9)[a]	2.7 (1-4)	13.2	6.8	37 (32-45)
Coconut fat	3.8[c] (2-5)	2.2 (1.7-3)	tr	2.0	33 (28-39)
Corn oil	10.6[c] (7-15)	4.0[c] (2-7)	18.6	8.3	43 (31-56)
Tallow	5.6 (4-8)	2.7 (1-4)	8.9	2.5	43 (29-58)

[a] Values in parentheses represent range of N-nitrosamines levels obtained for five bellies per treatment.

[b] Cook-out from five bellies per treatment was combined and analyzed in triplicate.

[c] Values are significantly different from the control at the $p < 0.05$ level as per students' t-test (Gill, 1979).

The influence of dietary supplementation on the fatty acid composition of adipose tissue is summarized in Table 8. Supplementation of the diet with corn oil tended to increase the degree of unsaturation of the adipose tissue. Specifically, there was an approximate two-fold increase in the linoleic acid content of bacon adipose tissue from the corn oil treatment. Coconut fat-supplementation increased the degree of saturation in the adipose tissue, particularly with respect to oleic acid. The increase in total saturation paralleled the formation of greatly reduced levels of NPYR in the bacon. Conversely, the fatty acid composition of the adipose tissue of bacon from the pigs fed the corn oil diet correlated well with the higher levels of NPYR observed in this study, an indication that increasing unsaturation of the lipid enhances N-nitrosamine formation in bacon. Tallow, when fed to pigs did not alter appreciably the fatty acid profile of the adipose tissue of the bacon. N-Nitrosamine analyses also revealed no significant differences in NPYR and NDMA in bacon from pigs fed the tallow-supplemented diets.

The level of unsaturation in the bacon adipose tissue was further substantiated by the iodine values for each of the treatment groups (Table 9). A higher iodine value indicates a higher level of unsaturation. The bacon adipose tissue from the corn oil-fed group had an iodine value of 83.2 compared to a value of 60.0 for the control group. The bacon adipose tissue from the tallow-fed group and the coconut fat-fed group had iodine values of 62.4 and 56.2, respectively. The levels of unsaturation found in the bacon adipose tissue from these various treatments correlate well with N-nitrosamine formation in the fried bacon. The higher the level of unsaturation, the higher the level of N-nitrosamine formed.

Table 8. Fatty acid composition of adipose tissue of bacon produced from pigs fed various supplemented diets [a]

Diet/ Treatment	Fatty acid composition (%)									Total Unsat.	Sat/Unsat. ratio
	C12	C14	C16	C18	Total Sat.	C16:1	C18:1	C18:2	C18:3		
Control	--	1.5	21.0	11.7	34.2	2.6	50.0	13.5	--	66.1	0.52
Coconut fat	2.8	6.9	25.1	10.2	45.0	5.5	37.9	10.1	0.3	53.8	0.84
Corn oil	--	1.1	21.1	7.9	30.1	1.4	38.1	29.8	0.6	69.9	0.43
Tallow	--	1.2	17.2	14.0	32.4	2.7	51.9	12.9	--	67.5	0.48

[a] Average fatty acid data from five pigs per treatment.

Table 9. Iodine values of bacon adipose tissue[a] from pigs fed various diets

Treatment	Iodine Value [a]
Control	60.0
Tallow	62.4
Corn Oil	83.2
Coconut	56.2

[a] Values represent the average of duplicate analyses

These results are interesting in terms of the possible relationship between N-nitrosamine formation and the relative unsaturation of bacon adipose tissue. Mottram et al. (1977) showed that nitrosation occurs mainly in the adipose tissue and have postulated that the non-polar lipids provide an environment conducive to N-nitrosamine formation. Goutefongea et al. (1977) studied the interaction of nitrite with adipose tissue and suggested that nitrite reacts with unsaturated carbon-carbon bonds. Walters et al. (1979) also reported that unsaturated lipids can react with nitric oxide in a similar manner to simpler olefins such as cyclohexene. They demonstrated that pseudonitrosites (α-nitrosonitrite esters) of unsaturated triglycerides transnitrosate to secondary amines and suggested that similar derivatives of unsaturated lipids may be involved in N-nitrosamine formation in adipose tissue.

Mirvish and Sams (1983) recently demonstrated that a nitrosating agent can be produced from the reaction of methyl linoleate with nitrogen dioxide and that the reaction occurs at a slower rate with methyl stearate and methyl oleate. These authors speculated that active nitrite esters might arise by simple addition of N_2O_4 to an ethylene group to produce a nitrosate or by a more complex series of free radical reactions beginning with electron abstraction by nitrogen dioxide (Pryor and Lightsey, 1981). This reaction not only initiates the autoxidation of the alkene in the presence of oxygen or air, but it also leads to the production of nitrous acid rather than of a product containing a nitro group attached to a carbon atom. The nitrous acid can react with secondary amines to produce N-nitrosamines. Pryor and Lightsey (1981) also showed that the rate of nitrosation of the amine increased directly with the number of double bonds in the reaction medium, an indication

that nitrous acid formed by the NO_2-unsaturated ester reactions is participating in the nitrosation of the amine.

With regard to N-nitrosamine formation in bacon, it is well established that back bacon contains lower levels of NPYR than does bacon produced from pork bellies (Walters et al., 1979). Evans and Ranken (1975) have reported a mean ratio of saturated to unsaturated fatty acids in back pork fat of 0.86, whereas a ratio of 0.52 was obtained for the control bacon adipose tissue in the present study (Table 2). The addition of coconut fat to the pig diet elevated this ratio to 0.84 and resulted in a reduction of NPYR concentrations in the fried bacon samples to levels normally encountered in the leaner bacon (Mottram et al., 1977). Mottram et al. (1977) found also that the cooking of bacon lean and bacon fat separately in corn oil led to a marked enhancement of NPYR production in the former but not in the latter portion of the cured meat. These studies all lend credence to the postulate that NPYR formation during the frying of bacon could proceed through the intermediate formation of pseudonitrosite derivatives of unsaturated lipids.

II. The Effect of Frying Atmosphere on N-Nitrosamine Formation in Bacon

The effect of frying atmosphere on N-nitrosamine formation in bacon was investigated by frying commercially prepared bacon in atmospheres of air or nitrogen. In the initial study, bacon was fried in a skillet enclosed in a glove box and the vapors trapped in a series of traps. The results of the N-nitrosamine analysis of the cooking vapors collected during the bacon frying are summarized in Table 10. Average NDMA levels of 4.9 and 0.7 μg/kg were obtained for air and nitrogen, respectively, these figures representing the average of three fryings. NPYR levels were

Table 10. The effect of frying atmosphere on N-nitrosamine concentrations (μg/kg) in the cooking vapors produced during the frying of bacon (glove box study)[a]

N-Nitrosamine	Air	Nitrogen	Percent Reduction
NDMA	4.9	0.7	86
NPYR	8.9	1.4	84

[a] Values represent the average of three fryings.

8.9 and 1.4 μg/kg for atmospheres of air and nitrogen, respectively. These data represent reductions of 86 and 84 percent, for NDMA and NPYR, respectively, when bacon is fried in nitrogen.

A more extensive study was undertaken to determine the effect of oxygen exclusion from the frying atmosphere on N-nitrosamine formation in bacon (Table 11). Absence of air resulted in a marked reduction in both NDMA and NPYR levels. The greatest amount of inhibition (85%) was observed in the NDMA concentrations found in the vapor of the bacon fried under nitrogen. The NDMA levels present in bacon fried under nitrogen were much less (approximately 50%) than those detected in similar samples fried in air while the reduction in the NDMA level in the cook-out fat was minimal. The effect of frying atmosphere on NPYR formation in bacon was also very pronounced (Table 11). Frying under nitrogen resulted in NPYR reductions of 80, 69 and 72 percent in the cooking vapor, bacon and cook-out fat, respectively.

To further substantiate the role of oxygen in N-nitrosamine formation in fried bacon, air was eliminated (as far as possible) from the frying system at different times during the frying process. In the initial frying, air was eliminated (or reduced) after a frying time of 4 min by purging the frying system with nitrogen. The bacon was subsequently fried in the nitrogen atmosphere for an additional 10 min (Table 11). Further fryings were performed in which nitrogen was introduced into the frying system after 8 and 12 minutes of the frying cycle.

Results indicate that N-nitrosamine formation in bacon is influenced by the frying atmosphere (Table 11). In general, frying bacon in an inert (nitrogen) atmosphere resulted in much lower levels of N-nitrosamine. The only exception was in the NDMA levels in fried bacon which actually

Table 11. The effect of frying conditions on N-nitrosamine formation in bacon.

Time (min) and conditions of frying	NDMA (μg/kg) [a]			NPYR (μg/kg) [a]		
	Vapor	Bacon	Cook-out Fat	Vapor	Bacon	Cook-out Fat
0-Air 14-N2	1.3	2.1	1.2	2.6	3.6	6.3
14-Air 0-N2	9.5	4.3	1.3	12.9	11.5	22.1
4-Air [b] 10-N2	0.8	3.8	1.0	1.5	1.8	6.3
8-Air [b] 6-N2	2.9	2.1	2.4	4.1	3.9	7.5
12-Air [b] 2-N2	5.7	1.3	2.7	8.4	9.7	10.8

[a] Values represent the average of three fryings.

[b] The bacon was initially fried in air and then under nitrogen. Total frying time was 14 min.

decreased as the amount of time of air frying increased. However, NDMA levels in the frying vapors and cook-out fat increased as the air-frying time increased. Similarly, NPYR levels in the cooking vapors, fried bacon, and cook-out fat consistently increased as the length of frying time in air increased.

These experiments show that there is a definite involvement of oxygen in N-nitrosamine formation in bacon. The data also indicate that if oxygen is excluded from the frying environment, particularly toward the end of the frying process, N-nitrosamine formation is greatly reduced. These observations are consistent with those of Bharucha et al. (1979) who reported that both NDMA and NPYR formation take place towards the end of frying process. These investigators suggested that "N-nitrosamine formation during frying of bacon occurs essentially, if not entirely, in the fat phase, after the bulk of water is removed and therefore by a radical rather than an ionic mechanism." Bharucha et al. (1979) further speculated that the removal of water from the adipose tissue during the frying process allows the temperature to increase, and that N_2O_3 undergoes dissociation at these higher temperatures ($>100^{o}C$) to nitric oxide and NO_2. Nitric oxide in the presence of oxygen can become a powerful nitrosating agent particularly in an environment conducive to N-nitrosamine formation, such as in adipose tissue.

It is interesting to compare the results of Bharucha et al. (1979) with those of the present study in terms of the mechanism of NPYR formation in bacon. Bharucha et al. (1979) reported that the maximum amount of N-nitrosamine was produced when bacon is fried or grilled for about 12 min. During the initial 4 min of heating (cold frying pan set at the $360^{o}F$ setting), very little N-nitrosamine was found in the cook-out

fat, after which it increased sharply reaching a maximum at approximately 12 min and then declined. During the first few minutes of frying, the temperature barely exceeded the boiling point of water. They found that N-nitrosamine formation in bacon was accelerated only after the major portion of the water in the system was expelled and when temperatures were greater than 100°C. It is plausible to assume that during the early stages of frying, the water vapor produced during the frying process creates a relatively inert atmosphere in the frying system, and thus reduces N-nitrosamine formation in a similar manner to nitrogen in the present study. Here, NPYR formation appeared to increase rapidly after frying in air for 8 min of the frying cycle. There was approximately a 250 percent increase in NPYR in bacon when fried in air for 12 min of the frying process.

The results of this study support the postulate that nitrosation in bacon occurs in the fat phase and presumably by a radical rather than an ionic mechanism as proposed by Bharucha et al. (1979). The oxygen-dependent increase in a N-nitrosamine levels is consistent with a radical mechanism in which the oxidation of nitric oxide to a higher oxide of nitrogen may be a key step in N-nitrosamine formation in bacon (Dennis et al., 1982).

III. The Effect of Liquid Smoke Preparations on N-Nitrosamine Formation in Bacon

The effect of liquid smoke preparations on N-nitrosamine formation in bacon was investigated. Liquid smoke, when used in the manufacture of bacon, can be applied by either atomization onto the surface of the pork bellies or by incorporating the liquid smoke into the pumping pickle. Atomization of the liquid smoke onto the surface of the bellies occurs in the smokehouse, whereas the liquid smoke contained in the pickle is pumped into the green bellies before the smoking operation. The two different methods of liquid smoke application to pork bellies were investigated in order to determine their effect on N-nitrosamine formation in the finished bacon.

Two types of liquid smokes, Royal Smoke A (Treatment A) and Charsol C-10 (Treatment B), were applied to the surface of three pork bellies per treatment. Control samples (Treatment C) were similarly processed except that they were not exposed to liquid smoke. Results of N-nitrosamine analyses and residual nitrite determinations are presented in Table 12.

The atomization of both liquid smokes onto the surface of the pork bellies had no significant ($P<0.05$) effect upon NPYR formation in the fried product. A slight, although not significant decrease in NPYR levels in the fried bacon from Treatment A was observed; however, this may be due to the slight depletion of residual nitrite. Treatment B had similar NPYR and residual nitrite concentrations as the control samples. The effect of the atomized liquid smoke preparations on NDMA formation in fried bacon differed among the two liquid smoke samples tested. Fried bacon from Treatment A showed a significant ($P<0.05$) decrease in NDMA levels when compared to the control bacon. However, bacon prepared from Treatment B contained NDMA levels comparable to those of the control bacon. The

Table 12. Effect of atomized liquid smoke preparations on N-nitrosamine formation in fried bacon.

Treatment [a]	NDMA (μg/kg)	NPYR (μg/kg)	Residual NO2 (mg/kg)
A	0.6 [b] (ND-1.1)	11.7 (6.1-14.3)	29.4 (27-31)
B	2.7 (1.7-3.4)	13.6 (6.6-17.6)	45.6 (33-58)
C	2.5 (1.3-3.2)	13.0 (12.1-13.8)	45.0 (41-48)

[a] Treatment A; Royal Smoke A
Treatment B; Charsol C-10
Treatment C; Control

[b] Values are significantly different from the control at the ($P < 0.05$) level as per students' t-test (Gill, 1978).

decrease in NDMA levels observed in Treatment A bacon could, again, be partially explained by the residual nitrite depletion.

These results are in agreement with the finding of Theiler et al. (1983) who found that the surface application of liquid smoke preparations had little or no effect on N-nitrosamine formation in bacon, and in particular NPYR. They reported that liquid smoke must be incorporated into the pork belly, i.e., introduced via the curing brine, in order to achieve significant reductions in NPYR formation. However, Theiler et al. (1983) did not report the effect of liquid smokes on NDMA formation, although it would be expected that similar trends would have been observed as in the present study.

The second part of the present investigation related to the effect of pickle-solublized liquid smoke on N-nitrosamine formation in bacon. Two liquid smoke preparations, Royal Smoke H-neutralized (Treatment H) and Aro-Smoke P-50 (Treatment P), were solubilized in the curing pickle and stitch pumped at three different concentrations into pork bellies. Three bellies were used per treatment (Table 5). The pork bellies were then placed in the smokehouse for the duration of the cook cycle as described by Reddy et al. (1982). The results of the N-nitrosamine analyses and nitrite determinations are presented in Table 13. The control samples were not exposed to the liquid smoke preparations.

The two liquid smoke samples differed in their ability to influence N-nitrosamine levels in fried bacon. Treatment H at all three liquid smoke concentrations had no sigificant ($P<0.05$) effect upon both NDMA and NPYR levels when compared to the control. A slight decrease in NDMA levels was observed in the second (H2) and third (H3) liquid smoke concentrations compared to the lower initial level (H1) used. Increasing the levels of this liquid smoke had no significant ($P<0.05$) effect upon NPYR levels in the fried bacon.

Table 13. Effect of pickle-solublized liquid smoke preparations on N-nitrosamine formation in fried bacon.

Treatment [a]	NDMA (μg/kg) [b]	NPYR (μg/kg) [b]	Residual NO_2 (mg/kg)
H 1	2.9 (16-4.9)	13.1 (8.2-18.1)	44.4 (40-49)
H 2	2.1 (ND-2.9)	11.2 (6.2-16.0)	66.9 (59-75)
H 3	2.0 (1.5-2.5)	13.6 (7.6-18.9)	95.0 (88-100)
P 1	6.4 (ND-13.7)	22.9 (10.6-42.1)	85.0 (73-97)
P 2	2.0 (1.2-2.6)	28.8 [c] (22.5-43.1)	98.3 (90-98)
P 3	1.9 (1.1-3.2)	13.2 (11.9-14.5)	69.4 (59-80)
Control	2.5 (1.3-3.2)	13.0 (12.1-13.8)	45.0 (41-48)

[a] Treatment H: Royal Smoke H - neutralized; H 1 - 0.94%, H 2 - 1.88%, H 3 - 2.81%; Treatment P: Aro Smoke P-50; P1 - 1.25%, P2-1.56%, P3 - 2.50%.

[b] Values in parentheses represent range of N-nitrosamine levels obtained for three bellies per treatment.

[c] Values are significantly different from the control at the $P<0.05$ level as per students t-test (Gill, 1978).

The second liquid smoke preparation (Treatment P) at all three concentrations tested did not significantly effect ($P<0.05$) N-nitrosamine formation when compared to the control. The only exception to this was observed in the NPYR levels detected in the bacon which was stitch pumped with the second concentration (P2) of this liquid smoke. A significant ($P<0.05$) increase in NPYR levels was observed in this fried bacon. The third concentration (P3) of this liquid smoke did not influence NPYR formation when compared to the control. A slight increase in NPYR levels were found in the bacon prepared with the initial concentration (P1) of liquid smoke. However, this increase was not significant ($P<0.05$) due to the marked variation in NPYR levels detected.

The effect of liquid smoke (Treatment P) on NDMA formation was also not significant ($P<0.05$) when compared to the control. The increase in NDMA levels detected in the fried bacon from Treatment P1 was not significant due to the wide variation in N-nitrosamine levels detected. The higher concentrations (P2 and P3) of this liquid smoke did not influence NDMA formation when compared to the control bacon.

The two liquid smoke preparations used in this study appeared to influence residual nitrite levels in the finished bacon prior to frying. Increasing levels of liquid smoke in Treatment H resulted in a concomitant increase in residual nitrite. A more variable response was observed for Treatment P. A slight increase in residual nitrite occurred at the second concentration, whereas a noticeable decrease was observed at the highest concentration tested (P3). Except for the initial concentration of Treatment H, liquid smoke appeared to elevate the residual nitrite levels in bacon when compared to the control samples.

The addition of liquid smoke preparations to the pickle can influence residual nitrite levels. Theiler et al. (1982) reported that because most liquid smoke preparations are acidic in nature, they can be buffered so as to not drastically reduce nitrite levels when added to the pickle. Royal Smoke H is neutralized liquid smoke, whereas Aro-Smoke P-50 is unneutralized. Increasing the levels of the neutralized liquid smoke (Treatment H) actually caused an increase in residual nitrite, whereas increasing the levels of the unneutralized liquid smoke (Treatment P) caused a slight increase at the second concentration (P2) and a marked decrease at the highest level (P3) used when compared to the control. The decrease in residual nitrite seen at the third concentration is expected; however, the slight increase at the second concentration appears contradictory. It is quite possible that variations in the pork belly composition can account for the observed results. Woolford and Cassens (1977) reported that the lean portion of bacon usually contains more than three times the amount of added nitrite found in the adipose portion. However, numerous researchers have reported that NPYR formation is associated with the bacon adipose tissue and not with the lean (Fiddler et al., 1974; Patterson et al., 1976; Mottram et al., 1977).

Pensabene et al. (1977) observed that NPYR and NDMA are most highly correlated with residual and added nitrite and to a lesser degree with compositional factors. Due to the numerous complicating factors surrounding N-nitrosamine formation and residual nitrite a more extensive study of various liquid smoke preparations is required.

The influence of pickle-solubilized smoke preparations on both NDMA and NPYR formation is interesting in terms of the relationship of C-nitrosophenols and N-nitrosamine formation in bacon. Phenols, naturally

present in liquid smoke, react more rapidly with nitrite than do secondary amines (Challis, 1973). One would expect a decrease in N-nitrosamine formation when phenols are present due to a depletion of nitrite. It has been reported that certain phenolic constituents of smoked foods inhibit the nitrosation of morpholine (Issenberg and Virk, 1974). Other literature data (Knowles et al., 1975; Gilbert et al., 1975) suggest that competitive C-nitrosation of phenols in contrast to nitrosation of secondary amines can be used to explain the mechanism of N-nitrosamine inhibition. However, Davies et al. (1979) have reported that C-nitrosophenols can actually enhance the formation of N-nitrosamines, specifically NPYR. These authors observed that p-nitroso-o-cresol, an initial product of phenol nitrosation, catalyzes the nitrosation of pyrrolidine. Furthermore, Pignatelli et al. (1980) have shown that 1,3-dihydroxyphenols (e.g. resorcinol) are powerful catalysts of N-nitrosamine formation whereas 1,2-and 1,4-dihydroxyphenols have an inhibitory effect.

The influence of these two liquid smoke preparations on residual nitrite, and to a lesser extent NPYR formation, indicates there are marked differences between the two. Treatment H had no effect upon N-nitrosamine formation whereas residual nitrite concentration increased upon increased addition of liquid smoke. Treatment P, on the other hand, tended to accelerate NPYR formation at the lower concentrations, whereas it had a more variable response on residual nitrite. An explanation for these differences may be in the level of phenols found in the respective liquid smoke preparations. The phenolic concentration of the Royal Smoke H preparation was 7.5 mg/ml as compared to the Aro-Smoke P-50 sample which contained 39 mg phenols/ml (Sleeth et al., 1982). Additionally, these two

liquid smoke preparations differ in the recommended amounts that are to be added to the brine. This represents major treatment differences with respect to the amount of phenols pumped into the bellies. Treatments H1, H2, and H3 were stitch-pumped to give phenol concentrations of 7.5, 14.9 and 22.4 mg/kg of finished product, respectively. In contrast, Treatments P1, P2, and P3 were stitch-pumped to give phenol concentrations of 52, 65, and 104 mg/kg of finished product respectively. The different effects that these two liquid smoke preparations had on N-nitrosamine formation in bacon is most likely due to the vast differences in phenol concentration. Sleeth et al. (1982) reported that when Aro-Smoke was pumped into pork bellies to give a phenol concentration of 100 mg/kg of finished product, a 57% reduction in NPYR resulted. Although, the present studies failed to show a reduction in NPYR with treatment P3 (phenolic concentration 100 mg/kg) when compared to the control, a reduction in NPYR levels was observed over the two lower treatments, P1 and P2. It is quite possible that a certain phenolic concentration is required for either enhancement or inhibition of N-nitrosamine formation. The level of phenols present in bacon prepared using Royal Smoke H appears to be too low to affect N-nitrosamine formation. On the other hand, the greater level of phenols present in the bacon prepared with Aro-Smoke P-50 appears to be sufficient to influence N-nitrosamine formation. The phenolic concentrations present in the bacon from Treatments P1 and P2 tend to accelerate NPYR formation, whereas the level in Treatment P3 causes a decrease. This study indicates that a more extensive investigation dealing with the role of liquid smoke on N-nitrosamine formation is needed. Moreover, further studies are necessary to investigate the role of selected phenols at various concentrations on N-nitrosamine formation in bacon.

IV. The Effect of Phenols on N-Nitrosamine Formation in Bacon

Model system studies have shown that phenols naturally present in liquid smoke preparations can catalyze N-nitrosamine formation (Challis and Bartlett, 1975; Walker et al., 1979). However, there is a paucity of data regarding the catalytic or inhibitory properties of phenols when applied to bacon through the smoking process. Pignatelli et al. (1980) observed that 1,3-dihydroxyphenols (e.g., resorcinol) catalyzed the formation of N-nitrosamines, whereas 1,2- and 1,4-dihydroxyphenols were inhibitory. The inhibition caused by these phenolic compounds results from their ability to reduce the nitrosating agent, dinitrogen trioxide, to nitric oxide, an ineffectual nitrosating species (National Academy of Sciences, 1981). Resorcinol catalyzes N-nitrosamine formation due to its ability to rapidly form a nitroso intermediate which can interact with more dinitrogen trioxide to generate a powerful nitrosating agent (Pignatelli et al., 1981).

The majority of the studies investigating the influence of polyhydroxylated phenols (polyphenols) on N-nitrosamine formation have been performed using aqueous model systems. The effect of these compounds on N-nitrosamine formation in bacon systems may be quite different. For this reason, two phenolic compounds (resorcinol and catechol) reported to have opposite effects upon N-nitrosamine formation in model systems, were investigated as to their influence in bacon systems. The phenols were solubilized in a brine solution and stitch-pumped into pork bellies at two different concentrations.

In addition to the phenolic compounds, a carbonyl compound, methyl ethyl ketone, was also solubilized in the pickle in order to approximate the phenol/carbonyl ratio normally found in liquid smoke preparations (Sleeth et al., 1982). The initial concentration of the phenols and methyl ethyl ketone

approximated this ratio. The second concentration was approximately double the ratio normally found in liquid smokes. The average values for N-nitrosamine levels and residual nitrite concentrations are presented in Table 14. The control bacon was not exposed to any phenols or smoke treatments.

The results of these bacon studies differ from those of earlier investigations where model systems were employed. Catechol, at the initial concentration had no significant effect ($P < 0.05$) on both NDMA and NPYR formation when compared to the control bacon. At the second phenol/carbonyl ratio (2:1), catechol had different effects upon the formation of the two N-nitrosamines when compared to the control bacon. Catechol did not affect NDMA formation, whereas a significant increase ($P < 0.05$) in NPYR levels was observed. Catechol had been previously reported to inhibit N-nitrosamine formation (Pignatelli et al., 1980) in model system studies.

Resorcinol, when used at a concentration similar to that found in liquid smoke preparations, did not significantly ($P < 0.05$) influence NDMA and NPYR formation in bacon. Similarly, no significant effect ($P < 0.05$) on the formation of these two N-nitrosamines was observed in the bacon samples stitch-pumped with the higher concentration of resorcinol. These observations also differ somewhat from those of Pignatelli et al. (1980). These authors reported that resorcinol catalyzes the formation of N-nitrosamines in aqueous model systems. The present studies using bacon systems showed no influence of resorcinol on either NDMA or NPYR formation when compared to the control bacon samples.

The residual nitrite levels in the bacon samples treated with resorcinol and catechol were much higher than those in the control

Table 14. The effect of selected phenols on N-nitrosamine formation in bacon

Treatment	Fried Bacon (μg/kg)[a] NDMA	NPYR	Residual Nitrite (mg/kg)
Catechol 1	3.3 (1.9-4.5)	16.1 (8.5-22.8)	103 (95-108)
Catechol 2	1.4 (ND-2.6)	18.2[b] (10.3-23.3)	93 (75-105)
Resorcinol 1	1.1 (ND-3.0)	14.3 (7.3-24.5)	80 (67-100)
Resorcinol 2	1.5 (1.2-1.9)	11.1 (8.0-13.9)	62 (55-85)
Control	2.5 (1.3-3.2)	13.0 (12.1-13.8)	45 (42-49)

[a] Values represent duplicate analyses of three bellies per treatment; values in parentheses represent range of N-nitrosamine levels obtained.

[b]Values are significantly different from the control at the $P < 0.05$ level as per students t-test (Gill, 1978).

samples. These data may indicate that phenolic compounds have a protective effect on nitrite and slow down their rate of depletion in the bacon samples. However, it is quite possible that this phenomenon is an artifact of these studies or that it reflects inadequate stitch-pumping procedures.

The different effects of resorcinol and catechol on N-nitrosamine formation in bacon systems and model systems may be partially explained by the differences between these two systems. In the model systems, the reactions took place in an aqueous medium which is quite different from the bacon systems employed in the present study. The constituents present in pork bellies may be responsible for the different results obtained in this study. Phenolic compounds, due to their hydrophilicity, should remain in the aqueous portion of the cured belly. Their inability to influence N-nitrosamine formation may be due to the fact that N-nitrosamine formation occurs primarily in the adipose tissue (Mottram et al., 1977) where these phenols are not present. This is in agreement with Bharucha et al. (1980) who reported that there is very little difference in N-nitrosamine formation between smoked and unsmoked bacon. These authors suggested that C-nitrosophenols are not implicated in N-nitrosamine formation in bacon. This supports the results of an earlier study by Bharucha et al. (1979) who postulated that in order for a compound to effectively inhibit N-nitrosamine formation in bacon it must be lipophilic. Further studies are needed to investigate the effect of phenolic compounds on N-nitrosamine formation in bacon systems. This could be accomplished using a similar method described by Sen et al. (1976) whereby phenolic compounds could be sprayed on individual bacon slices before frying to study their influence on N-nitrosamine formation.

Because of the ability of certain phenolic compounds to influence N-nitrosamine formation in aqueous model systems (Challis and Bartlett, 1975; Walker et al., 1979; Pignatelli et al., 1980), it is interesting to speculate on their possible role in in vivo nitrosation reactions. An aqueous environment does exist in the stomach and small intestine, and it is possible that the ingestion of smoked food products could influence N-nitrosamine formation in the body. Lijinsky et al. (1970) reported that amines can be nitrosated in the aqueous environment of the stomach and intestines. Ohshima and Bartsch (1981) reported the endogenous nitrosation of proline in humans upon ingestion of beet juice (as a high nitrate source) and proline. Similarly, Ladd et al. (1983) observed that proline was endogenously nitrosated in humans after ingesting beet juice and proline. These authors also reported that cigarette smokers produced approximately 2.5 times as much N-nitrosoproline (NPRO) as non-smokers. They concluded that the higher level of salivary thiocyanate found in people who smoke was responsible for the increased rate of endogenous nitrosation in this group compared to non-smokers. These experiments indicate that certain compounds, upon ingestion, can influence in vivo nitrosation. Little is known about the effect of ingested phenolic compounds on in vivo nitrosating. Studies investigating the influence of these compounds on in vivo nitrosation are needed.

The Effect of Nitrogen Oxides on N-Nitrosamine Formation in Model Systems

The concern over the formation of N-nitrosamines and precursors is well documented (National Academy of Sciences, 1981). The usual route of investigations deal with endogenous and exogenous sources of both nitrite and nitrosatable amines. However, the role of nitrogen oxides in the nitrosation of secondary amines has only recently been recognized. These

nitrogen oxides can act as nitrosating agents and may subsequently form N-nitrosamines (Challis et al., 1978; Challis and Kyrotopoulos, 1978, 1979).

The effect of nitrogen oxides on N-nitrosamine formation in model systems was investigated. Nitrogen dioxide (NO_2) and nitric oxide (NO) were mixed with either air or nitrogen to give a final concentration of 200 ppm, and were bubbled through oleic acid or linoleic acid solutions containing pyrrolidine (PYR) for 30 minutes. The subsequent mixture was then analyzed for the presence of NPYR. Results of these analyses are presented in Table 15.

The mixture of NO_2 and nitrogen produced a greater level of NPYR when bubbled through the linoleic acid solution when compared to the oleic acid solution. The reactions of NO_2 in nitrogen with pyrrolidine in linoleic and oleic acids produced NPYR at levels of 12.3 and 4.0 μg, respectively. Similarly, NO_2 in air produced 1.4 μg NPYR when reacted with the linoleic acid solution as compared to 1.1 μg NPYR when reacted with pyrrolidine in the oleic acid solution. The amount of NPYR produced in these model systems was much greater when NO_2 was mixed with nitrogen as opposed to mixing with air.

These model system studies indicate that by increasing the number of ethylenic groups in the reaction medium, an increase in the nitrosation of PYR results. The difference between the results for the oleate and the linoleate solutions paralleled the increase in the number of ethylene groups in the reaction medium. This is in agreement with the finding of Pryor and Lightsey (1981) who investigated the reactions of nitrogen dioxide with pyrrolidine in methyl oleate and methyl linoleate systems.

Table 15. Nitrosation of pyrrolidine in fatty acid model systems by nitrogen oxides

Fatty Acid	Composition	NPYR[a] (μg)
Oleic	NO/N_2	2.6
Linoleic	NO/N_2	4.5
Oleic	NO/air	981.0
Linoleic	NO/air	3567.4
Oleic	NO_2/N_2	4.0
Linoleic	NO_2/N_2	12.3
Oleic	NO_2/air	1.1
Linoleic	NO_2/air	1.4

[a] Values represent the average of duplicate analyses.

These authors showed that the reaction initiates the autoxidation of the alkene and it leads to the production of nitrous acid rather than of a product containing a nitro group attached to a carbon atom. The nitrous acid can react with secondary amines to produce N-nitrosamines (Pryor and Lightsey, 1981). Additionally, it was also shown that the rate of nitrosation of the amine increases directly with the number of double bonds in the reaction medium, and indication that nitrous acid formed by the NO_2-unsaturated ester reactions is participating in the nitrosation of the amine. Mirvish and Sanes (1983) reported that the reaction of nitrogen dioxide in air with methyl linoleate produced a nitrosating agent (NSA) that could reaction with morpholine in vitro to produce NMOR. They also observed that the NSA yield from linoleic acid was four times that from methyl oleate and seven times that from methyl stearate. It was concluded that the NSA produced by NO_2 consisted of nitrosites (α-nitronitrite esters) which were produced by addition of NO_2 (or its dimer, N_2O_4) to ethylenic groups of unsaturated fatty acids, or by a more complex series of free radical reactions, beginning with electron abstraction by NO_2 (Pryor and Lightsey, 1981). The latter type of reaction would mainly occur with polyunsaturated fatty acids.

Other oxides of nitrogen have been reported to be nitrosating agents, especially in the presence of air or metal catalysts (NAS, 1981). Challis and Kyrtopoulos (1978) reported nitric oxide reacts slowly with secondary amines under anaerobic conditions and that this reaction can be accelerated by introducing air into the reaction environment. The influence of nitric oxide on NPYR formation in fatty acid systems was investigated.

Nitric oxide (NO) was mixed with either air or nitrogen to give a final concentration of 100 ppm and bubbled through oleic acid and linoleic acid solutions containing pyrrolidine in a similar manner to that described for NO_2. The results of the NPYR analyses are presented in Table 15. As expected, the gaseous mixture of NO and nitrogen produced more NPYR when bubbled through the linoleic acid solution as compared to the oleic acid solution. A concentration of 2.6 μg of NPYR was formed in the oleic acid solution, whereas 4.5 μg of NPYR was produced in the linoleic acid mixture. A similar trend was observed when NO was mixed with air and bubbled through these fatty acid solutions. The linoleic acid solution formed more NPYR than did the oleic acid solution. A concentration of 3567.4 μg of NPYR was formed in the linoleic acid solution, whereas 981.0 μg of NPYR were detected in the oleic acid solution. Similarly to NO_2, the increase in NPYR formation by increasing the number of double bonds in the reaction medium occurred when NO was mixed with either air or nitrogen, although it was much greater when NO was mixed with air. Theoretically, NO mixed with nitrogen is a very poor nitrosating agent. However, in these studies, NPYR was formed with this gaseous mixture and was most likely due to residual air left in the reaction medium from incomplete purging of the system with nitrogen.

Frouin (1976) has hypothesized that much of the nitrite added to cured meat is rapidly converted to various forms of bound nitric oxide, i.e., by reaction with proteins, thiols, hydroxyls, carboxyls, reducing agents and heme pigments. Dennis et al. (1982) reported that N-nitrosamine formation from bound nitric oxide may occur either by direct transnitrosation of the secondary amines or by an indirect process involving the initial release of the nitric oxide moiety. It is well recognized that nitric oxide is a

poor nitrosating agent but in the presence of air, it is rapidly converted to higher oxides of nitrogen, which are powerful nitrosating agents (NAS, 1981). The data presented in this study support this statement. Nitric oxide, when mixed with air, became a powerful nitrosating agent in these model system studies. Additionally, by increasing the number of double bonds in the reaction environment an increase in NPYR formation was observed.

To correlate these observations to the data reported in the previous feeding trial studies, pig fat obtained from the feeding study was utilized as the lipid media. Leaf fat was extracted with hexane to remove the component triglycerides. Pyrrolidine was then added to the extracted fat and the gaseous mixture of nitric oxide in air (100 ppm) was bubbled through the model system. The nitric oxide/air mixture was used because of its ability to greatly enhance NPYR formation in fatty acid model systems. The results are presented in Table 16.

The NO/air mixture formed 10.4 μg NPYR when bubbled through the fat obtained from the control group of pigs. The fat from the corn oil-and coconut fat-fed groups formed 16.8 μg and 12.3 μg of NPYR, respectively. On the other hand, the fat from the tallow-fed group formed a much lower amount of NPYR, with a concentration of 8.2 μg being obtained.

The results obtained from these model system studies using the leaf fat from the feeding trials differ somewhat from the results of the bacon frying study using the bacon from these feeding trials. As expected, the fat obtained from the pigs fed the corn-oil enriched diet produced the highest amount of NPYR. Fatty acid analysis of the leaf fat from this treatment revealed a mean ratio of saturated to unsaturated fatty acids of 0.55 compared to 0.89 for the control group (Table 16). In other words,

Table 16. Nitrosation of pyrrolidine by nitrogen oxides in model systems composed of fat from pigs fed various diets [a,b]

Fat Source/Diet	Gas Composition	NPYR (μg)	Sat./Unsat. ratio
Control	NO/air	10.4	0.89
Tallow	NO/air	8.2	0.73
Corn Oil	NO/air	16.8	0.55
Coconut Fat	NO/air	12.3	1.33

[a]The model system contained 10^{-3}M PYR and the concentration of nitric oxide in air was 100 ppm and bubbled through at a constant rate of 115 ml/min for one-half hour at 45°C.

[b]Values represent the average of duplicate analysis.

the greatest amount of NPYR was formed in the adipose tissue with the highest level of unsaturation. The results obtained from the coconut fat-fed group and the tallow-fed group differed from this premise. The fat from the coconut fat-fed group formed more NPYR than the control but had a saturated to unsaturated fatty acid ratio of 1.33. At this ratio, one would have expected a much lower amount of NPYR to form compared to the control. This was not the case. Similarly, the fat from the tallow-fed group formed a lesser amount of NPYR compared to the control, but had a saturated to unsaturated fatty acid ratio of 0.73.

Nevertheless, this study generally indicates that a higher level of unsaturation in the adipose tissue can lead to an enhancement of N-nitrosamine formation. The results from the feeding trial study in conjunction with those from the model system studies adds credence to this theory.

SUMMARY AND CONCLUSIONS

Numerous factors have been shown to influence N-nitrosamine formation in bacon. This study has indicated that the level of fatty acid unsaturation in bacon adipose tissue and also the atmosphere in which bacon is fried can also influence the formation of these compounds. Additionally, the smoking process does not appear to affect N-nitrosamine formation possibly due to the fact that the compounds present in smoke originally thought to influence nitrosation reactions are water-soluble and nitrosation reactions take place in the adipose tissue of bacon. In another phase of this investigation, nitrogen oxides were reacted with pyrrolidine in various fatty acid reaction medium to evaluate the role of unsaturation in N-nitrosamine formation.

In the first phase of the study, different diets were fed to swine in order to alter the fatty acid profile in the belly adipose tissue. Bacon was prepared from pork bellies of swine fed various diets and fried after two weeks of refrigerated storage. The data indicated that the level of fatty acid unsaturation present in the bacon adipose tissue correlated well with both NPYR and NDMA formation in the fried bacon. These observations lend credence to the postulate that N-nitrosamine formation during the frying of bacon could proceed through the intermediate formation of pseudonitrosite derivatives of unsaturated lipids.

In the second stage of the study, the influence of frying atmosphere on N-nitrosamine formation in fried bacon was investigated. Results indicate that there is a definite involvement of oxygen in N-nitrosamine formation in bacon. These data also indicate that if oxygen is excluded from the frying environment, particularly toward the end of the frying process, N-nitrosamine formation is greatly reduced. These observations are consistent with the postulate that N-nitrosamine formation during bacon frying occurs essentially, if not entirely, in the fat phase after the bulk of the water is removed and therefore by a radical rather than an ionic mechanism.

In the third phase of the study, the effects of liquid smoke preparations on N-nitrosamine formation in bacon were evaluated. The results of this investigation indicate that liquid smoke, particularly when atomized on the surface of cured pork bellies, does not appear to influence N-nitrosamine formation in the fried product.

In the fourth phase of this study, the influence of selected phenols on N-nitrosamine formation in bacon systems was investigated. The results generally indicate that these phenolic compounds, when applied to cured pork bellies via the brine, do not effect N-nitrosamine formation in fried bacon. Their inability to influence N-nitrosamine formation may be due to the fact that N-nitrosamine formation occurs primarily in the adipose tissue where these phenols are not likely to be present in high concentration. These observations support the postulate that in order for a compound to effectively inhibit N-nitrosamine formation in bacon it must be lipophilic. However, it is quite possible that these phenolic compounds could contribute to <u>in vivo</u> nitrosation in the aqueous environment of the stomach and intestines. Ladd et al. (1983) have shown

that certain compounds when ingested could contribute to the *in vivo* formation of N-nitrosamines.

In the fifth phase of this investigation the reaction between nitrogen oxides and pyrrolidine in unsaturated fatty acid systems was studied. The results of these model system studies indicate that by increasing the number of ethylenic groups in the reaction medium, the increase in the rate of nitrosation of PYR resulted. In addition, it was shown that the rate of nitrosation of PYR increases directly with the number of double bonds in the reaction medium, an indication that nitrous acid formed by the NO_2-unsaturated ester reactions is participating in the nitrosation of the amine. These observations are supported by Mirvish and Sams (1983) who found that nitrogen dioxide when reacted with methyl linoleate produced more nitrosating agents than when reacted with methyl oleate. These studies add credence to the postulate that a nitrosating agent can arise from the interaction of nitrogen dioxide and unsaturated fatty acids.

As a result of these investigations, several conclusions pertaining to the factors which influence N-nitrosamine formation in bacon can be drawn. These can be summarized as follows:

1. The degree of unsaturation in bacon adipose tissue parallels the formation of N-nitrosamines (NDMA and NPYR) in fried bacon.

2. The mechanism of N-nitrosamine formation in fried bacon is oxygen-dependent. This oxygen-dependency in consistent with a radical mechanism in which the oxidation of nitric oxide to a higher oxide of nitrogen is a key step in N-nitrosamine formation in bacon.

3. Liquid smoke preparations do not appear to influence N-nitrosamine formation in bacon due to their hydrophilic characteristics.

4. Selected phenols which have been reported to influence N-nitrosamine formation in model systems do not appear to have the same effect in bacon systems.

5. Nitrogen oxides can form NPYR and the rate of nitrosation of NPYR increases directly with the number of double bonds in the reaction medium. These findings indicate that nitrous acid formed by the NO_2-unsaturated ester reactions is participating in the nitrosation of the amine.

FUTURE AREAS OF RESEARCH

This study on factors influencing N-nitrosamine formation in bacon has raised several questions which merit further investigation. Specific areas which should be addressed include:

1) An investigation into the effect of varying the duration of feeding coconut fat to pigs and subsequently its effect on fatty acid composition of pork belly adipose tissue.

2) Further study of the effects of liquid smoke preparations in N-nitrosamine formation in bacon.

3) Further clarification of the influence of selected phenols at various concentrations on N-nitrosamine formation in bacon.

4) An investigation of the ability of residual phenols and nitrite in smoked cured meats to initiate and/or accelerate *in vivo* nitrosation reactions. Specifically, their role in the presence of N-nitroso-thiazolidine carboxylic acid in human urine requires clarification.

APPENDIX

Effect of Diet on the Fatty Acid Composition of Pork Fat

The effect of various fat-containing diets on the fatty acid composition of pork fat at different carcass locations was also investigated as a consequence of the feeding trial. Diets consisting of 15% added corn oil, coconut fat, or tallow were prepared by adding the respective fat to a basic (control) ration and were fed to pigs for 5 weeks. The pigs were slaughtered and the carcasses were allowed to chill at $2^{o}C$ for 48 hr. before fat samples were collected from six different carcass locations on each pig. These included the back fat at three different locations (1) above the third rib (2) above the last rib (3) above the latissimus dorsi, the leaf fat, the fat from the top of the ham, and the inter-muscular fat between the shoulders. Fatty acid analyses were carried out as previously described. The fatty acid composition of the fat taken from these various locations are presented in the following Tables A1 through A6.

The influence of dietary fat on the pattern of fatty acid deposition in non-ruminants has been summarized by Pearson et al. (1977). According to these authors, non-ruminants readily incorporate the unsaturated fatty acids of the diet into the depot fats.

Table A1. Fatty acid composition of the intermuscular fat of pigs fed various supplemented diets

Fatty acid composition (percent)[a]	Diet			
	Control	Corn Oil	Tallow	Coconut Fat
C12	0.12	---	---	4.63
C14	1.72	1.41	1.84	8.31
C16	23.45	15.60	21.23	22.48
C18	12.60	8.90	12.01	11.78
Total Saturated	37.89	25.91	35.08	47.2
C16:1	3.62	2.30	3.81	4.10
C18:1	44.83	35.17	49.40	34.53
C18:2	13.02	34.53	9.05	13.09
C18:3	0.27	0.10	1.40	0.18
Total Unsaturated	61.74	72.10	63.66	51.90
Sat./Unsat. ratio	0.61	0.36	0.55	0.91

[a] Average fatty acid data from five pigs per treatment.

Table A2. Fatty acid composition of the leaf fat of pigs fed various supplemented diets

Fatty acid composition (percent)[a]	Diet			
	Control	Corn Oil	Tallow	Coconut Fat
C12	---	---	---	3.68
C14	1.70	1.35	1.71	8.74
C16	26.55	20.94	24.02	29.21
C18	18.40	13.08	15.94	15.41
Total Saturated	46.65	35.37	41.67	57.04
C16:1	2.18	1.55	3.01	3.18
C18:1	37.46	30.79	44.39	29.77
C18:2	12.03	30.76	8.47	9.41
C18:3	1.03	0.70	1.23	0.46
Total Unsaturated	52.70	63.80	57.10	42.82
Sat./Unsat. ratio	0.89	0.55	0.73	1.33

[a] Average fatty acid data from five pigs per treatment.

Table A3. Fatty acid composition of the backfat (over the third rib) of pigs fed various supplemented diets

Fatty acid composition (percent)[a]	Diet			
	Control	Corn Oil	Tallow	Coconut Fat
C12	---	---	---	3.80
C14	0.77	0.67	0.84	7.72
C16	16.03	13.60	15.88	20.15
C18	8.80	8.50	10.18	10.13
Total Saturated	25.60	22.77	26.90	41.80
C16:1	3.67	1.80	5.38	5.10
C18:1	48.03	36.86	52.88	38.37
C18:2	22.53	37.97	15.75	13.97
C18:3	0.10	0.53	0.50	0.30
Total Unsaturated	74.33	77.16	74.51	57.74
Sat./Unsat. ratio	0.34	0.30	0.36	0.73

[a] Average fatty acid data from five pigs per treatment.

Table A4. Fatty acid composition of the backfat (over the last rib) of pigs fed various supplemented diets

Fatty acid composition (percent)[a]	Diet			
	Control	Corn Oil	Tallow	Coconut Fat
C12	---	---	---	3.30
C14	1.55	0.96	1.12	6.67
C16	17.40	13.65	16.54	19.32
C18	11.32	8.22	9.32	9.96
Total Saturated	30.27	22.83	26.98	39.25
C16:1	3.96	1.77	4.90	4.34
C18:1	47.16	38.72	54.22	40.52
C18:2	18.14	36.15	13.10	15.60
C18:3	0.60	0.70	0.38	---
Total Unsaturated	69.86	77.34	72.60	60.46
Sat./Unsat. ratio	0.43	0.30	0.37	0.65

[a] Average fatty acid data from five pigs per treatment.

Table A5. Fatty acid composition of the backfat (over the latissimus dorsi) of pigs fed various supplemented diets

Fatty acid composition (percent)[a]	Diet			
	Control	Corn Oil	Tallow	Coconut Fat
C12	---	---	---	3.30
C14	1.00	0.90	1.00	7.00
C16	14.98	14.00	15.78	18.70
C18	8.85	8.45	8.95	9.93
Total Saturated	24.83	23.35	25.73	38.93
C16:1	4.48	2.43	3.73	6.10
C18:1	49.83	37.15	53.60	39.73
C18:2	20.48	36.08	18.15	15.40
C18:3	---	0.05		
Total Unsaturated	74.79	76.73	75.48	61.23
Sat./Unsat. ratio	0.33	0.30	0.34	0.64

[a] Average fatty acid data from five pigs per treatment.

Table A6. Fatty acid composition of the ham fat of pigs fed various supplemented diets

Fatty acid composition (percent)[a]	Diet			
	Control	Corn Oil	Tallow	Coconut Fat
C12	0.10	---	---	2.90
C14	1.90	1.38	1.37	6.97
C16	15.10	16.43	12.87	22.13
C18	9.00	7.53	7.50	8.62
Total Saturated	26.10	25.34	21.74	40.62
C16:1	4.20	3.43	5.70	5.48
C18:1	46.50	38.48	51.97	41.22
C18:2	22.80	31.43	19.53	11.07
C18:3	0.10	1.03	1.10	0.67
Total Unsaturated	73.60	74.37	78.3	58.44
Sat./Unsat. ratio	0.35	0.34	0.28	0.70

[a] Average fatty acid data from five pigs per treatment.

BIBLIOGRAPHY

Amundson, C.M., Sebranek, J.G., Rust, R.E., Kraft, A.A., Wagner, M.K. and Robach, M.C. 1982a. Effect of belly composition on sorbate-cured bacon. J. Food Sci. 47:218.

Amundson, C.M., Sebranek, J.G., Kraft, A.A, Rust, R.E., Wagner, M.K. and Robach, M.C. 1982b. Effect of belly handling (chilled, frozen, preriogor) and smoked delay on regular and sorbate-cured bacon. J. Food Sci. 47:222.

A.O.A.C. 1975. Official Methods of Analysis, 12 edition, Association of Official Analytical Chemists, Washington, D.C.

A.O.C.S. 1973. Official Method Da 15-48. American Oil Chemists' Society, Champaign, IL.

Archer, M.C., Clark, S.D., Thilly, J.E. and Tannenbaum, S.R. 1971. Environmental nitroso compounds: reaction of nitrite with creatine and creatinine. Science 174:1341.

Archer, M.C., Tannenbaum, S.R., Fan, T.Y. and Weisman, M. 1975. Reaction of nitrite with ascorbate and its relation to nitrosamine formation. J. Natl. Cancer Inst. 54:1203.

Ashton, M.R. 1970. The occurrence of nitrates and nitrites in foods. The British Food Manufacturing Industries Research Association, Lit. Surv. No. 7.

Bailey, M.E. and Swain, J. 1973. Influence of nitrite on meat flavor. Proc. Meat Industry Research Conf., American Meat Institute Foundation, Chicago, IL, p. 29.

Bharucha, K.R., Cross, C.K. and Rubin, L.J. 1979. Mechanism of N-nitroso-pyrrolidine formation in bacon. J. Agric. Food Chem. 27:63.

Bharucha, K.R., Cross, C.K. and Rubin, L.J. 1980. Long chain acetals of ascorbic and erythorbic acids as antinitrosamine agents for bacon. J. Agric. Food Chem. 28:1274.

Birdsall, J.J. 1981. Human Dietary Nitrite Intake: and, Results of Animal Feeding Studies of Nitrite. Paper presented at the public meeting of the Committee on Nitrates, Nitrites and Alternative Curing Agents in Food, January 22, 1981, National Research Council, National Academy of Sciences, Washington, D.C. p. 16.

Bokhoven, C. and Niessen, H.J. 1961. Amounts of oxides of nitrogen and carbon monoxide in cigarette smoke, with and without inhalation. Nature 192:458.

Brooks, J., Haines, R.B., Marion, T. and Pace, J. 1940. The function of nitrate, nitrite and bacteria in the curing of bacon and hams. Special Report 49. Food Investigation Board, Dept. Sci. Ind. Res. Gt. Britain.

Burden, E.H.W.J. 1961. The toxicology of nitrates and nitrites with particular reference to the potability of water supplies. Analyst 68:429.

Cantoni, C., Bianchi, M.A., Rennon, P. and D'Aubert, S. 1969. Putrefaction of sausages. Archiso Veterinario Italiano 20:245.

Cassens, R.G., Greaser, M.L., Ito, T. and Lee, M. 1979. Reactions on nitrite in meat. Food Technol. 33(7):46.

Castell, C.H., Smith, B. and Neal, W. 1971. Production of dimethylamine in muscle of several species of gadoid fish during frozen storage, especially in relation to presence of dark muscle. J. Fish Res. Bd. Can. 23:1.

Challis, B.C. and Lawson, A.J. (1971). The chemistry of nitroso-compounds Part II. The nitrosation of phenol and anisole. J. Chem. Soc. B:770-775.

Challis, B.C. 1973. Rapid nitrosation of phenols and its implications for health hazards from dietary nitrite. Nature 244:466.

Challis, B.C. and Bartlett, C.D. 1975. Possible carcinogenic effects of coffee constituents. Nature 254:532.

Challis, B.C. and Kyrtopoulos, S.A. 1977. Rapid formation of carcinogenic N-nitrosamines in aqueous alkaline solution. Br. J. Cancer 35:693.

Challis, B.C., Edwards, A., Hunna, R.R., Kyrtopoulos, S.A. and Outram, J.R. 1978. Rapid formation of N-nitrosamines from nitrogen oxides under neutral and alkaline conditions. pp. 127-142 in E.A. Walker, L. Griciute, M. Castegnaro and R.E. Lyle, eds. Environmental Aspects of N-nitroso Compounds. IARC Sci. Publ. No. 19. IARC, Lyon France.

Challis, B.C. and Kyrtopoulos, S.A. 1978. The chemistry of nitroso compounds. Part 12. The mechanism of nitrosation of aqueous piperdien by gaseous dinitrogen tetraoxide and dinitrogen trioxide in aqueous alkaline solutions. Evidence for the existence of molecular isomers of dinitrogen tetraoxide and dinitrogen trioxide. J. Chem. Soc. Perkin Trans. II:1296.

Challis, B.C. and Kyrtopoulos, S.A. 1979. The chemistry of nitroso-compounds. Part II. Nitrosation of amines by the two-phase interaction of amines of solution with gaseous oxides of nitrogen. J. Chem. Soc. Perkin Trans. I:299.

Christiansen, L.N., Johnston, R.W., Kawkker, D.A., Howard, J.W. and Aunan, W.J. 1973. Effects of nitrite and nitrate on toxin production by Clostridium botulinum and on N-nitrosamine formation in perishable canned comminuted cured meat. Appl. Microbiol. 25:357.

Clifford, M.N., Tang, S.L. and Eyog, A.A. 1980. Smoking of foods. Process. Biochem. 15: 8-11, 17, 26.

Coleman, M.H. 1978. A model system for the formation of N-nitroso-pyrrolidine in grilled or fried bacon. J. Food Technol. 13:55.

Comly, H.H. 1945. Cyanosis in infants caused by nitrates in well water. J. Am. Med. Assoc. 129:112.

Cottrell, R.C., Young, P.J., Walters, D.G., Phillips, J.C., Lake, B.G. and Gangolli, S.D. 1979. Studies of the metabolism of N-nitrosopyrrolidine in the rat. Toxicol. Appl. Pharmacol. 51:101.

Crosby, N.T., Foreman, J.K., Palframan, J.F. and Sawyer, R. 1972. Estimation of steam-volatile N-nitrosamines in foods at the 1 μg/kg level. Nature 238:342.

Crosby, N.T. and Sawyer, R. 1976. N-Nitrosamines. A review of chemical and biological properties and their estimation in food stuffs. Adv. Food Res. 22:1.

Dahn, L., Loewe, L. and Bunton, C.A. 1960. Uber die Oxydation von Abscorbinaure durch salpetriqe Saure. Teil VI: Ubersicht und Dis Kussion der Ergebnisse. Helvet. Chim. Acta. 43:320.

Davies, R. and McWeeny, D. 1977. Catalytic effect of nitrosophenols on N-nitrosamine formation. Nature. 266:657.

Davies, R., Dennis, M.J., Massey, R.C. and McWeeny, D.C. 1978. Some effects of phenol and thiol-nitrosation. Reactions on N-nitrosamine formation. In: Environmental Aspects of N-Nitroso Compounds, IARC Scientific Publications No. 19. International Agency for Research on Cancer, Lyon, France, p. 183.

Davies, R., Massey, R.C. and McWeeny, D.J. 1980. The catalysis of the N-nitrosation of secondary amines by nitrosophenols. Food Chem. 6:115.

Dennis, M.J., Massey, R.C. and McWeeny, D.J. 1982. The effect of oxygen on nitrosamine formation in bacon. Z. Lebensm. Unters. Forsch. 174:114.

Dierick, N., VandeKerCkhove, and Demeyer, D. 1974. Changes in nonprotein nitrogen compounds during dry sausage ripening. J. Food Sci. 39:301.

Douglass, M.L., Kabacoff, B.L., Anderson, G.A. and Cheng, M.C. 1978. The chemistry of nitrosamine formation, inhibition and destruction. J. Soc. Cosmet. Chem. 29:581.

Druckrey, H., Preussmann, R. and Ivankovic, S. 1969. Nitroso compounds in organotropic and transplacental carcinogenesis. Ann. N.Y. Acad. Sci. 163:676.

Dudley, R. 1979. How to keep your bacon out of trouble. Meat Ind. 26(2):24.

Ehrenberg, L., Hussain, S., Saleh, M.N. and Lundquist, U. 1980. Nitrous esters - A genetical hazard from nitrogen oxides (NO_x)? Hereditis 92:127-130.

Eisenbrand, G., Janzowski, C. and Preussmann, R. 1976. Analysis formation and occurence of volatile and non-volatile N-nitroso compounds - recent results. Paper presented at the Second Symposium on Nitrite in Meat Products, Zeist, The Netherlands.

Eisenbrand, C., Spigelhalder, B., Janzowski, C., Kann, J. and Preussmann, R. 1977. Volatile and non-volatile N-nitroso compounds in foods and other environmental media. Presented at the 5th IARC Meeting on Analysis and Formation of N-Nitroso Compounds, August 22-24, 1977, University of New Hampshire, Durham, N.H.

Ender, F. and Ceh, L. 1971. Conditions and chemical reaction mechanisms by which nitrosamines may be formed in biological products with reference to their possible occurence in food products. Z. Lebensm. Unters. Forsch. 145:133.

Erlandsson, G.B. 1981. Fate of nitrite. Paper submitted to the Committee on Nitrites and Alternative Curing Agents in Foods, Executive Office Assembly of Life Sciences, National Research Council. National Academy of Sciences, Washington, D.C., p. 4.

Evans, G.G. and Ranken, M.D. 1975. Fat cooking losses from non-emulsified meat products. J. Fd. Technol. 10:63.

Fan, T.Y. and Tannenbaum, S.R. 1973. Natural inhibitors of nitrosation reactions: the concept of available nitrite. J. Food Sci. 38:1067.

Federal Register, 1975. 40; p. 52614.

Fiddler, W., Pensabene, J.W., Doerr, R.C. and Wasserman, A.E. 1972. Formation of N-nitrosodimethylamine from naturally occurring quanternary ammonium compounds and tertiary amines. Nature 236:307.

Fiddler, W., Pensabene, J.W., Fagan, J.C., Thorne, E.J., Piotrowski, E.G. and Wasserman, A.E. 1974. The role of lean and adipose tissue on the formation of nitrosopyrrolidine in fried bacon. J. Food Sci. 39:1070.

Fiddler, W., Pensabene, J.W., Piotrowski, E.A., Phillip, J.G., Keating, J., Mergens, W.J., and Newmark, H.L. 1978. Inhibition of formation of volatile nitrosamines in fried bacon by the use of cure-solubilized α-tocopherol. J. Agric. Food Chem. 26:653.

Fine, D.H. 1977. An assessment of human exposure to N-nitroso compounds. Presented at the 5th IARC meeting on Analysis and Formation of N-Nitroso Compounds, August 22-24, 1977, University of New Hampshire, Durham, N.H.

Fooladi, M.H., Pearson, A.M., Coleman, T.H. and Merkel, R.A. 1979. The role of nitrite in preventing development of warmed-over flavor. Food Chem. 4:283.

Foster, W.W. and Simpson, T.H. 1961. Studies of the smoking process for foods. I. The importance of vapors. J. Sci. Food Agric. 12:363.

Frouin, A. 1976. Nitrates and nitrites: reinterpretation of analytical data by means of bound nitric oxide. Proc. 2nd. Int. Symp. Nitrite in Meat Products. PUDOC, Zeist, Netherlands, pp. 115-120.

Fujinaka, N., Masuda, Y., and Kuratsune, M. 1976. Methylguanidine content in food. Gann 67:679.

Gilbert, J. and Knowles, M.E. 1975. The chemistry of smoked foods. A review. J. Food Tech. 10:245.

Gill, J.L. 1978. Design and Analysis of Experiments in the Animal and Medical Sciences. Vol. 1 Ames, Iowa: The Iowa State University Press.

Golovnya, R.V. and Zhuravleva, I.L. 1970. Amines in volatile substances of Russian cheese. Malochn. Prom. 38:8.

Golovnya, R.V. 1976. Analysis of volatile amines contained in foodstuffs as possible precursors of N-nitroso compounds. In: Environmental N-Nitroso Compounds Analysis and Formation. Walker, E.A., Bogovski, P. and Griciute, L. (eds.), International Agency on Cancer, Lyon. Scientific Publication No,. 14, p. 237.

Gorbatov, V.M., Krylova, N.N., Voloinskaya, V.P., Lyaskovskaya, Yu, N., Bazarova, K.I., Khlamova, R.I. & Yakovleva, G. Yu. (1971). Liquid smokes for use in cured meats. Food Technol. 25(1):71-77.

Gough, T.A. and Goodhead, K. 1975. Occurence of volatile nitrosamines in spice mixes. J. Sci. Food Agric. 26:1473.

Gough, T.A. and Walters, C.L. 1976. Volatile nitrosamines in fried bacon. In: Environmental N-Nitroso Compounds: Analysis and Formation. Walker, E.A., Bogovski, P. and Griciute, L., ed. Lyon, France, IARC Sci. Pub. No. 14, pp. 195.

Gough, T.A., McPhail, M.F., Webb, K.S., Wood, B.J. and Coleman, R.F. 1977. An examination of some foodstuffs for the presence of volatile nitrosamines. J. Sci. Food Agric. 28:345.

Goutefongea, R., Cassens, R.G. and Woolford, G. 1977. Distribution of sodium nitrite in adipose tissue during curing. J. Food Sci. 42:1637.

Gray, J.I. and Dugan, L.R. 1975. Inhibition of N-nitrosamine formation in model food systems. J. Food Sci. 40:981.

Gray, J.I. 1976. N-Nitrosamines and their precursors in bacon: A review. J. Milk Food Technol. 39:686.

Gray, J.I., Collins, M.E. and Russell, L.F. 1977. Formation of N-nitroso-hydoxypyrrolidine in model and cured meat systems. Can. Inst. Food Sci. Technol. J. 10:36.

Gray, J.I. and Collins, M.E. 1977a. A comparison of proline and putrescine as precursors of N-nitrosopyrrolidine in nitrite-treated pork systems. J. Food Sci. 42:1034.

Gray, J.I. and Collins, M.E. 1977b. The development of free proline during the storage of green pork bellies. Can. Inst. Food Sci. Technol. J. 10:97.

Gray, J.I., Collins, M.E. and MacDonald, B. 1978. Precursors of dimethylnitrosamine in fried bacon. J. Food Prot. 41:31.

Gray, J.I. and Randall, C.J. 1979. The nitrite/N-nitrosamine problem in meats: An update. J. Food Prot. 42:168.

Gray, J.I., Irvine, D.M. and Kakuda, Y. 1979. Nitrates and N-nitrosamines in cheese. J. Food Prot. 42:263.

Gray, J.I. , Bussey, D.M., Dawson, L.E., Price, J.F., Stevenson, K.E., Owens, J.L., and Robach, M.C. 1981. Investigation into the formation of N-nitrosamines in heated chicken frankfurters. J. Food Sci. 46(6):1817.

Gray, J.I. 1981. Formation of N-nitroso compounds in foods. In: N-Nitroso Compounds. Scanlan, R.A. and Tannenbaum, S.R. (ed.), Am. Chem. Soc. Symp. Series 174, Washington, D.C. p. 165.

Greenberg, R.A. 1976. Nitrosopyrrolidine in United States cured meat products. Proc. 2nd Int. Symp. Nitrite Meat Prod., Zeist, Pudoc. Wageningen, p. 203.

Hansen, T., Iwaoka, W.T., Green, L. and Tannenbaum, S.R. 1977. Analysis of N-nitrosoproline in raw bacon. Further evidence that nitrosoproline is not a major precursor of nitrosopyrrolidine. J. Agric. Food Chem. 25:1423.

Hanst, P.L., Spence, J.W., and Miller, M. 1977. Atmospheric chemistry of N-nitrosodimethylamine. Environ. Sci. Technol. 11 403.

Hartmann, T. 1967. Biosynthesis of volatile amines in apple-fruit by aldehyde amination. Z. Ptlanzenphysiol. 51:368.

Hartman, P.E. 1982. Nitrates and nitrites: ingestion, pharmacodynamics and toxicology. In: Chemical Mutagens, deSerres, F.J. and Hollaender, A. (eds.), Plenum Press, New York, vol. (7):

Havery, D.C., Fazio, T. and Howard, J.W. 1978. Trends in levels of N-nitrosopyrrolidine in fried bacon. J. Assoc. Off. Anal. Chem. 61:1379.

Havery, D.C., Kline, D.A., Miletta, E.M., Joe, F.L. and Fazio, T. 1976. Survey of food products for volatile N-nitrosamines. J. Assoc. Off. Anal. Chem. 59:540.

Hecker, L.I., Elespuru, R.K. and Farrelly, J.G. 1979. The mutagenicity of nitrosopyrrolidine is related to its metabolism. Mutation. Res. 62:213.

Heisler, E.G., Siciliano, J., Krulick, S., Feinberg, J. and Schwartz, J.H. 1974. Changes in nitrate and nitrite content, and search for nitrosamines in storage-abused spinach and beets. J. Agric. Food Chem. 22:1029.

Herdlicka and Janicek. 1964. Volatile amines as components of toasted oat flakes. Nature (London) 49:1201.

Herring, H.K. 1973. Effects of nitrite and other factors on the physico-chemical characteristics on nitrosamine formation in bacon. Proc. Meat Industry Research Conf., American Meat Institute Foundation, Chicago, IL, p. 47.

Hisatune, I.C. 1961. Thermodynamic properties of some oxides of nitrogen. J. Phys. Chem. 65:2249.

Hollenbeck, C.M., Moore, D.G. and Wendorf, W.L. 1973. Some facets of the smoke flavoring process. Proceedings of the Meat Industry Research Conference. American Meat Institute Foundation, Chicago, IL.

Huxel, E.T., Scanlan, R.A., and Libbey, L.M. 1974. Formation of N-nitroso-pyrrolidine from pyrrolidine ring containing compounds at elevated temperatures. J. Agric. Food Chem. Vol. 22, 4:698.

Hwang, L.S. and Rosen, J.D. 1976. Nitrosopyrrolidine formation in fried bacon. J. Agric. Food Chem. 24:1152.

International Agency for Research on Cancer. 1978. Environmental Carginogens: Selected Methods of Analysis. Analysis of Volatile Nitrosamines in Food. IARC Scientific Publication No. 18. International Agency for Research on Cancer, Lyon, France, vol. 1, p. 212.

Issenberg, P. and Virk, M. 1974. Quoted by, Issenberg, P. 1976. Nitrite, nitrosamines and cancer. Fed. Proc. 35:1322.

Janzowski, C., Eisenbrand, G. and Preussmann, R. 1978. Occurrence of N-nitrosamino acids in cured meat products and their effect on formation of N-nitrosamines during heating. Food Cosmet. Toxicol. 16:343.

Jones, K. 1973. The chemistry of nitrogen. In: Comprehensive Inorganic Chemistry, Vol. 2. Bailar, J.C., Emeteus, H.J., Hyholm, R. and Trotman-Dickenson, A.F. (eds.). Pergamon Press, New York, Chapter 19, p. 372.

Kakuda, Y. and Gray, J.I. 1980. N-Nitrosamides and their precursors in food systems. I. Formation of N-substituted amides. J. Agr. Food Chem. 28:580.

Kamm, J.J., Dashman, T., Conney, A.H. and Burns, J.J. 1973. Protective effect of ascorbic acid on hepatotoxicity caused by nitrite plus aminopyrine. Proc. Natl. Acad. Sci. 70:747.

Kamm, J.J., Dashman, T., Conney, A.H. and Burns, J.J. 1975. Effect of ascorbic-acid on amine-nitrite toxicity. Ann. N.Y. Acad. Sci. 258:169.

Kamm, J.J., Dashman, T., Newmark, H.L. and Mergens, W.J. 1977. Inhibition of amine-nitrite hepatotoxicity by alpha-tocopherol. Toxicol. Appl. Pharm. 41:575.

Kawabata, T., Ohshima, H., Uibu, J., Nakamura, M., Matsui, M. and Hamano, M. 1979. Occurrence, formation and precursors of N-nitroso compounds in Japanese diet. In: Naturally Occurring Carcinogens. Mutagens and Modulators of Carcinogenesis. Miller, E.C., Miller, J.A., Hironi, I., Sugimura, T. and Takayama, S. (eds.), Japan Scientific Society Press, Tokyo, Japan and University Park Press, Baltimore, Maryland, p. 195.

Kawamura, T., Sakai, K., Miyazawa, F., Wada, H., Ito, Y. and Tanimura, A. 1971. Nitrosamines in foods. V. Distribution of secondary amines in foods. (2). Agric. Biol. Chem. (Tokyo) 12:p. 394.

Knowles, M.E. 1974. Nitrosation of phenols in smoked bacon. Nature (London) 239:672.

Knowles, M.E., Gilbert, J. and McWeeny, D.J. 1975. Phenols in smoked cured meats. Phenolic composition of commercial liquid smoke preparations and derived bacon. J. Sci. Food Agric. 26:189.

Koppang, N. and Rimeslatten, H. 1976. Toxic and carcinogenic effects of nitrosodimethylamine in milk. In: Environmental N-Nitroso Compounds Analysis and Formation, Walker, E.A., Gogovski, P. and Griciute, L. (Eds.), International Agency for Research on Cancer, Lyon, Scientific Publication No. 14, p. 443.

Kristoffersen, T. 1963. Cheese flavor in perspective. Manuf. Milk Prod. J. 54:12.

Ladd, K.F., Newmark, H.L. and Archer, M.C. 1983. Increased endogenous nitrosation in smokers. Paper presented at the 8th International Meeting on N-Nitroso Compounds - Occurrence and Biological Effects, Banff, Canada.

Lakritz, L., Spinelli, A.M. and Wasserman, A.E. 1975. Determination of amines in fresh and processed pork. J. Agric. Food Chem. 23:344.

Lakritz, L., Spinelli, A.M. and Wasserman, A.E. 1976. Effect of storage on the concentration of proline and other free amino acids in pork bellies. J. Food Sci. 41:879.

Landmann, W.A. and Batzer, O.F. 1966. Influence of processing procedures on the chemistry of meat flavors. J. Agric. Food Chem. 14:210.

Lijinsky, W., Keefer, L. and Loo, J. 1970. The preparation and properties of some nitrosamino acids. Tetrahedron. 26:5137.

Lijinksy, W. 1977. Nitrosamines and nitrosamides in the etiology of gastrointestinal cancer. Cancer 40:2446.

Lin, J.K. and Lue, R.Y. 1979. Studies on the chemical toxicants in Chinese foods. II. The levels of nitrite and nitrate in edible vegetable and secondary amines in fish and shrimp. Natn. Sci. Council Monthly (R.O.C.) 7(5):485.

Lin, J.K. and Yen, J.Y. 1980. Changes in the nitrate and nitrite contents of fresh vegetables during cultivation and post-harvest storage. Food Cosmet. Toxicol. 18:597.

Lucke, F.K. and Leistner, L. 1979. Temperaturanforderungen an Freischerzeugnisse mit vermindestem nitsitgehalt. Die Fleischerei. d30:692.

MacDonald, B., Gray, J.I. , Kakuda, Y. and Lee, M.L. 1980. Role of nitrite in cured meat flavor: Chemical analysis. J. Food Sci. 45:889.

Maga, J.A. 1978. Amines in foods. C.R.C. Critical Rev. Food Technol. 8:373.

Magee, P.N., Montesano, T. and Preussmann, R. 1976. N-Nitroso compounds and related carcinogens. In: Chemical Carcinogens, Searle, C.E. (Ed.), American Chemical Society, New York, p. 490.

Maier, H.G. 1970. Volatile flavoring substances in food stuffs. Angew Chem. 9:917.

Marion, L. 1950. The pyrridine alkaloids. In: The Alkaloid Chemistry and Physiology, Maske, R.H.F. and Holmes, H.L. (eds.), Academic Press, Inc., New York, p. 167.

Maynard, D.N., Baker, A.V., Minotti, P.L, and Peck, N.H. 1976. Nitrate accumulation in vegetables. Adv. Agron. 28:71.

Mergens, W.J., Kamm, J.J., Newmark, H.L., Fiddler, W. and Pensabene, J.W. 1978. Alpha-tocopherol: Uses in preventing nitrosamine formation. In: Environmental Aspects of N-Nitroso Compounds. Walker, E.A., Castegnaro, M., Griuite, L., and Lyle, R.E. (eds.), IARC Publication No. 19. Lyon, France, p. 199.

Mergens, W.J. and Newmark, H.L. 1979. The use of alpha-tocopherol in bacon processing - an update. Proc. Meat Industry Research Conf., America Meat Institute Foundation, Chicago, IL, p. 79.

Mergens, W.J. and Newmark, H.L. 1980. Antioxidants as blocking agents against nitrosamine formation. In: Autoxidation in Foods and Biological Systems, Simic, M. and Karel, M., (eds.), Plenum Press, New York and London, p. 387.

Mirvish, S.S. 1970. Kinetics of dimethylamine nitrosation in relation to nitrosamine carcinogenesis. J. Natl. Cancer Inst. 44:633.

Mirvish, S.S. 1971. Kinetics of nitrosamide formation from alkylureas, N-alkylurethanes, and alkylguanidines: Possible implications for the etiology of human gastric cancer. J. Nat. Cancer Inst. 46:1183.

Mirvish, S.S. 1972a. Kinetics of nitrosation reaction in relation to tumorigenesis experiments with nitrite plus amines or ureas. In: N-Nitroso compounds in the Environment, Bogovski, P., Preussmann, R. and Walker, E.A. (eds.), International Agency for Research on Cancer, Lyon, Scientific Publication No. 3, p. 104.

Mirvish, S.S., Wallcave, L., Eagen, M. and Shubik, P. 1972b. Ascorbate-nitrite reaction: possible means of blocking the formation of carcinogenic N-nitroso-compounds. Science 177:65.

Mirvish, S.S. 1975. Formation of N-nitroso-compounds: chemistry, kinetics and in vivo occurrence. Toxicol. Appl. Pharmacol. 31:325.

Mirvish, S.S. 1977. N-Nitroso compounds: their chemical and in vivo formation and possible importance as environmental carcinogens. J. Toxicol. Environ. Health 2:1267.

Mirvish, S.S. and Cairnes, D.D. 1981. Identification of the compound in a fish product yielding methylurea (MU) on nitrosation-denitrosation as creatinine (CRN). Proc. Am. Assoc. Cancer Res.

Mirvish, S.S. 1981. Inhibition of the formation of carcinogenic N-nitroso compounds by ascorbic acid and other compounds. In: Cancer; Achievements, Challenges, Prospects, Birchenall, J.H. (ed.).

Mirvish, S.L. and Sams, J.P. 1983. A nitrosating agent from the reaction of atmosphere nitrogen dioxide (NO_2) with methyl linoleate: comparison with a product from the skins of NO_2-exposed compounds, Banff, Alberta, Canada, September 4-10. Paper presented at the 8th International Meeting on N-Nitroso Compounds, Banff, Alberta, Canada.

Mohler, K., and Hallermayer, E. 1973. Buldung von Nitrosaminen aus Lecithin und Nitrit. Z. Lebensm. Unters-Forsch. 151:52.

Morrison, W.R. and Smith, L.M. 1964. Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol. J. Lipid Res. 5:600.

Morruzzi, G. and Caldavera, C.M. 1964. Occurence of polyamines in the germs of cereals. Arch. Biochem. Biophys. 105:209.

Mottram, D.S., Patterson, R.C., Rhodes, D. and Gough, T. 1975. Influence of ascorbic acid and pH on the formation of N-nitrosodimethylamine in cured pork containing added dimethylamine. J. Sci. Food Agric. 26:47.

Mottram, D.S. and Patterson, R.L.S. 1977. The effect of ascorbate reductants on N-nitrosamine formation in a model system resembling bacon fat. J. Sci. Food Agric. 28:352.

Mottram, D.S., Patterson, R.L.S., Edwards, R.A. and Gough, T.A. 1977. The preferential formation of volatile N-nitrosamine in the fat of fried bacon. J. Sci. Fd. Agric. 28:1025.

Mulders, E.J. 1973. Volatile components from the non-enzymatic browning reaction of the cysteine, cysteine-ribose system. Z. Lebensm. Unters. Forsch. 152:193.

Nakamura, M., Baba, N., Nakaska, T., Wada, Y., Ishibishi, T. and Kawabata, T. 1976. Pathways of formation of N-nitrosopyrrolidine in fried bacon. J. Food Sci. 41:874.

National Academy of Sciences. 1981. The Health Effects of Nitrate, Nitrite and N-Nitroso Compounds. National Academy Press. Washington, D.C.

Newmark, H.L. and Mergens, W.J. 1981a. Blocking Nitrosamine Formation using Ascorbic Acid and -Tocopherol. In: Gastronintestinal Cancer, Endogenous Factors, Bruce, W.R., Correa, P., Lipkin, M., Tannenbaum, S.R. and Wilkins, T.D. (eds.) Cold Spring Harbor Laboratory, p. 267.

Nitrite Safety Council. 1980. A survey of nitrosamines in sausages and dry-cured meat products. Food Technol. 34(7):45.

Oda, H., Tsubone, H., Suzuki, A., Ichinose, T., and Kubota, K. 1981. Alterations of nitrite and nitrate concentrations in the blood of mice exposed to nitrogen dioxide. Environ. Res. 25:294.

Ohshima, H. and Bartsch, H. 1981. Quantitative estimation of endogenous nitrosation in humans by monitoring N-nitrosoproline excreted in the urine. Cancer Res. 41:3658.

Olajos, E.J. 1977. Biological interactions of N-nitroso compounds. A review. Ecotox. Environ. Safety 1:175.

Owens, J.L. and Kinast, O.E. 1980. An improved procedure for the determination of volatile N-nitrosamines in bacon grease by using the mineral oil distillation-thermal energy analyzer method. J. Agr. Food Chem. 28:1262.

Parks, N.J., Krohn, K.A., Mathis, C.A., Chasko, J.H., Geiger, K.R., Gregor, M.E. and Peeks, N.F. 1981. Nitrogen-13-labeled nitrite and nitrate: Distribution and metabolism after intratracheal administration. Science 212:58.

Patterson, R.L.S. and Mottram, D.S. 1974. The occurence of volatile amines in uncured and cured pork meat and their possible role in nitrosamine formation in bacon. J. Sci. Food Agric. 25:1419.

Patterson, R.L.S. and Edwards, R.A. 1975. Volatile amines production in uncured pork during storage. J. Sci. Food Agric. 26:1371.

Patterson, R.L.S., Taylor, A.A., Mottram, D.S. and Gough, T.A. 1976. Localised occurence of N-nitrosopyrrolidine in fried bacon. J. Sci. Food Agric. 27:257.

Pearson, A.M., Love, J.D. and Shorland, F.B. 1977. Warmed-over flavor in meat, poultry and fish. Adv. Food Res. 23:1.

Pegg, A.E. 1977. Formation and metabolism of alkylated nucleosides. Possible role in carcinogenesis by nitroso compounds and alkylating agents. Adv. Cancer Res. 25:195.

Pensabene, J.W., Fiddler, W., Gates, R.A., Fagan, J.C. and Wasserman, A.E. 1974. Effect of frying and other cooking conditions on nitrosopyrrolidine formation in bacon. J. Food Sci. 39:314.

Pensabene, J.W., Fiddler, W., Doerr, R.C., Lakritz, L. and Wasserman, A.E. 1975. Formation of dimethylnitrosamine from commercial lecithin and its components in a model system. J. Agric. Food Chem. 23:979.

Pensabene, J.W., Fiddler, Mergens, W. and Wasserman, A.E. 1978. Effect of α-tocopherol formulations on the inhibition of nitrosopyrrolidine formation in model systems. J. Food Sci. 43:801.

Pensabene, J.W., Feinberg, J.I., Piotrowski, E.G. and Fiddler, W. 1979. Occurrence and determination of N-nitrosoproline and N-nitrosopyrrolidine in cured meat products. J. Agr. Food Chem. 27:842.

Pensabene, J.W., Fiddler, W., Miller, A.J. and Phillips, J.G. 1980. Effect of preprocessing procedures for green bellies on N-nitrosopyrrolidine formation in bacon. J. Agric. Food Chem. 28:966.

Pignatelli, B., Friensen, M. and Walker, E.A. 1980. The role of phenols in catalysis of nitrosamine formation. In: N-Nitroso Compounds; Analysis, Formation and Occurence. IARC Scientific Publications No. 31, International Agency for Research on Cancer, Lyon, France, p. 95.

Preussmann, R., Schmaehl, E., Eisenbrand, G. and Port, R. 1976. Dose-response study with N-nitrosopyrrolidine and some comments on risk evaluation of environmental N-nitroso compounds. Proc. 2nd Int. Symp. Nitrite Meat Prod., Zeist, Pudoc, Wegeningen, p. 261.

Preussmann, R., Eisenbrand, G. and Spigelhalder, B. 1979. Occurrence and formation of N-nitroso compounds in the environment and in vivo. In: Environmental Carcinogenesis, Emmelot, P. and Kriek, E. (eds.). Elsevier/North-Holland Biomedical Press, Amsterdam, Holland.

Pyror, W.A. and Lightsey, J.W. 1981. Mechanisms of nitrogen dioxide reactions: Initiation of lipid peroxidation and the production of nitrous acid. Science 214:435.

Reddy, S.K., Gray, J.I., Price, J.F. and Wilkens, W.F. 1982. Inhibition of N-nitrosopyrrolidine in dry cured bacon by α-tocopherol-coated salt systems. J. Food Sci. 47:1598.

Rice, S.L., Eitenmiller, R.R. and Koehler, P.E. 1976. Biologically active amines in food: A review. J. Milk Food Technol. 39(5):353.

Robach, M.C., Owens, J.L., Panquette, Sofos, J.N. and Busta, F.F. 1980. Effects of various concentrations of sodium nitrite and potassium sorbate on nitrosamine formation in commercially prepared bacon. J. Food Sci. 45:1280.

Ruiter, A. 1973. Determination of volatile amines and amine oxides in food products. Proc. Int. Symp. Nitrite Meat Products, Zeist, Pudoc. Wageningen, p. 37.

Sander, J. 1967. Kann nitrit in der menschlichen Nahrung Ursacheeiner Krebsentstehund durch Nitrosaminbildungsein? Arch. Hyg. Bakteriol. 151:22.

Sander, J. and Swhweinsberg, F. 1972. In vivo and in vitro experiments on the formation of N-nitroso compounds from amines or amides and nitrate or nitrite. In: N-Nitroso Compounds Analysis and Formation, Bogovski, P., Preussman, R. and Walker, E.A. (eds.). International Agency for Research on Cancer, Lyon, Scientific Publication No. 3, p. 97.

Scanlan, R.A., Lohlen, S.M., Bills, D.D. and Libby, L.M. 1974. Formation dimethylnitrosamine from dimethylamine and trimethylamine at elevated temperatures. J. Agr. Food Chem. 22:149.

Scanlan, R.A. 1975. N-Nitrosamines in foods. Crit. Rev. Food Technol. 5:357.

Schmahl, D. 1980. Risk assessment of N-nitroso compounds for human health. Oncology 37:193.

Sebranek, J.G. 1979. Advances in the technology of nitrite use and consideration of alternatives. Food Technol. 33(7):58.

Sen, N.P., Smith, D.C. and Schwinghamer, L. 1969. Formation of N-nitroamines from secondary amines and nitrite in human and animal gastric juice. Food Cosmet Toxicol. 7:301.

Sen, N.P., Miles, W.F., Donaldson, B., Panalaks, T. and Iyengar, J.R. 1973a. Formation of nitrosamines in a meat curing mixture. Nature (London) 245:104.

Sen, N.P., Donaldson, B., Iyengar, J.R. and Panalaks, T. 1973b. Nitrosopyrrolidine and dimethylnitrosamine in bacon. Nature 241:473.

Sen, N.P. and Donaldson, B. 1974. The effect of ascorbic acid and glutathione on the formation of nitrosopiperazines from piperazine adipate and nitrite. In: N-Nitroso Compounds in the Environment. Bogovski, P., Walker, E.A. and Davis, W. (eds.). IARC Scientific Publication. No. 9.

Sen, N.P. and McKinley, W.P. 1974. Meat curing agents as one source of nitrosamines. Proc. IV Int. Congress Food Sci. and Technol. Vol. III, p. 476.

Sen, N.P., Iyengar, J.R., Donaldson, B. and Panalaks, T. 1974. Effect of sodium nitrite concentration on the formation of nitrosopyrrolidine and dimethylnitrosamine in fried bacon. J. Agric. Food Chem. 22:540.

Sen, N.P., Donaldson, B., Seaman, S., Iyengar, J.R. and Miles, W.F. 1976a. Inhibition of nitrosamine formation in fried bacon by propyl gallate and L-ascorbyl palmitate. J. Agric. Food Chem. 24:397.

Sen, N.P., Iyengar, J.R., Miles, W.F. and Panalaks, T. 1976c. Nitrosamines in cured meat products. In: Environmental N-Nitroso Compounds Analysis and Formation, Walker, E.A., Bogovski, P., Griciute, L. (eds.), International Agency for Research on Cancer, Lyon, France.

Sen, N.P., Seaman, S., and Miles, W.F. 1976b. Dimethylnitrosamine and nitrosopyrrolidine in fumes produced during the frying of bacon. Food Cosmet. Toxicol. 14:167.

Sen, N.P., Donaldson, B., Seaman, S., Iyengar, J.R. and Miles, W.F. 1977. Recent studies in Canada on the analysis and occurence of volatile and non-volatile N-nitroso compounds in foods. Presented at the 5th IARC meeting on Analysis and Formation of N-Nitroso Compounds, August 22-24, 1977. University of New Hampshire, Durham, N.H.

Sen, N.P., Seaman, S., and Miles, W.F. 1979. Volatile nitrosamines in various cured meat products: Effect of cooking and recent trends. J. Agr. Food Chem. 27:1354.

Sen, N.P. 1980. Nitrosamines. In: Safety of Foods. Graham, H.D. (ed.), AVI Publishing Co., Westport, Conn., p. 319.

Shewan, J.M. 1951. The chemistry and metabolism of the nitrogenous extractives in fish. Biochem. Soc. Symp. (Cambridge, England), No. 6, p. 28.

Singer, G.M. and Lijinsky, W. 1976. Naturally occurring nitrosatable compounds. I. Secondary amines in food stuffs. J. Agric. Food Chem. 24:550.

Sink, J.D. and Hsu, L.A. 1977. Chemical effects of smoke processing on frankfurter manufacture and storage characteristics. J. Food Sci. 42:1489.

Sleeth, R.B., Theiler, R.F. and Rendek, R.B. 1982. Process for preparing cooked bacon having reduced levels of N-nitrosamines. U.S. Patent No. 4315948.

Spiegelhader, B., Eisenbrand, G. and Preussmann, R. 1976. Influence of dietary nitrate on nitrite content of human saliva: possible relevance to in vivo formation of N-nitroso compounds. Food Cosmet. Toxicol. 14:545.

Spinelli, A.M., Lakritz, L. and Wasserman, A.E. 1974. Effects of processing on the amine content of pork bellies. J. Agric. Food Chem. 22:1026.

Spinelli-Gugger, A.M., Lakritz, L. and Wasserman, A.E. 1980. Effect of processing on the amino acid composition and nitrosamine formation in pork belly adipose tissue. J. Agric. Food Chem. 28:1980.

Spinelli-Gugger, A.M., Lakritz, L. and Wasserman, A.E. and Gates, R.A. 1981. Nitrosamine precursors in the adipose tissue of pork bellies. J. Food Sci. 46:1136.

Tabor, H., Rosenthal, S.M. and Tabor, S.M. 1958. The biosynthesis of spermidine and spermine from putrescine and methionine. J. Biol. Chem. 233:1026.

Tanaka, K., Chung, K.C. and Hayatsu, H. 1978. Inhibition of nitrosamine formation in vitro by sorbic acid. Food Cosmet. Toxicol. 16:209.

Tannenbaum, S.R., Sinskey, A.J., Weisman, M. and Bishop, W. 1974. Nitrite in human saliva; its possible relation to nitrosamine formation. J. Nat. Cancer Inst. 53:79.

Tannenbaum, S.R., Weisman, M. and Fett, D. 1976. The effect of nitrate intake on nitrite formation in human saliva. Food Cosmet. Toxicol. 14:549.

Tannenbaum, S.R. 1978. Nitrite, Nitrate Synthesis in Human Body. In: Food Chem. News. 19, Jan. 9 issue, p. 52.

Tannenbaum, S.R., Fett, D., Young, V.R., Land, D.D. and Bruce, W.R. 1978. Nitrite and nitrate are formed by endogenous synthesis in the human intestine. Science 200:1487.

Tarr, H.L.A. 1940. Specificity of transamine-oxidase. J. Fish Res. Bd. Can. 5:187.

Theiler, R.F., Aspelund, T.G., Sato, K. and Miller, A.F. 1981a. Model system studies on N-nitrosamine formation in cured meats: the effect of slice thickness. J. Food Sci. 46:691.

Theiler, R.F., Sato, K., Aspelund, T.G. and Miller, A.F. 1981b. Model system studies on N-nitrosamine formation in cured meats: The effect of curing solution ingredients. J. Food Sci. 46:996.

Theiler, R.F., Sato, K., Aspelund, T.G. and Miller, A.F. 1984. Inhibition of N-nitrosamine formation in a cured ground pork belly model system. J. Food Sci. 49:341.

Tompkin, R.B., Christiansen, L.N. and Shaparis, A.B. 1978. The effect of iron on botulinal inhibition in perishable canned cured meat. J. Food Technol. 13:521.

Usher, C.D. and Telling, G.M. 1975. Analysis of nitrate and nitrite in food stuffs: a critical review. J. SCi. Food Agric. 26:1793.

Velisek, J. and Davidek, J. 1974. Formation of aliphatic amines by oxidation of some brown pigments. Z. Lebensm. Unters. Forsch. 154:157.

Velisek, J., Davidek, J., Klein, S., Karaskova, Vykoukova, I. 1975. The nitrosation products of creatine and creatinine in model systems. Z. Lebensm. Unters. Forsch. 159:97.

Voight, M.N. and Eitenmiller, R.R. 1974. Fluorescent quantitation of biologically active amines in foods with 7-chloro-4-nitrobenzofurazan. J. Food Sci. 39:420.

Walker, E.A., Pignatelli, B. and Castengnaro, M. 1979. Catalytic effect of p-nitrosophenol on the nitrosation of diethylamine. J. Agr. Food Chem. 27:393.

Walters, C.L., R.J. Hart, and S. Perse. 1979. The possible role of lipid pseudonitrosites in nitrosamine formation in fried bacon. Z. Lebensm. Unters. Forsch. 168:177.

Walters, C.L. 1980. Nitrosamines in meat products, A chapter, In: Meat Science, Lawrie, R.A. (ed.), 3rd edition, Pergamon Press, Oxford, p. 195.

Wartheson, J.J., Bills, D.D., Scanlan, R.A. and Libbey, L.M. 1976. N-Nitrosopyrrolidine collected as a volatile during heating-induced formation in nitrite containing pork. J. Agric. Food Chem. 24:892.

Wasserman, A.E. 1966. Organoleptic evaluation of three phenols present in wood smoke. J. Food Sci. 31:1005.

White, J.W. Jr. 1975. Relative significance of dietary sources of nitrate and nitrite. J. Agric. Food Chem. 23:886.

White, J.W., Jr. 1976. Relative significance of dietary sources of nitrate and nitrite. J. Agric. Food Chem. 24:202.

Wishnok, J.S. 1979. Quantitative aspects of exposure and mechanism in N-nitrosamine carcinogenesis. In: N-nitrosamines. Anselme, J.P. (ed.), Am. Chem. Soc. Symp. Series 101, Washington, D.C., p. 154.

Witter, J.P., Gatley, S.J. and Balish, E. 1979. Distribution of nitrogen-13 from labeled nitrate. ($^{13}NO_3^-$) in human and rats. Science 204:411.

Woolford, G. and Cassens, R.G. 1977. The fate of sodium nitrite in bacon. J. Food Sci. 42:586.

World Health Organization. 1978. Nitrates, Nitrites, and N-Nitroso Compounds: Environmental Health Criterion No. 5. World Health Organization, Geneva, Switzerland. Available from WHO Publications Center, Albany, New York, p. 107.